Phytochemical Investigation of methanolic extract of *Polygonum lanatum*

(Fam- Polygonaceae)

B. PHRM Project

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June 2015

EAST WEST UNIVERSITY

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A project report submitted to the department of Pharmacy, East West University in conformity with the requirements for the Degree of Bachelor of Pharmacy

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In the name of

Almighty Allah,

The most Gracious

&

The most Merciful

This Book is dedicated to

My Parents

And

Teachers

Acknowledgement

I am deeply thankful and grateful to the **Almighty Allah** for helping me in all critical situations to reach at this end stage of my undergraduate research program.

Then, I would like to express my most sincerely appreciation to **Dr. Muniruddin Ahmed**, Professor & Advisor, Department of Pharmacy, East West University. Dhaka. I feel so fortunate and pleasant to have him as my research supervisor.He impressed me not only by his profound and intelligent knowledge, but also by his generosity, wisdom, patience, and the capability to grasp the key points from millions of minutiae. I would not have brought this dissertation to reality without his countless encouragement, support and kind direction.

Then I have to confess the contribution of **Dr. Repon Kumer Saha**, Assistant Professor, Department of Pharmacy, East West University, Dhaka, who brought me into an amazing world where I have learned many advanced techniques. His serious attitudes in work, extensive knowledge, eagerness to teach updated techniques inspired me in every steps of my research work.

I express my sincere thanks to **Dr. Chowdhury Faiz Hossain**, Professor and Chairperson, Department of Pharmacy, East West University, Dhaka for extending his guidance and sincere support in many ways.

I owe my deepest gratitude to all of my **Respected Teachers** of the Department of Pharmacy, East West University, Dhaka for their continuous support, affection and sincere advice to complete my investigation.

I also acknowledge the support and help of **Laboratory assistants and Employees** of the Department of Pharmacy, East West University, Dhaka in doing my research work.

Lastly my special regards and gratitude go to **Shawqi vai, my Parents, my research partner Riduan** and **my roommates Rabby and Raju** for their support and appreciation throughout my work.

Sabbir Ahmed

CERTIFICATE

This research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy (B.Pharm) was carried out by Sabbir Ahmed (ID # 2011-3-70-031).

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CERTIFICATE

This is to certify that the thesis "Phytochemical Investigation of methanolic extract of *Polygonumlanatam (*Fam- Polygonaceae*)"* is submitted to the Department Of Pharmacy, East West University, Aftabnagar, Dhaka in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B.Pharm) was carried out by Sabbir Ahmed (ID # 2011-3-70-031) under my guidance and supervision and that no part of the thesis has been submitted for any other degree. I further certify that all the sources of information and laboratory facilities availed of this connection is duly acknowledged.

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Abstract

The objective of this research is to study the phytochemical properties of methanolic extracts of *Polygonum lanatum*, a plant of Polygonacea family. The phytochemical evaluation was carried out by performing several test TLCs, preparative TLC, & IR test. *Polygonum lanatum,* a tropical herb, is commonly known as Bishkatali. The plant belongs to family Polygonacea, possessing analgesic, diuretic and anti-inflammatory activity. The plant is distributed in Southeast Asia. Isolation of sterols, terpenoids, flavonoid glycosides, coumaryl glycoside, lignans, quinones, 6-methoxyplumbagin was resulted through the phytochemical investigation on this plant. The leaves are slightly bitter, cure inflammation, skin diseases, laxative, bronchitis, nervous affection, leucoderma, biliousness and good in small pox. The plant possesses nutritional value including-carotene, saponins, cholesterol, glucoside, enhydrin and so on. It is reported that plant possesses Antioxidant, Hepatoprotective, CNS Depressant, Analgesic and Antidiarrheal activity. Thin-layer chromatography (TLC) is a mature and very established technique, frequently used in many fields of applications ranging from natural product analysis to chemical or pharmaceutical applications. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. TLC & IR test performed to investigate bioactive natural compounds from *P. lanatam*. Some bands were observed through those tests.

Chapter 1: Introduction

1.1 General Introduction

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various. (Farnsworth and Fabricant, 2001)

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people. Herbal medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. The potential of medicinal plants can be assessed by finding new chemical entities of wide structural diversity. These new chemical substances can also serve as templates for producing more effective drugs through semi-synthetic and total synthetic procedure. According to World Health Organization (WHO), about 74% of 119 plant-derived pharmaceutical medicines or biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses (Mukeshwar and Mousumi, 2011). In Bangladesh, ninety percent of the medicinal plants are wild sourced. Out of approximately 5,000 species of indigenous

and naturalized phanerogamic and pteridophytic plants growing in the country, more than a thousand of them, including many food, vegetable, beverage, spice & ornamental plants, contain medicinally useful chemical substances (Mia, 1990).

Growing in the forests, jungles, wastelands, and along roadsides the types of medicinal plants in Bangladesh are varied. A total of 546 medicinal plants that occur in the country have been counted so far. However, this list is not exhaustive since it is believed that many other medicinal plants also grow there, but have not yet been discovered or identified (Yusuf et al., 1994).

The word drug itself comes from the Dutch word "droog" (via the French word Drogue), which means 'dry' because most drugs (medicines) came from dried plants. Some examples are quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Medicinal plants are used as a source of drugs for the treatment of various human and livestock health disorder all over the world from ancient time to the present day. A total of 25,000 species of flowering plants are referred to as medicinal plants. The World health Organization (WHO) enlisted some 21,000 plants species (Penso et al., 1980).

The present global herbal market is worth about US$ 62 billion per annum. The annual growth of herbal market is about 15% and the global herbal market by 2050 is expected to be about US$ 5 trillion (WHO, 2002).

In Bangladesh, about 500 plant species have been identified as medicinal plant because of their therapeutic properties. In the meantime, a large number of industries (400 herbal factories) have been established in the country for producing Ayurvedic and Unani medicines. It has been estimated that Bangladesh has a market of about 100 core taka worth herbal products annually. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for their treatment of various diseases. However, scientific studies have been conducted only to a limited extent with few medicinal plants. (Ghani, 2003)

1.2 Rationale and objective of the work

Medicinal components from plants play many important roles in traditional medicine. It is estimated that there are about 2,500,000 species of higher plants and the majority of these have not yet been investigated in detail for their pharmacological activities (Ram et al., 2003).

In developing countries, about 80% of the population relies on traditional medicine for their primary health care (Matu and Staden, 2003).

Since Bangladesh has a vast resource of medical plants and majority of our population has to rely upon indigenous system of medication from economic point of view. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicine usually exists side by side with western forms of health care. (Munguti, 1997)

Identification and isolation of the active constituents from traditionally used phyto-therapy can ensure the health care of the poor people. In addition, herbal k2drugs could be scientifically modified for better pharmacological activity and to establish safe and effective drugs and the rationality of the present study lies in meeting the challenge of developing herbal medicines, which needs a systematic research on indigenous medicinal plants for the welfare of the humanity. There are several familiar approaches for lead searching from the plants and the isolated bioactive compounds are utilized in three basic ways (Cox *et al*., 1994).

These can be stated as Unmodified natural plant products where ethno medial uses suggested clinical efficacy, e.g., digitalis. Unmodified natural products of which the therapeutic efficacy was only remotely suggested by indigenous plant use, e.g. vincristine. Modified natural or synthetic substances based on a natural product used in folk medicine, e.g., aspirin.

1.3 Phytochemistry:

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs—examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. Toxic plants even have use in pharmaceutical development.

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs.

Alkaloids are a class of chemical compounds containing a nitrogen ring. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimeric galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arhythmia compound quinidine; the anti-asthma therapeutic ephedrine; and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

Polyphenols (also known as phenolics) are compounds contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.

Glycosides are molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore.

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, which are often strong smelling and thus may have had a protective function. They are the major components of resin, and of turpentine produced from resin. (The name "terpene" is derived from the word "turpentine"). Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

A consortium of plant molecular researchers at Washington State University, the Donald Danforth Plant Science Center, the National Center for Genome Resources, and the University of Illinois at Chicago began an NIH-sponsored study of over thirty medicinal

plant species late 2009. The initial work, to develop a sequence reference for the transcriptome of each, has led to the development of the Medicinal Plant Transcriptomics Database.

1.4 Medicinal Plant

"Medicinal Plants *are plants that provide people with medicines - to prevent disease, maintain health or cure ailments." In one form or another, they benefit virtually everyone on Earth. No exact definition of a Medicinal Plant is possible. There are related issues, such as for nutrition, toiletry, bodily care, incense and ritual healing.*

1.4.1 History of medicinal plants:

The use of plants as medicine predates written human history. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. The use of herbs and spices in cuisine developed in part as a response to the threat of food-borne pathogens. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected. In all cultures vegetables are spiced less than meat, presumably because they are more resistant to spoilage. Angiosperms (flowering plants) were the original source of most plant medicines. (Sherman et al, 2001)

Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed, have medicinal properties.

A large amount of archaeological evidence exists which indicates that humans were using medicinal plants during the Paleolithic, approximately 60,000 years ago. Furthermore, animals such as non-human primates, monarch butterflies and sheep are also known to ingest medicinal plants to treat illness. Plant samples gathered from prehistoric burial sites are an example of the evidence supporting the claim that Paleolithic peoples had knowledge of herbal medicine. For instance, a 60 000-year-old Neanderthal burial site,

"Shanidar IV", in northern Iraq has yielded large amounts of pollen from 8 plant species, 7 of which are used now as herbal remedies. (Edward et al, 1986)

The deliberate placement of flowers has been challenged. Paul Pettitt has stated that the "deliberate placement of flowers has now been convincingly eliminated", noting that "A recent examination of the microfauna from the strata into which the grave was cut suggests that the pollen was deposited by the burrowing rodent Meriones tersicus, which is common in the Shanidar microfauna and whose burrowing activity can be observed today". Also medicinal herbs were found in the personal effects of Ötzi the Iceman, whose body was frozen in the Ötztal Alps for more than 5,000 years. These herbs appear to have been used to treat the parasites found in his intestines. (Solecki and Ralph, 1975)

Ancient time: In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who created clay tablets with lists of hundreds of medicinal plants (such as myrrh and opium). In 1500 B.C., the Ancient Egyptians wrote the Ebers Papyrus, which contains information on over 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake.

In India, Ayurveda medicine has used many herbs such as turmeric possibly as early as 1900 BC. Earliest Sanskrit writings such as the Rig Veda, and Atharva Veda are some of the earliest available documents detailing the medical knowledge that formed the basis of the Ayurveda system. Many other herbs and minerals used in Ayurveda were later described by ancient Indian herbalists such as Charaka and Sushruta during the 1st millennium BC. The Sushruta Samhita attributed to Sushruta in the 6th century BC describes 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources.

The mythological Chinese emperor Shennong is said to have written the first Chinese pharmacopoeia, the "Shennong Ben Cao Jing". The "Shennong Ben Cao Jing" lists 365 medicinal plants and their uses - including Ephedra (the shrub that introduced the drug ephedrine to modern medicine), hemp, and chaulmoogra (one of the first effective treatments for leprosy). Succeeding generations augmented on the Shennong Bencao

Jing, as in the Yaoxing Lun (Treatise on the Nature of Medicinal Herbs), a 7th-century Tang Dynasty treatise on herbal medicine.

The earliest known Greek herbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have survived intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates (e.g. De herbis et curis) and - especially - Galen (e.g. Therapeutics), provided the pattern for later western medicine. Sometime between 50 and 68 A.D., a Greek physician known as Pedanius Dioscorides wrote Περὶ ὕλης ἰατρικῆς (commonly known by its Latin title De Materia Medica), a compendium of more than 600 plants, 35 animal products, and ninety minerals. De Materia Medica remained the authoritative reference of herbalism into the 17th century. Similarly important for herbalists and botanists of later centuries was Theophrastus' Historia Plantarum, written in the 4th century BC, which was the first systematization of the botanical world. (Sumner and Judith, 2000)

1.4.2 Contribution of medicinal plants

All culture from ancient timk6es to the present day has used plants as sources of medicine. Today the majority of the world's population continues to rely on medicinal plants for the health care needs. Cultural global demand for herbal product is vast and growing. The habitants that supports medicinal plant are being rapidly degraded or destroyed for instance by over grazing or conversion to agricultural land. Many plants synthesize substance that is useful to the maintenance of health in human and other animals. These include aromatic substance, most of which are phenols or their oxygen substituted derivatives such as tannins. Many are secondary metabolites .of which at least 12000 have been isolated –a number estimated to be less than 10% of the total. In many cases, substances such as alkaloids serve as plant defense mechanism against predation by microorganism, insects, and herbivores. Many of the herbs and species used by humans to season food yield useful medicinal compounds. (Falco et al., 2003)

Plants up regulate and down regulate their biochemick2al paths in response to the local mix of herbivores, pollinators and microorganisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. It is the secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs.

Some examples are given below:-

Table 1.1: The list of the medicinal substance derived from plants.

Drugs /chemical Name	Action /Clinical use	Plant source
Atropine	Antichlolinergic	*Atropa belladonna*
Arecoline	antihelmintic	*Areca catechu*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubifacient	Cinnamonum acemphore
Codeine	Analgesic, Sedative	*Papaver somniferum* linn.
Digitoxin,Digoxin	Cardiotonic	Digitalis purpurea Lin. Digitailis lanata
Ergotametrine,Ergotamine, Ergotoxin	Vasoconstrictor,ergotamine,	Claviceps purpurea. Tul
Hyosine Hyosinamine	Parasympatholytic,Mydriatic,anti spasmodic	Datura , hyocyamus, scopolia ,

1.4.3 Medicinal plant users

It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either in part or entirely. For many this is out of necessity, since many cannot afford the high costs of pharmaceutical drugs. Growing numbers of American health care consumers are turning to plant medicines for many reasons - low cost and seeking natural alternatives with fewer side effects are commonly cited.

1.4.4 Reasons behind popularity of plant medicine

1.4.4.1 Historic use:

Based upon the traditions of Europeans, Chinese, Egyptians, American Indians, and other cultures, Information on plant medicines handed down over the centuries. Currently anthropologists and other scientists are investigating newly discovered cultures (visits with shamans in Amazonia and Belize, for example), to determine what plants are being used - this "ethnobotany" has introduced a number of new compounds into pharmaceutical research (Joy et al., 1998)

1.4.4.2 Extrapolation

Extrapolation can be referred to the process by which a biological test is conducted thinking that some activity in vitro (in the test tube or in the lab) implies major activity in humans. The reasoning involved in predicting a conclusion or making a logical judgment on the basis of circumstantial evidence and prior conclusions rather than on the basis of direct observation.

1.4.4.3 Phytomedicinal prospecting

Screening plants for biological activity. Huge projects are currently underway by such organizations as INBio, Costa Rica's National Institute of Biodiversity. INBio is cataloging all species of plants and animals in the country – estimated to be around

500,000! To complete this ambitious task they train "community taxonomists" to identify plants and animals (Christopher et al., 2008).

1.4.4.4 Current Legal Status in the US

US currently have an "open market" for anything labeled as a "nutritional supplement". This is both good and bad - it means "caveat emptor" - let the buyer beware! Unfortunately the buyer may occasionally be gambling with their life if they are not making knowledgeable choices. Since "nutritional supplements" are not sold as a treatment for a specific problem, they buyer must be informed. The bad aspect of this open market approach is that even products with proven health benefits may not be marketed for that use unless the producer has gone through the FDA protocols for determining safety and efficacy. The best example, perhaps, of this restriction is that it would be absolutely illegal to sell prune juice as a treatment for constipation. While no one would argue that it is safe and effective, no one has gone through the rigorous FDA approval process. A current controversy surrounds the sale of products containing natural sources of ephedrine. Ephedrine is an adrenaline-like compound found in some plants, especially ephedra species such as Ma Huang and Mormon Tea. This stimulant is found in "thermogenic" weight loss products and also sold as a "legal amphetamine" under such names as "Herbal Ecstasy". A number of people who have taken excessive amounts of this drug have died. The FDA is currently mulling over the idea of taking it off the market. Manufacturers are hurrying to change the labels and provide warnings in an effort to prevent FDA action.

1.4.4.5 Current Industry Problems

Is the "natural" product you are buying really what it says it is??? This problem has long plagued the industry. When some dried root or leaf products sell for $80 per pound, the temptation to add a little extra something along the way goes from the harvesters to the middlemen to the retailers - all of whom are paid by the pound.

There have been studies of retail ginseng, for example, which show numerous purchased products with no ginseng content whatsoever! Perhaps these products rely on the power of suggestion! Looking at this more innocently, a peasant in the Amazon may not know one plant from a similar-looking cousin. The industry is establishing standards in an effort to improve its credibility – some products, such as ginkgo, are now standardized and offer some uniformity.

1.4.5 A Few examples which might be useful

Garlic:

Garlic has been shown to reduce cholesterol about 12% and to reduce platelet adhesiveness (the reason an aspirin a day is recommended to reduce the risk of stroke and heart attack). Huge controversy exists over what forms (other than fresh) provide sufficient allicin (the active ingredient) activity. Fresh garlic tends to cause heartburn and social consequences. Garlic preparations in oil have been found to be relatively useless in some studies. Coated tablets are probably good - the "odorless" kind. Some experts, however, say "if it doesn't stink, it doesn't work". The equivalent of 4 to 5 cloves per day is recommended. A 1992 German study of 18 commercial preparations showed significant allicin activity in only five.

Ginkgo:

Flavone glycosides ("ginkgolides") have been shown to reduce capillary fragility and decrease blood cell transit from inflamed capillaries. They have been shown to reduce "PAF" - platelet activating factor". These glycosides are also potent anti-oxidants. Ginkgo preparations are used in Germany to treat "reduced intellectual capacity and vigilance" associated with aging, as well as to treat peripheral vascular disease. Available in Germany in liquid, tablet, and injectable forms, in 1988 over 5.2 million prescriptions were written – even though it is also available over-the-counter. Germany's Commission E, established to study plant medicines, declared ginkgo as safe and effective with very

few side-effects (occasional headache). It should be taken three times daily with meals. HYPE ALERT: Ginkgo is being widely advertised as a product which will improve mental functioning of anyone who takes it - this is NOT TRUE! Studies in the elderly have shown some benefit, whereas studies in middle-aged and younger people have not shown any significant benefit.

Cranberry Juice or Extract:

This and related *Uva Ursi* extract has been shown to inhibit the activity of "adhesin", which is needed for the pili, or tiny hairs, on the *E coli* bacteria to stick to the cells lining the urinary tract. They may therefore be useful in the treatment and prevention of bladder infections with those bacteria. **(**Mollik, 2010)

Valerian:

The roots and rhizomes of *Valeriana officianalis* have been used as a sleep aid for perhaps over one thousand years. Its effectiveness as a minor sedative or calmative continues to be confirmed. It is safe and effective and a good recommendation for those with minor difficulties or in whom an addictive alternative might be risky. It is frequently combined with hops and/or passionflower, two other safe and effective sedating herbs. Historical note: when the Latin name of a plant has the term *officianalis* in it, it means that it was grown in the monks' garden and kept in the office for medicinal uses.

1.5 Plant based prophylactic agents

Plant based drugs are organic compounds derived from plants that alter normal body functions. Plant drugs are distinct from plant based nutrients. Apart from nutrients and molecules that have direct effect on human body, there are several non-nutritive organic compounds, in plants, having no apparent detrimental action on human body when consumed with food in the natural concentration. Many compounds belong to this group act as prophylactic agent (Prophylaxis: treatment given or action taken to prevent disease

(Simpson and Weiner, 2010)) in the system and help to protect the body from several degenerative diseases caused, especially by oxidative stress in consequent to metabolism. A cross cultural population comparison study showed that plasma levels of antioxidant molecules viz. vitamin C, β carotene, vitamin E and selenium were significantly higher in men, 40-49 aged, from Switzerland and Italy compared to their counterpart in Finland and Scotland (Gey, 1987). In that study, the author highlighted that there is an antioxidant index with respect to antioxidant molecule level in each subjects. The antioxidant molecule's levels in such subjects correlates with a positive antioxidant index and this relation was inversely related to mortality rates by ischemic heart diseases (IHD). The antioxidant hypothesis evolved from this study with the proposal that high intake of dietary antioxidants prevent oxidation of plasma and thereby oxidative stress (Gey, 1987). In the last decade, considerable worldwide attention has been given to plant phenolic antioxidants including flavonoids and various other phytochemicals found in many fruits and vegetables, red wine, etc. for their protective effect against the damage from oxidative stress. The literature on antioxidants has expanded tremendously because of accumulation of evidence that they may contribute to the recognized extra nutritional benefits of food and beverages containing phenolic compounds (Frankel and German, 2006; Kroon and Williamson, 2005). Prophylactic activities of phytochemicals are attributed to their antioxidant/radical scavenging activities to retard oxidative stress caused by free radicals in vivo.

Free radicals are highly reactive chemical entities normally produced but often

Over produced in all higher organisms. When free radicals are excessively produced, it can damage biomolecules viz. fatty acid, protein and DNA and can be one among many reasons for the early incidence of degenerative disease.

1.6 Etiology of chronic degenerative diseases; special attention to free radicals:

With increase in life expectancy, chronic degenerative diseases have become by far the principal cause of death world over. The highest mortality (26.3%) was attributed to cardiovascular diseases (CVD). The next highest mortality was due to malignant neoplasms (24.1%). Other causes were; chronic lower respiratory diseases (4.9%), Diabetes mellitus (3%), and Alzheimer's disease (1.8 %) followed by others (Heron, 2010). In most cases, the etiology is highly complicated and multi-factorial such as genetic, environmental, occupational, dietary habit, lifestyle etc. In all above said factors, free radical production is a common phenomenon that speeds up the onset and progression of degenerative diseases. Free radical theory of aging argues that oxygen free radicals produced during normal respiration would cause cumulative damage which would eventually lead to organism's loss of functionality and ultimately death (Harman, 1972; Harman, 1992). However evidences to substantiate this hypothesis lack at present. During the last 3 decades huge body of literatures have been published that correlate free radicals and onset of cancer, CVD, diabetes, cataract etc. It can be presumed that free radicals along with several other risk factors may accelerate the onset and progression of several degenerative diseases. One of the approaches thus could be to use free radical scavengers / antioxidants to prevent or retard onset and progression.

1.7 Free radical mediated oxidation of biomolecules

The biomolecules are more prone to oxidative stress. These undergo structural and subsequent functional changes when exposed to oxidative stress. The changes lead to the abnormalities in the physiological homeostasis and leads to many pathological conditions.

1.8 Lipid peroxidation: Lipid is probably the most studied substrate for oxidation by free radicals in biological system. Oxidation of lipid in no enzymatic mode is mainly

by free radicals and ROS. The free radicals that induce lipid oxidation are superoxide radical, peroxide radical and nitric oxide. Superoxide does not abstract hydrogen atom even from very reactive bis-allylic methylene groups (Afanas'ev, 1989; Bielski et al., 1983), although it's conjugated acid HOO• is more active in hydrogen atom abstraction and probably capable of initiating the lipid peroxidation (Bielski et al., 1983). Though the superoxide is too inert to initiate lipid peroxidation, it can initiate lipid oxidation through other ways such as by reduction of ferric to ferrous ion that catalyzes the Fenton's reaction. Subsequent studies showed that formation of hydroxyl radicals, even if it take place during lipid peroxidation, are of no real importance and that have been reported by several authors (Bast and Steeghs, 1986; Beloqui and Cederbaum, 1986; Gutteridge, 1982; Vile and Winterbourn, 1987). The possibility of hydroxyl radical dependent lipid peroxidation was studied earlier and reported that hydroxyl radicals are involved in the NADPH dependent microsomal lipid peroxidation (Lai and Piette, 1977). Peroxy radicals especially neutral, positively and negatively charged alkyl peroxyl radicals are more efficient initiators of LDL oxidation compared to that of superoxide (Bedard et al., 2001). NO is also incapable of abstracting hydrogen atom from unsaturated substrates similar to superoxide, but forms various other reactive species capable of initiating lipid oxidation. The pro-oxidant effect of NO depends on the relative concentration of NO and oxygen, the direct interaction of NO with free radicals formed in the lipid peroxidation and conversion of NO into peroxy nitrites or other reactive NO metabolites (Bloodsworth et al., 2000; O'Donnell et al., 1997; Rubbo et al., 1994).

1.9 Amino acid oxidation: Amino acids are also more sensitive to free radicals and ROS damage. Oxidation of amino acid moieties of functional and structural protein leads to the inactivation of enzymes, receptors, hormones, loss of structural integrity etc. Free radicals cause fragmentation of protein and cross linking of amino acids by hydroxyl radicals in the absence of dioxygen.(Dean et al., 1986).

Alpha-position of the simple aliphatic amino acid or amino acid residue in the polypeptide chain is more prone to the hydroxyl radical-mediated abstraction of hydrogen atom. As the number of carbon atoms in an amino acid increase, that amino acid would be more prone to free radical mediated cross link with other aliphatic amino acids. In the case of aromatic amino acids, the ring is the primary site of attack leading to ring scission, and in the case of tyrosine, to the formation of Tyr-Tyr cross-linked dimmers (Stadtman, 1993).

Davies et al. reported that superoxide radical alone does not damage amino acids but, in the presence of hydroxyl radicals, it causes several fold damage to the protein compared to that of the damage caused by hydroxyl radical alone (Davies, 1987).

ROS mediated oxidation of amino acids can also lead to hydroxylation of aromatic groups and aliphatic amino acid side chains, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulfoxidation of methionine residues, chlorination of aromatic groups and primary amino groups, and to conversion of some amino acid residues to carbonyl derivatives (Stadtman and Levine, 2003)

1.10 D'A oxidation: DNA is another target for ROS, RNS, free radicals, transition metals, etc. Reactions of hydroxyl radicals with DNA have been thoroughly studied based on the effect of ionizing radiations on DNA. One important attribute of free radical mediated damage to DNA molecule is the multiple ways of free radical attack. Mostly the DNA damage happens through base modification (Aruoma et al., 1989; Halliwell and Aruoma, 1991).

Another important mode of DNA damage is the oxidative stress induced strand breaks.

Hydroxyl radicals can easily abstract hydrogen atom from the ribose of sugarphosphate backbone and causes single strand break (Breen and Murphy, 1995).

Though the single strand breaks are not very harmful, double strand breaks can cause cell death. The double strand breaks are formed because of multiple hydroxyl radical attack (Ward, 1985). Mitochondrial DNA is more sensitive to

ROS compared to nDNA. Both xanthine oxidase and menadione generated oxygen radicals caused severe damage to mtDNA with no significant effect on nDNA (Grishko et al., 2001). This theory is supported by the evidence that the lack of histone proteins in the mtDNA makes this more susceptible to ROS induced damage (Ballinger et al., 2000; Yakes and Houten, 1997). Reactive nitrogen species is also known to have the capacity to induce damage to DNA.

Amount of nitric oxides generated by interleukin-1-B-induced nitric oxide synthase is sufficient to cause damage to DNA in many cell line in vitro (Delaney et al., 1993).

1.11 Consequences of free radicals and ROS mediated oxidation of biomolecules

Free radicals, ROS and RNS cause irreversible damage to biomolecules such as fatty acid, amino acid, and DNA. Oxidative modification / damage of these molecules lead to the onset of several degenerative diseases such as cancer, vascular diseases, diabetes, inflammation etc.

1.11.1 Cancer: The possible role of free radicals in cancer has been discussed based on the discovery of excess production of free radicals in tumor cells (Saprin et al., 1965).

Further, the discovery of superoxide in biological system and superoxide dismutase attracted much attention towards the association of free radicals and carcinogenesis. Studies in expression level of SOD gene in normal and cancer cells pointed out this association because, the tumor cells express low levels of SOD (Oberley, 1982). In addition, Mn SOD is not expressed at all in cancer cells even at elevated level of superoxide (Oberley and Oberley, 1988).

Consequently, elevated superoxide cause DNA damage and thus initiate carcinogenesis (Nakamura et al., 1988). During initiation process, the involvement of free radicals were emphasized based on the fact that organic peroxides promotes carcinogenesis (Floyd, 1990). Further, ROS and RNS react with guanine and forms 8-OHdG (Kim et al., 2003).

The role of 8-OHdG in the process of carcinogenesis is well established (Floyd, 1990; Ogawa et al., 1995)

Wei and Frenkel, 1993) by its potential to mutate a few cancer related genes and transformation of proto-oncogenes to oncogenes (Cerutti, 1994).

Another study reported that enhanced production of hydroxyl radicals can initiate carcinogenesis, in particular, cologenic hydroxyl radical and other ROS generation is considered as one important factor for the cause of colorectal carcinoma (Babbs, 1990). The involvement of inflammation, especially inflammatory phagocytes, on the cancer promotion has been understood very long time back. During inflammation, stimulated macrophage induced DNA damage supposedly through the generation of free radicals (Chong et al., 1985).

The ROS, RNS, and various other carcinogens together may change the normal cellular genome to neoplastic one at the onset of cancer.

1.11.2 Diabetes: The involvement of free radicals in the development of diabetes is a core research area in the epidemiological studies of diabetes. Much study has been done on the free radicals in biology and diabetes independently. However, the number of comprehensive studies to understand the involvement of free radicals in the etiology of diabetes is very few. Type 1 diabetes is caused by destruction of pancreatic beta cells responsible for the production of insulin. In human the diabetogenic process is caused by immune destruction of beta cells; part of this process is apparently by white cell production of ROS. Well established evidence is the experimental diabetic inducing agents; alloxan and streptozotocin. Though the mechanism of action of these two compounds are different, both results in the production of ROS. The presence of ROS scavengers effectively inhibited the development of diabetes in these compound induced diabetic models (Josefsen et al 1988) demonstrated that the circulating monocytes in newly diagnosed type-1 diabetes were activated which could play a very important role in the destruction of β-cells of pancreas (Josefsen et al., 1994). These monocytes were reported to produce excess superoxide in patients with early hypertriglyceridemia and

diabetes (Hiramatsu and Arimori, 1988). In type-2 DM also the plasma redox balance is disturbed and oxidative stress is observed.

This is evidenced by several fold reduced plasma superoxide dismutase level and other endogenous antioxidants in type-2 DM patients compared to that of

nondiabetic control (Collier et al., 1990). Thus the oxidative stress caused at the onset of type-2 DM may promote the progression of pancreatic cell damage as well as leads to higher prevalence to mortality from CVD (MacRury et al., 1993).

Vascular diseases: Vascular diseases such as atherosclerosis, peripheral artery disease, hypertension, peripheral vascular disease etc. are caused by xenobiotics, physical inactivity, unhealthy diet etc. However the free radicals and ROS in the vascular system promote the onset as well as progression. The role of free radicals in the etiology of a few vascular diseases are presented in the following sections Ischemic reperfusion injury: Hypoxia and reoxygenation generally causes injury to cells (Li and Jackson, 2002). The major cause of circulatory shook, myocardial ischemia, and stroke are believed to be reoxygenation. During reoxygenation, large amount of ROS especially superoxide (Kim et al., 2002; Kowaltowski and Vercesi, 1999; McCord, 1985) and hydroxyl radicals (Werns et al., 1985) are formed and recognized as the cause of reoxygenation injury. Formation of ROS under this pathological conditions were established by several studies (Das et al., 1986; Hess et al., 1982; Werns et al., 1985).

1.11.3 Atherosclerosis: A large number of reports emphasize that excess superoxide play an important role in the onset of atherosclerosis and hence promote endothelial dysfunction (Kojda and Harrison, 1999). Moreover the oxidized proteins, lipids, LDL and nucleic acids as a result of plasma oxidative stress also promote the progression of vascular tissue damage (Beckman and Koppenol, 1996) and atherosclerotic plaque formation (Stocker et al., 2004).

Promotion of atherosclerosis as a result of reduced expression of extracellular SOD and mutation in endothelial is an important evidence for the role of ROS in vascular diseases (Faraci and Didion, 2004; Fukai et al., 2002; Landmesser et al., 2000).

1.11.4 Hypertension: The possible role of free radicals in the pathogenesis of atherosclerosis and hypertension has been suspected for long time. This was evidenced by low serum antioxidant capacity and hypertension (Salonen et al., 1988), high serum antioxidant capacity and low level of atherogenic protein (Nyyssonen et al., 1994). correlation between antioxidant supplement and normotension (Salonen et al., 1994) etc.

Inflammation: Under chronic inflammatory condition, a large number of ROS are produced. Superoxide thus produced stimulate the release of IL-1 from blood monocytes (Kasama et al., 1989). IL-1 act as feedback booster and in turn increases the formation of excess ROS in the vicinity (Babior et al., 1973).

The excess ROS thus produced oxidize lipoprotein, lipids, protein etc. and accelerate atherogenic processes in the vascular system (Zhang et al., 2002), induce carcinogenesis (Coussens and Werb, 2002), neurodegeneration (Akiyama et al., 2000) etc.

Life expectancy: Aging is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age. Among the several ageing theories proposed, the "free radical theory of aging" (Harman, 1956; Harman, 1992) has gained universal acceptance and is supported by the fact that the sum of the deleterious free radical reactions going on continuously throughout the cells and tissues constitutes the aging process (Sohal and Weindruch, 1996). The free radial theory is supported by the "rate of living" hypothesis, which links metabolic rate and subsequent free radical production with the short lifespan of organisms (Ku et al., 1993). Under vigorous metabolism, free radicals and ROS are produced and can damage proteins, DNA and lipids and this oxidation process accelerate aging process (Barja and Herrero, 2000; Sohal et al., 1995; Sohal and Weindruch, 1996). Caloric restriction and thereby reducing free radical production and oxidative stress has been shown to increase lifespan in animal studies (Agarwal and Rao, 1998; Sohal and Weindruch, 1996). The inverse relation between free radicals and oxidative stress versus longevity is reported by several studies (Buchan and

Sohal, 1981; Merry, 2004; Yan and Sohal, 2000). Other studies also supported this relation indirectly such as low cellular superoxide and hydrogen peroxide production, as a result of high antioxidant enzymes level, and maximum life span (Barja, 1998; Ku et al., 1993). Genetic studies viz. over expression of superoxide dismutase in transgenic flies, catalase enzyme in *C. elegans*, mitochondrial catalase enzyme in mice etc. support the relation between free radicals and longevity (Melov et al., 1995; Tower, 2000).

1.12 Scavenging of free radicals and other ROS

In a physiological system, free radicals are formed as a part of normal metabolism and by exogenous factors, and the antioxidant defense system continuously scavenges the excess oxidants, ROS and free radicals formed. The free radical production and its removal is taking place in a balanced condition. When the free radical production is more and a corresponding removal is not done, the system would undergo a state called oxidative stress. The oxidative stress can be cellular, tissue level or in organ level and can be an important cause for early onset of various degenerative diseases as discussed in the previous section. The antioxidant defense system plays a vital role in removal of excess free radicals and maintains a balanced redox state. One important line of defense is a system of enzymes, including glutathione peroxidase, superoxide dismutase and catalase, which decrease concentrations of the most harmful oxidants in the tissues. Several essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes. Hence, if the nutritional supply of these minerals is inadequate, enzymatic defenses against free radicals may be impaired (Bagchi and Puri, 1998).

The second line of defense against free radical damage is the presence of nonenzymatic antioxidants. Antioxidants are a group of substances which when present at lower concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative process, while often being oxidized themselves (Vaya and Aviram, 2001). Antioxidants are stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. Some such antioxidants, including glutathione,

ubiquinol and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although about 4000 antioxidants have been identified, the best known are vitamin E, vitamin C and the carotenoids. Many other non-nutrient food substances, generally phenolic or polyphenolic compounds, display antioxidant properties and, thus, may be important for health (Bagchi and Puri, 1998). Both antioxidant defense systems jointly scavenge the excess free radicals produced in vivo. Therefore the antioxidant defense system helps to maintain health and protect from early onset of free radical mediated degenerative diseases.

1.13 Endogenous antioxidants:

ROS contribute to the formation of various pathological conditions. To counteract the effects of ROS, the body is endowed with a protective mechanism consisting of enzymatic and non-enzymatic endogenous antioxidants. Up regulation of enzymatic antioxidants have been reported to minimize free radical production and oxidative stress mediated tissue damage and hence the onset and progression of degenerative disease (Li et al., 2006).

Endogenous antioxidants are capable of different activities and work synergistically with exogenous antioxidants contributing the overall protective effect to the individuals by preventing or delaying the onset and progression of various free radical contributed degenerative disease (Serafini, 2006).

Following section presents about various endogenous antioxidant and their importance.

1.13.1 Superoxide dismutase: Superoxide dismutase is the first antioxidant enzyme involved in the antioxidant defense system found in higher organism and microbes (Fridovich, 1983; Fridovich, 1986) and the major function is the removal of superoxide radicals formed by various reasons.

1.13.2 Catalase: Catalase is a common peroxidase enzyme found virtually in all aerobic organisms that breakdown hydrogen peroxide which is produced by superoxide dismutase enzyme. Hydrogen peroxide is an important starting molecule for the production of hydroxyl radicals by Fenton's reaction. Catalase decomposes hydrogen peroxide into water and oxygen and thereby prevents the damaging effect caused by hydroxyl radicals (Deisseroth and Dounce, 1970). Glutathione peroxidase: Glutathione peroxidase (GPX) is ubiquitous selenium containing antioxidant enzyme in all higher organisms that catalyze the decomposition of hydrogen peroxide to water by utilizing reduced glutathione as hydrogen atom source. There are several isozymes differentially located in various organs. GPX-1 is present in the cytoplasm of all mammalian cells and its preferred substrate is hydrogen peroxide. GPX-2 is an intestinal and extracellular enzyme. The GPX enzyme, which is more abundant in extracellular fluid, especially plasma, is isozyme-3. isozyme-4 also expressed in all cells in less abundance whose preferred substrate is lipid hydroperoxids (Muller et al., 2007).

1.13.3 Glutathione reductase: Glutathione reduct ase (GR) catalyzes the reduction of oxidized glutathione. Oxidation of glutathione by GPX forms two glutathione molecules, which are linked to form glutathione disulfide (GSSG), a stable molecule. The reduction of glutathione disulfide catalyzed by GR produces two molecules of GSH, which is one important substrate for GPX for decomposing hydrogen peroxide to water. The substrate, GSH, used from the pool during the detoxification of hydrogen peroxide is maintained by the glutathione reductase.

Thiols: Thiols contain highly active SH group and therefore having antioxidant property. The most studied endogenous antioxidant thiols are lipoic acid and glutathione. Lipoic acid and dihydrolipoic acids are present in most kind of cells. Properties and therapeutic effects of LA and DHLA are well reviewed (Fuchs et al., 1997; Sies, 1997). DHLA is an efficient scavenger of all oxygen radicals; however, LA is active only in the reaction with highly reactive hydroxyl radicals.

1.13.4 Ubiquinones: Ubiquinones are essential electron carriers in the mitochondrial electron transport chain. They shuttle electron from NADH and succinate dehydrogenase to the cytochrome b-c1 complex. There are two types of redox interaction, in which ubiquinone can manifest their antioxidant activity: the reaction with quinone and hydroquinone formation. The antioxidant activities of biquinone has been demonstrated in vitro and in vivo studies (Filipe et al., 2001; Robak et al., 1986; Silva et al., 2000)

1.13.5 Uric acid: Uric acid is another physiologically important antioxidant. Uric acid contains two active hydroxyl groups in the purine heterocycle. The physiological level of uric acid protects erythrocyte against free radical damage (Ames et al., 1981). It is also a major antioxidant in human airway mucosal surface (Peden et al., 1990).

1.13.6 'ADPH: NADPH is an indirect antioxidant due to its capacity to reduce various oxidized substrates. Recent study showed that NADPH possesses scavenging capacity against free radicals such as CO3•-, NO2•, ROO• and RO• (Kirsch and Groot, 2001).

1.13.7 Melatonin: Melatonin is a pineal hormone, which is synthesized from tryptophan. Melatonin is an effective scavenger of hydroxyl radicals, nitric oxide and peroxy nitrite (Reiter et al., 2000). It is an effective inhibitor of iron initiated peroxidation of brain phospholipids liposome (Marshall et al., 1996).

1.13.8 Exogenous antioxidants:

Similar to endogenous antioxidants, some exogenous dietary compounds can neutralize the free radicals as well as enhance the activities of endogenous antioxidants. When the system is under oxidative stress and the endogenous antioxidants are not sufficient enough to scavenge the free radicals and ROS, the dietary antioxidants may be required

to maintain optimal cellular functions (Rahman, 2007). Some important dietary antioxidants are presented below.

1.13.9 α-Tocopherol: α-Tocopherol is a lipid soluble phenolic antioxidant with an active hydroxyl group. Several authors reported the high antioxidant and antiradical activities of α-tocopherol (Burton et al., 1983; Doba et al., 1985; Lambelet and Loliger, 1984; Scarpa et al., 1984). However similar to many other antioxidants, α-tocopherol also shows pro-oxidant action under certain conditions (Terao and Matsushota, 1986; Upston et al., 1999; Weinberg et al., 2001).

1.13.10 Vitamin C: Ascorbic acid is a highly active free radical scavenger and strong reducing agent. Oxidation and reduction reactions of ascorbic acid with numerous oxidants and reductants are well studied (Afanas'ev, 1989). Other than its antioxidant properties pro-oxidant activities also well studied. It is known that the competition between antioxidant and pro-oxidant activities of ascorbic acid depends on the rate of reaction (Afanas'ev et al., 1987; McCay et al., 1978). Ascorbic acid at lower concentration enhanced lipid peroxidation but inhibited at higher concentration (Afanas'ev et al., 1989). Presence of other factor also promotes the pro-oxidant activity of ascorbic acid. In the presence of Fenton's reactants, ascorbic acid promotes the hydroxyl radical production by redox cycling of iron ion (Benherlal and Arumughan, 2008).

1.14 Polygonaceae family

The plant kingdom continues to be a foremost source of novel natural products with potential for use as drugs or pharmaceutical mediators. According to The World Health Organization, more than 80% of the world population in developing countries depends primarily on plants based medicines for basic healthcare needs. Polygonum is a member

of Polygonaceae family that contains, according to respective taxonomic treatments; ca. 300 species is distributed worldwide in temperate climates. They vary widely from prostrate herbaceous annual plants under 5 cm high, others erect herbaceous perennial plants growing to 3 - 4 m, and yet others perennial woody vines growing up to 20-30 m high in trees. Several are aquatic, growing as floating plants in ponds.

The leaves are 1- 30 cm long, and vary in shape between species from narrow lanceolate to oval, broad triangular, heart-shaped, or arrowhead forms. The stems are often red-speckled or reddish. Flowers are small, white, pink or greenish, appearance in summer in dark cluster from the leaf joints or stem apices. A number of Polygonum species are used as food and for traditional folk medicines such as cardiovascular protection, antiinflammation, neuroprotection and mitigation of biochemical processes involved in age-related neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

Chemical constituents recognized in the Polygonum species are flavonoid, triterpenoids, anthraquinones, coumarins, phenylpropanoids, lignans, sesquiterpenoids, stilbenoids, and tannins. Amongst them, flavonoids are the most common components found in Polygonum and have previously been used as chemotaxonomic markers of the genus, playing an important role in the systematics of Polygonaceae species. Present study is an attempt to compile an up-to-date and comprehensive review of the genus Polygonum that covers its traditional medicinal uses, chemistry and pharmacology. Many plants of this genus are pharmacologically known but chemically unknown and vice-versa, therefore, the scope of future research in this aspect.

1.14.1 *Polygonum lanatum* :

Polygonum lanatum (Syn. *P. lanigerum*; Bengali name- Bishkatali; Family- Polygonaceae) is a herb, distributed in Eastern India, Bangladesh, Burma, Indonesia, Nepal and the Philippines. It is reputed for its antiinflammatory, analgesic and diuretic activities (Saha et al., 2005).

1.14.1.1 Chemical constituents:

Previous phytochemical studies with Polygonum species revealed the occurrences of a number of sterols (Fukuyama et al., 1983), terpenoids (warburganal and related drimanetype sesquiterpenoids, Fukuyama et al., 1982), flavonoid glycosides (Ahmed et al., 1988; Khoda et al., 1990), coumaryl glycoside (hydropiperoside, Fukuyama et al., 1983), quercetin glycosides (Fukuyama et al., 1983), lignans (Kim et al., 1994) and quinones (Fukuyama et al., 1983; 6-methoxyplumbagin, Al- Hazimi and Haque, 2002).

Figure: *Polygonum Lanatam*

1.14.1.2 Botanical description:

Annual ascending herbs, to 140 cm tall, densely lanate or tomentose throughout; stems much branched; nodes enlarged. Leaves lanceolate, 7-20 cm long, 1.5-3.5 cm wide, apex acuminate, base cuneate to narrowly cuneate; petioles narrowly winged, 0.2-1 cm long; ocreae tubular, 2-2.5 cm long apex truncate, upper margins short ciliate or eciliate. Inflorescences 5 or 6-flowered fascicles in spike-like panicles, terminal or axillary,

densily flowered, interrupted below, 4-8 cm long; peduncles 2-5 cm long; bracts lanceolate, apex acuminate , upper margins ciliate. Flowers greenish-white or pink; pedicels included; perianth segments 4, obovate, 2-3 mm long, apex obtuse; stamens 5 or 6, styles deeply 2-cleft included; nectaries present. Achenes brown to dark , lenticular, smooth, shining, 2-3 mm long.

1.14.1.3 Taxonomy:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Polygonales

Family: Polygonaceae

Genus: Polygonum

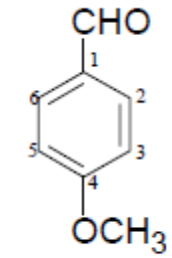
Species: *Polygonum lanatum*

1.14.1.4 Hazards:

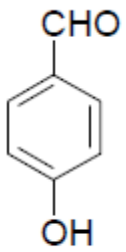
Although no specific mention has been made for this species, there have been reports that some members of this genus can cause photosensitivity in susceptible people. Many species also contain oxalic acid (the distinctive lemony flavor of sorrel) whilst not toxic this substance can bind up other minerals making them unavailable to the body and leading to mineral deficiency. Having said that, a number of common foods such as sorrel and rhubarb contain oxalic acid and the leaves of most members of this genus are nutritious and beneficial to eat in moderate quantities. Cooking the leaves will reduce their content of oxalic acid.

Chapter 2: Literature Review

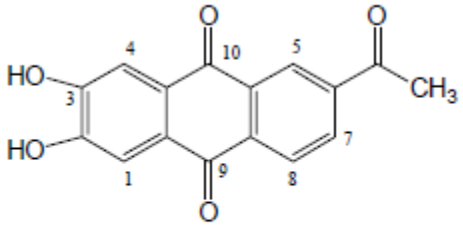
2.1 Chemical Constitutional review of *Polygonum Lanatum*:



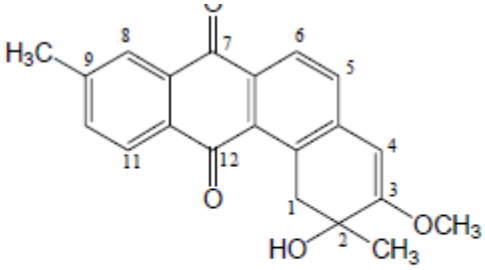
Compound (1)



Compound (2)



Compound (3)



Compound (4)

Compound (1) was obtained as colored crystal. It appeared as a dark quenching spot on the TLC plate under UV light at 254 nm and was soluble in dichloromethane and chloroform. The 1H NMR spectra of compound (1) was given in the experimental part. On this basis of the NMR spectroscopic data, the compound (1) was identified as panisaldehyde. Compound (2) was obtained as colorless gummy material. It appeared as dark unclenching spots on TLC plate under UV light at 254 nm and was soluble in CH2Cl2 and CHCl3. The 1H NMR spectrum of compound (2) showed signals for four methines, one aldehydic group and one hydroxyl group. The structure of compound (2) was elucidated by direct comparison of each spectral data with compound (1). Although the 1H NMR spectrum of compound (2) was in close correspondence to that of compound (1), some differences could be observed, such as the resonance at δ=3.89 in the 1H NMR could not be observed in compound (2). On the other hand one-proton

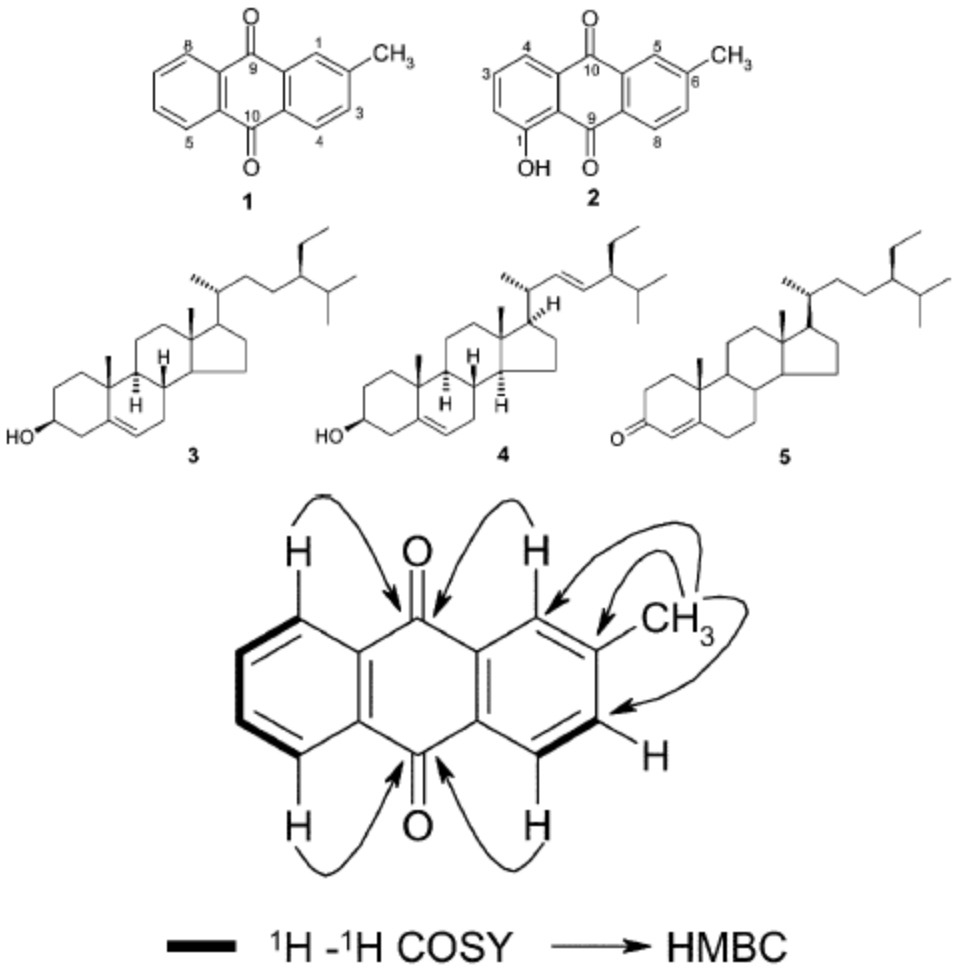
broad singlet at δ=7.51 was appeared in the 1H NMR spectrum of compound (2) corresponding to the 4-OH group. The 1H NMR spectrum of compound (2) showed two symmetric pairs of coupled of two doublets at δ 7.82 (H-2 and H-6) and at δ-7.35 (H-3 and H-5) assigning to a 1,4-disubsituted benzene ring present in compound (2). The presence of sharp one proton singlet at δ= 9.97 could be attributed to one aldehydic group.

So it is clear from all of these 1H NMR signals that the aldehydic and hydroxyl groups are the substituents at C-1 and C-4, respectively, of the 1,4-disubsituted benzene ring. On this basis compound (2) was identified as 4-hydroxy benzaldehyde. Compound (3) was obtained as pink gum. It appeared as a dark quenching spot on the TLC plate under UV light at 254 nm and was soluble in CH2Cl2 and CHCl3. The 1H NMR (400 MHz, CDCl3) spectrum of compound (3) has shown general features of substituted anthraquinone system. The 1H NMR spectrum has shown two singlets integrating for one proton each at δ=7.48 and δ=7.50 indicated a typical 1,2,4,5-tetra substituted aromatic ring and the position of the two singlet at δ=7.48 and δ=7.50 could be attributed to the two protons of C-1 and C-4 respectively. The two dublets (J=6.6 Hz), each integrating for one proton, centered at δ=8.07 and δ=8.15 could be assigned to the two o-coupled aromatic protons, located at C-7 and C-8 of the anthraquinone system. A one proton singlet at δ=8.54 was attributed for another aromatic proton at C-5. A singlet integrating for three protons at δ=2.62 indicated the presence of a methyl group. The relatively deshielded nature of this methyl group indicated that it might be the part of an acetyl group attached to the aromatic ring at C-6. On these NMR data compound (3) could be proposed as 6-acetyl-2,3-dihydroxy anthacene- 9,10-dione.

Compound (4) was obtained as gummy material. It appeared as a dark quenching spot on the TLC plate under UV light at 254 nm and was soluble in CH2Cl2 and CHCl3.

The 1H NMR spectra of compound (4) was given in the experimental part. On this basis of the NMR spectroscopic data, the compound (4) was identified as 2-hydroxy-3-methoxy-2,9-dimethyl-1,2-dihydrotetraphene-7,12-dione

2.2. Repeated chromatographic separation and purification of the n-hexane: Dichloromethane and methanolic extracts of the stem of *P. lanatum* provided a total of five compounds (1 - 5), the structures of which were determined by extensive NMR spectral analysis. The 13C NMR spectrum of compound 1 displayed 15 carbon resonances, including two carbonyl group signals at δ 183.0 and 183.5. The HSQC and DEPT experiments indicated that 8 out of the 15 carbons had attached protons. The DEPT 135 spectrum revealed the presence of seven aromatic methene carbons and a methyl group resonance. The 1H and 13C NMR spectral data of this compound demonstrated the general features of substituted anthraquinone skeleton (Chakraborty et al., 1978). The 1H NMR spectrum showed two multiplets, each integrating for two protons, at δ 7.78 and δ 8.30 indicative of a typical 1, 2-disubstituted aromatic ring. Two doublets (J = 8.0 Hz), each of one proton integrating, centered at δ 7.58 and δ 8.20 could be assigned to the two ortho-coupled aromatic protons. A one proton singlet at δ 8.09 was attributed to H-1. A singlet integrating for three protons at δ 2.53 suggested the presence of an aromatic methyl group, whose presence was confirmed from the 13C NMR signal at δ 21.9. Analysis of one- and twodimensional NMR spectra including COSY, HSQC and HMBC data disclosed the structural features of 1 in the figure. The position of the aromatic methyl



group at C-2 was unambiguously determined by HMBC correlations from the methyl proton to C-1, C-2 and C-3. On this basis, compound 1 was identified as 2-methylanthracene-9,10-dione, previously reported from Clausena heptaphylla (Chakraborty et al., 1978).

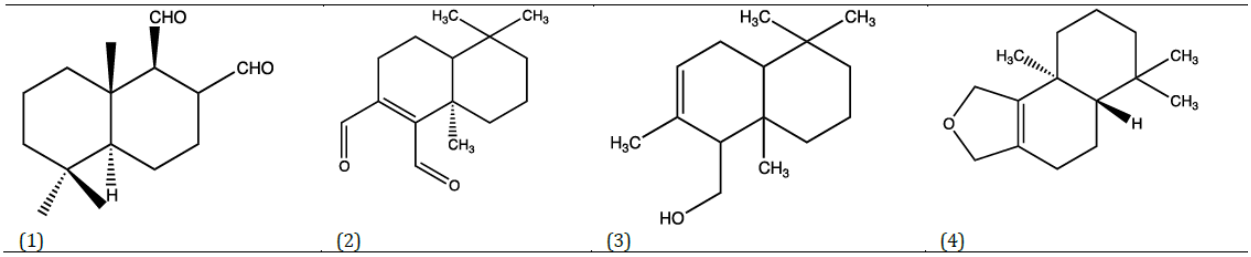
The 1H NMR spectral data of compound 2 was almost identical to that of 2-methylanthracene-9,10- dione (1) and 4-deoxyanthrakunthone (Khoda et al., 1990), suggesting a close structural similarity among these compounds. Thus, it revealed the presence of six protons in the aromatic region, a methyl group and a phenolic hydroxyl functionality. The presence of a one-proton broad signals at δ 8.30 could be ascribed to the aromatic proton H-5, while the triplet at δ 7.81 (J = 8.0 Hz) and a broad doublet at δ 7.65 were assigned to H-3 and H-2, respectively.

The doublets (J = 8.2 Hz), each integrating for one proton, centered at δ 7.60 and δ 8.21 could be assigned to ortho-coupled aromatic protons at C-7 and C-8, respectively. A singlet integrating for three protons at δ 2.54 indicated the presence of an aromatic methyl group at C-2. The sharp singlet at δ 12.67 could be ascribed to the chelated phenolic hydroxyl proton at C-1. On the basis of the above spectral features compound 2 characterized as 1-hydroxy-6-methylanthracene-9,10-dione (2), previously known to occur in Tectona grandis. The identity of compound 2 was further confirmed by comparison of its spectral data with published values (Bhargava et al., 1991). Compounds 3, 4 and 5 were identified as β- sitosterol, stigmasterol and sitosterone, respectively by comparison of their 1H NMR spectral data with reported values as well as by co-TLC with authentic samples.

2.3 Chemical constituents review of polygonum genus:

2.3.1 *Polygonum acuminatum*: Following structures have found

Polygodia (1), isopolygodial (2), drimenol (3), confertifolin (4). (Tamilselvan et al,2011)

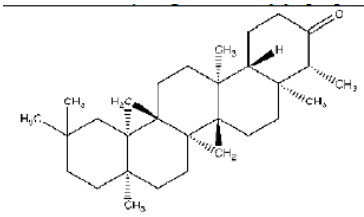


(1) (2) (3) (4)

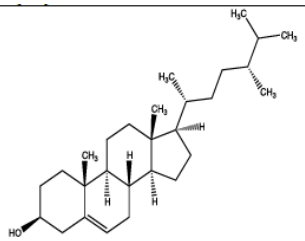
2.3.2 *Polygonum amplexicaule*: Following structures have found

Friedelin (1), *β* −sitosterol (2), simiarenone (3), angelicin (4), psoralen (5), palmitic acid (6), (-)-epicatechin (7), quercetin (8), Emodin-8-O-β-D-glucoside (9), flavan-3-ol (10), khellactone (11). 5, 6-dihydropyranobenzopyrone (12), amplexicine (13), catechin (14),

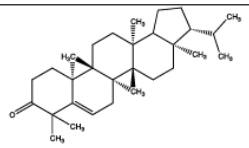
rutin (15), quercetin-3-O-β-D-galactopyranoside (16), chlorogenic acid (17), galloyl glucose (18), caffeic acid (19), gallic acid (20), scopletin (21). (Tamilselvan et al,2011)



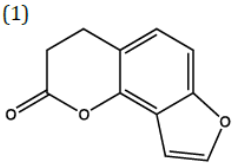
(1)



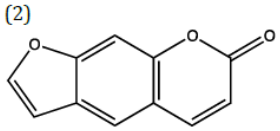
(2)



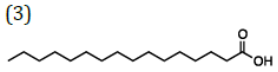
(3)



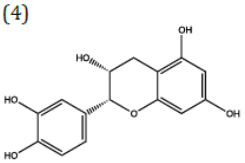
(4)



(5)



(6)



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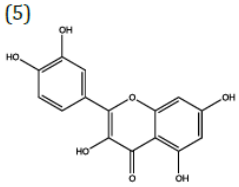
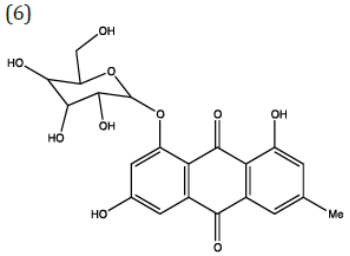


(8)



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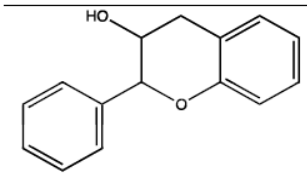
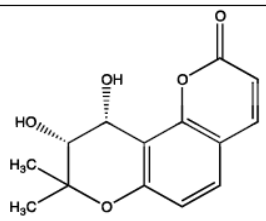


(10)



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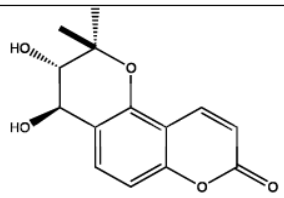

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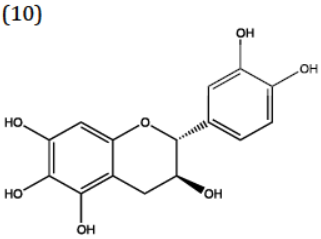

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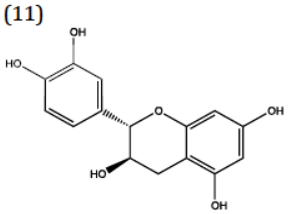

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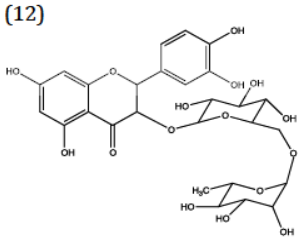

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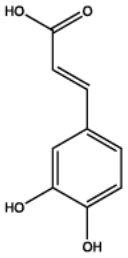

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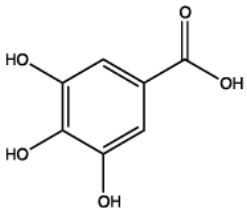

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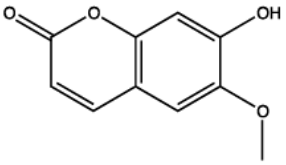
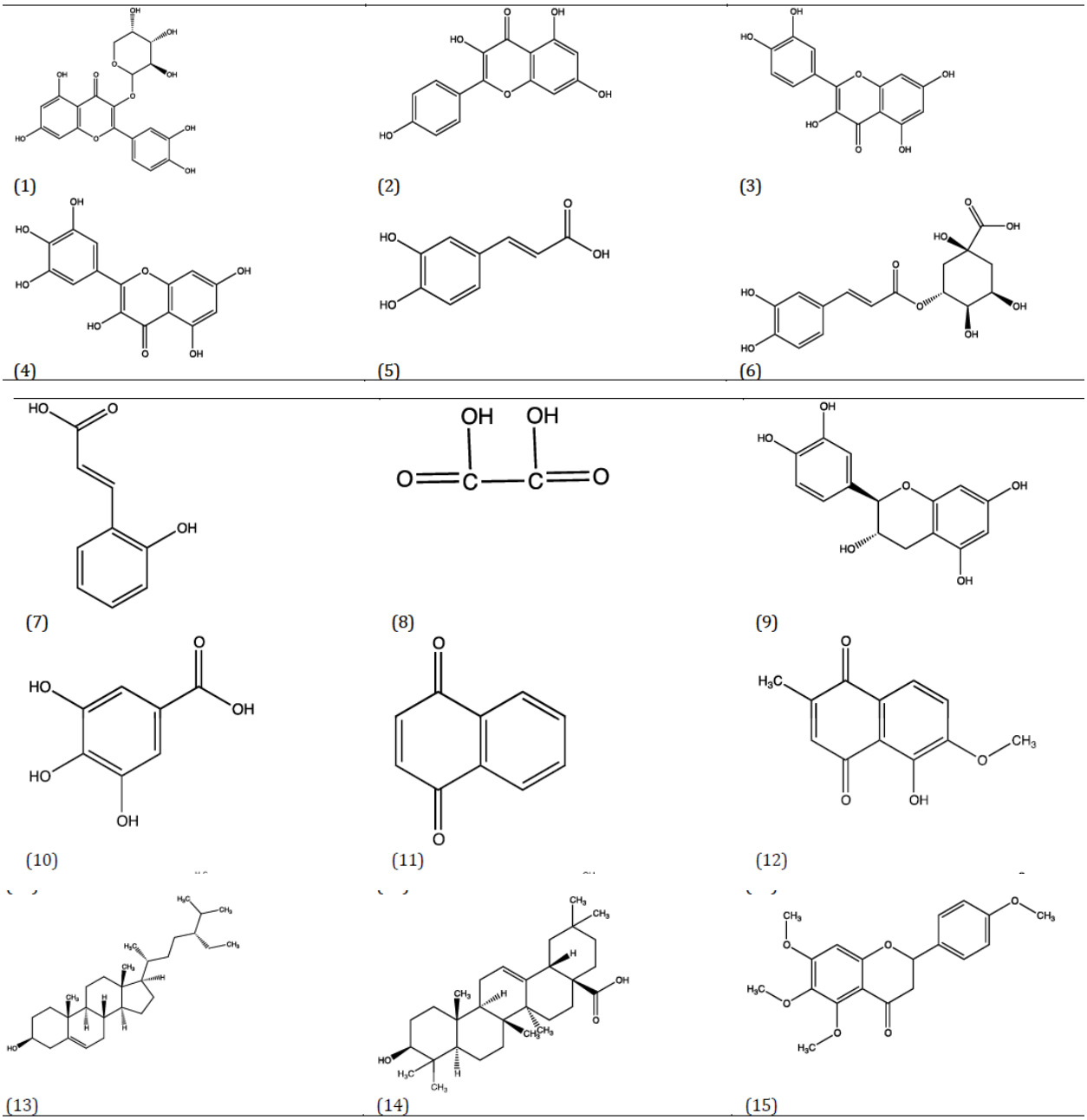


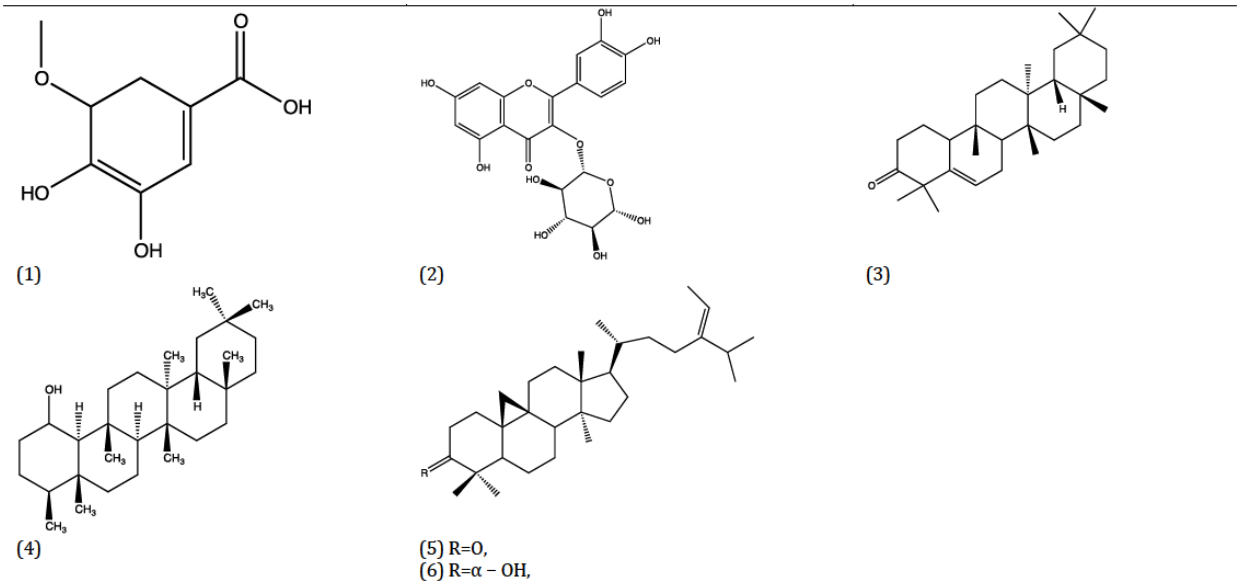
(21)

2.3.3 *Polygonum aviculare*: Following structures have been found Avicularin (1), kaempferol (2), quercetin (3), myricetin (4), cafferic acid (5), chlorogenic acid (6), coumaric acid (7), oxalic acid (8), D-catechin (9), gallic acid (10) [78], naphthoquinone (11), 6-methoxyplumbagin (12), β-sitosterol (13), oleanolic acid (14), 5,6,7,4'-tetramethoxyflavanone (15). [Pattanayak and Sunita, (2009)]



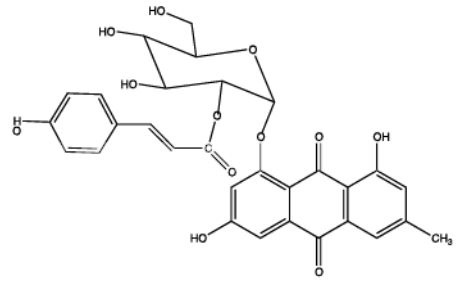
2.3.4 *Polygonum bistorta*

3-O-methyl-gallic acid (1), quercetin-3-O-β-D-glucopyranoside (2) [80], 5-glutinen-3-one (3), friedelanol (4), 24(E)-ethylidenecycloartanone (5), 24(E)-ethylidenecycloartan-3a-ol (6), cycloartane-3, 24-dione (7), 24-methylenecycloartanone (8), c-sitosterol (9), β-sitosterol (10), β - sitosterone (11), friedelin (12), 3-β -friedelinol (13). (Devi *et al*, 2010)

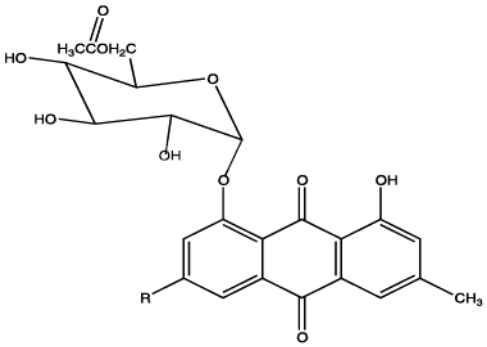


2.3.5 *Polygonum Cillinerve*

Emodin-8-β-D-(2″-O-coumarate) glucoside (1), emodin-8-β- D-(6´-O-acetyl) glucoside (2), physicon-8-β-D-(6´-O-acetyl) glucoside (3). (Tamilselvan et al,2011)



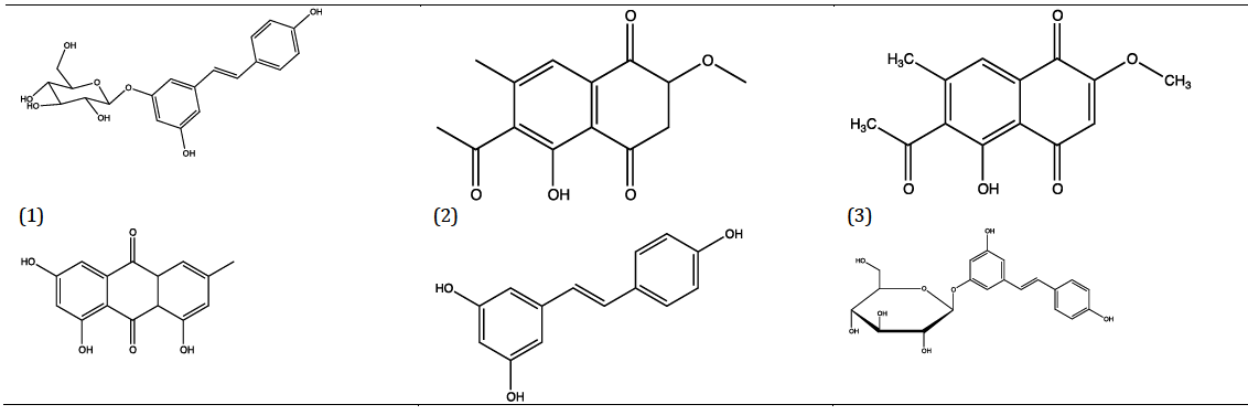
(1)



(2). R= OH,
(3). R = OCH_3

2.3.6 *Polygonum cuspidatum*

Piceid (5,4'-dihydroxystilbene-3-O-β-D-glucopyranoside) (1), 2-methoxy-6-acetyl-7-methyljuglone (2), 2- methoxystypandrone (3), emodin (4), resveratrol (5), polydatin (6), emodin-8-O-β-D-glucopyranoside (7), (E)-3, 5, 12- trihydroxystilbene-3-O- β-D-glucopyranoside-2'- (3", 4", 5"- trihydroxybenzoate) (8), (+)- catechin-3-O-gallate (9), resveratroloside (10), 3,5,5-trihydroxystilbene-3-O- (6"-galloy)- glucoside (11), emodin-1-O-glucoside (12), torachrysone-8- Oglucoside (13), emodin-8-O-glucoside (14), emodin-8-O-(6'- malonyl)-glucosideb (15), torachrysone-8-O-(6'-acetyl)- glucoside (16), physcion-8-O-glucoside (17), physcion-8-O-(6'- acetyl)-glucoside (18). (Tamilselvan et al, 2011)



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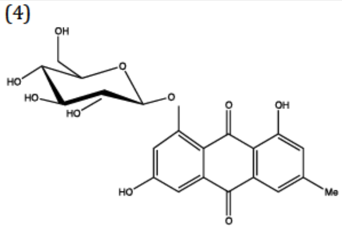

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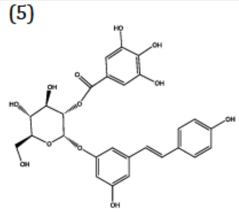

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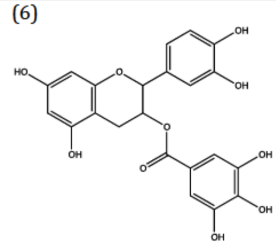

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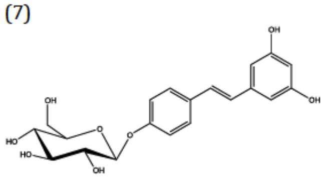

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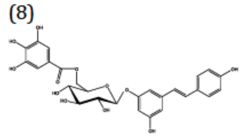

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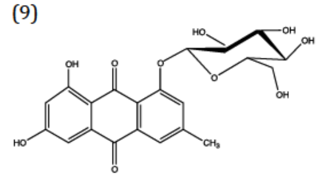

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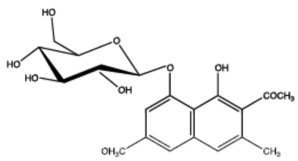

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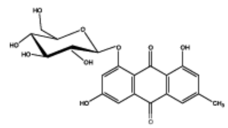

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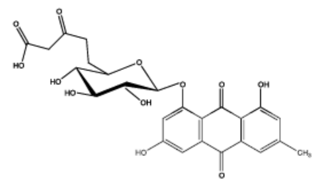

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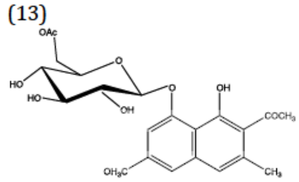

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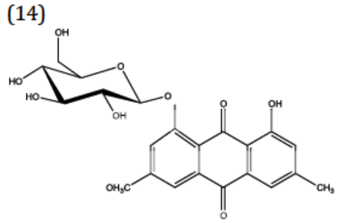

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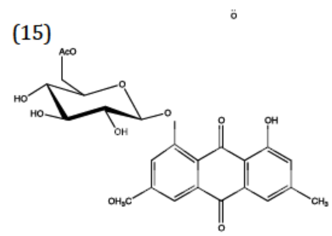


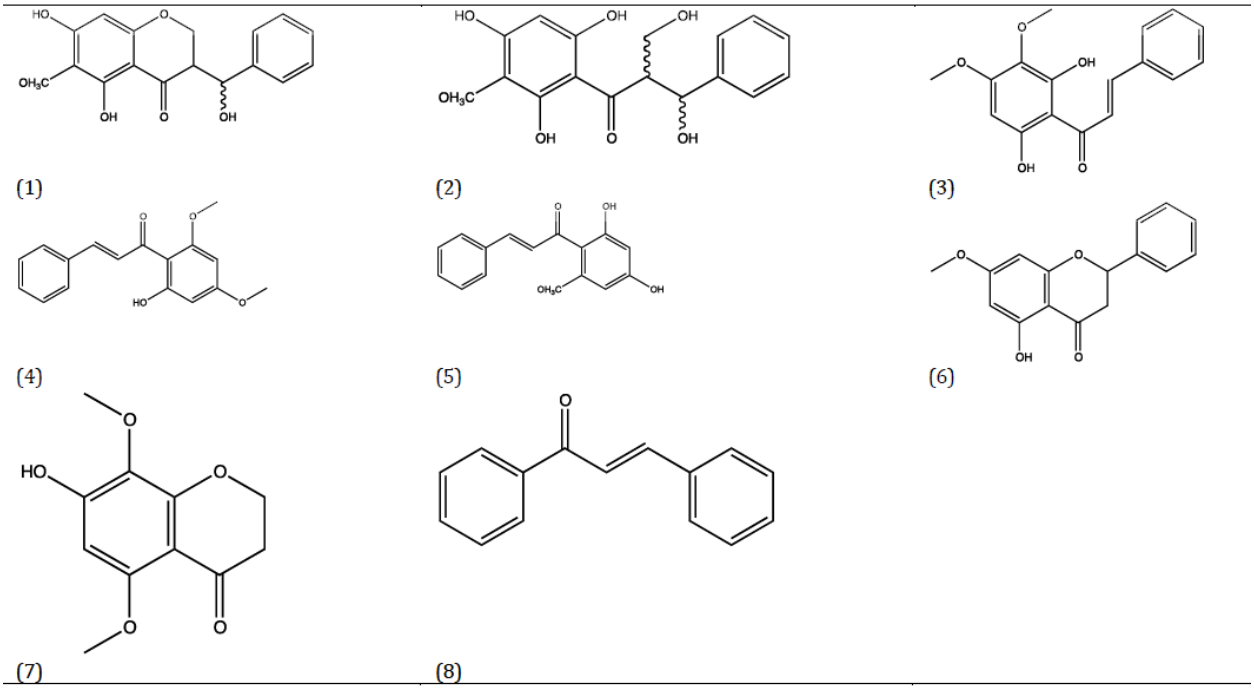
(16) (17) (18)

2.3.7 *Polygonum ferrugineum*

5,7-dihydroxy-6-methoxy-3- (9-hydroxy-phenylmethyl)- chroman-4-one (1), 2’4’6’-trihydroxy-30-methoxy-ahydroxymethyl- b-hydroxy-dihydrochalcone (2), pashanone (3), flavokawin B (4), cardamonin or alpinetin chalcone (5),

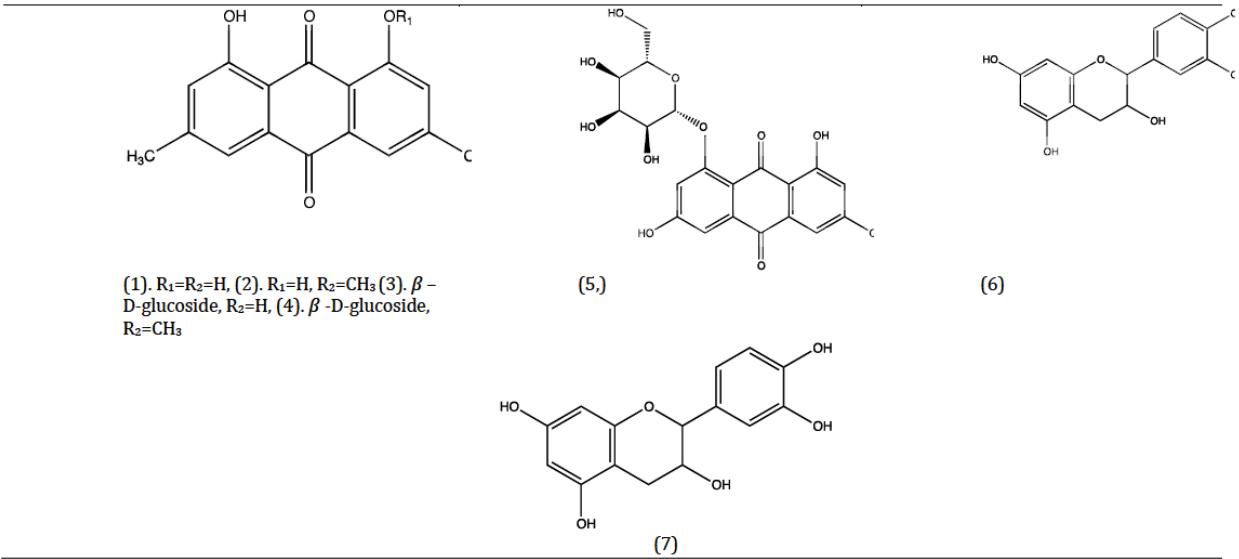
pinostrobin (6) 5,8-dimethoxy-7-hydroxychroman-4-one (7),

chalcone (8). [Pattanayak and Sunita, (2009)]



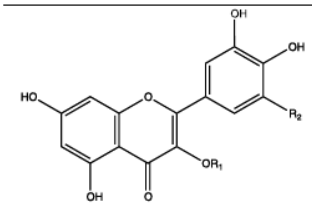
2.3.8 *Polygonum hypoleucum*

Emodin (1), physcion (62A) (2), emodin 1-O-β-D-glucoside (49A) (3), physcion 1-O β-D-glucoside (50A)(4), emodin-8-O-β-D-glucopyranoside (5), (+)-catechin (6), (-)-epicatechin (7). (Tamilselvan et al,2011)

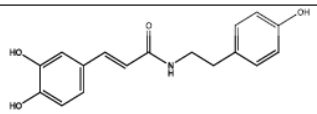


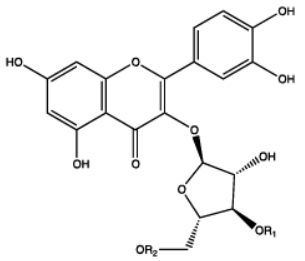
2.3.9 Polygonum hyrcanicum

Quercetin (1), myricetin (2), N-trans-caffeoyltyramine (3), quercetin 3-O-α-L-(3”,5”-diacetyl-arabinofuranoside) (4), quercetin 3-O-α-L-(3”-acetyl-arabinofuranoside) (5), myricetin 3-O-α-L-arabinofuranoside (6), myricetin 3-O-α-L- (3”,5”-diacetyl-arabinofuranoside) (7), (+) catechin (8), (-) gallocatechin (9), myricetin 3-O-β-D-galactopyranoside (10) myricetin 3-O-α-L-rhamnopyranoside (myricitrin) (11), quercetin 3-O-β-D-galactopyranoside (12), quercetin,3-O-α-Larabinofuranoside (avicularin) (13), tyrosol (14), p-coumaric acid (15), ferulic acid (16), N-cis-feruloyltyramine (16), N-trans-pcoumaroyltyramine (17), N-trans-3,4- dimethoxycinnamoyldopamine (18). (Tamilselvan et al, 2011)

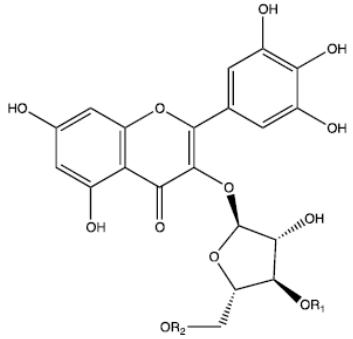


(1). R_1=H, R_2=H, (3)
(2). R_1=H, R_2=OH,
(10). R_1= β-D-galactopyranoside, R_2=OH,
(11). R_1= β α-L-rhamnopyranoside, R_2=OH,
(12).R_1= β-D-galactopyranoside, R_2=H.

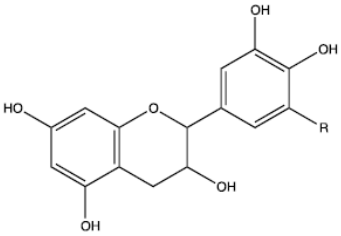




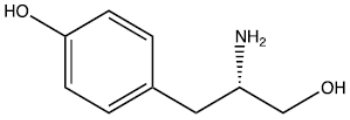
(4). R_1=-COOH$_3$, R_2=COCH$_3$,
(5). R_1=-COCH$_3$, R_2=H,
(13). R_1=H R_2=H,



(6). R_1=H R_2=H,
(7). R_1=-COOH3, R_2=COCH3,



(8). R=H
(9). R=OH



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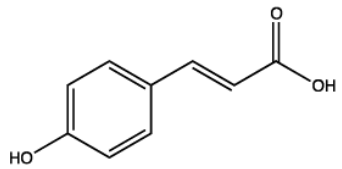

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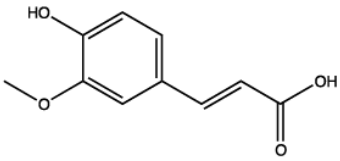

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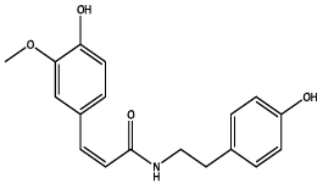

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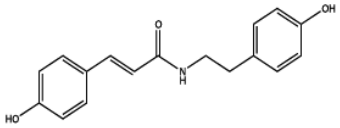

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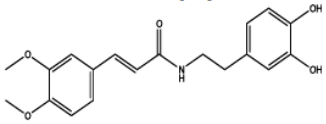
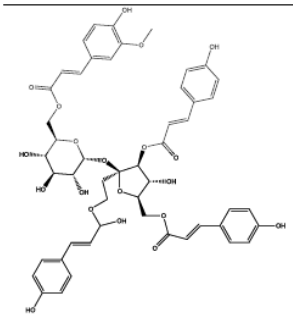


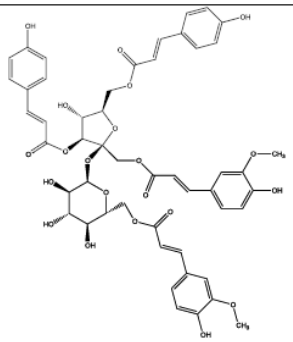
(19)

2.3.10 *Polygonum lapathifolium*

Vanicoside B (1), lapathoside A (2), 5-hydroxy-7-methoxy flavanone (pinostrobin) (3). (Devi *et al*, 2010)



(1)



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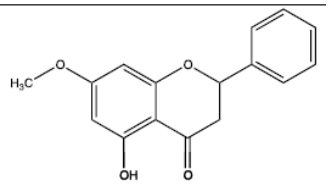
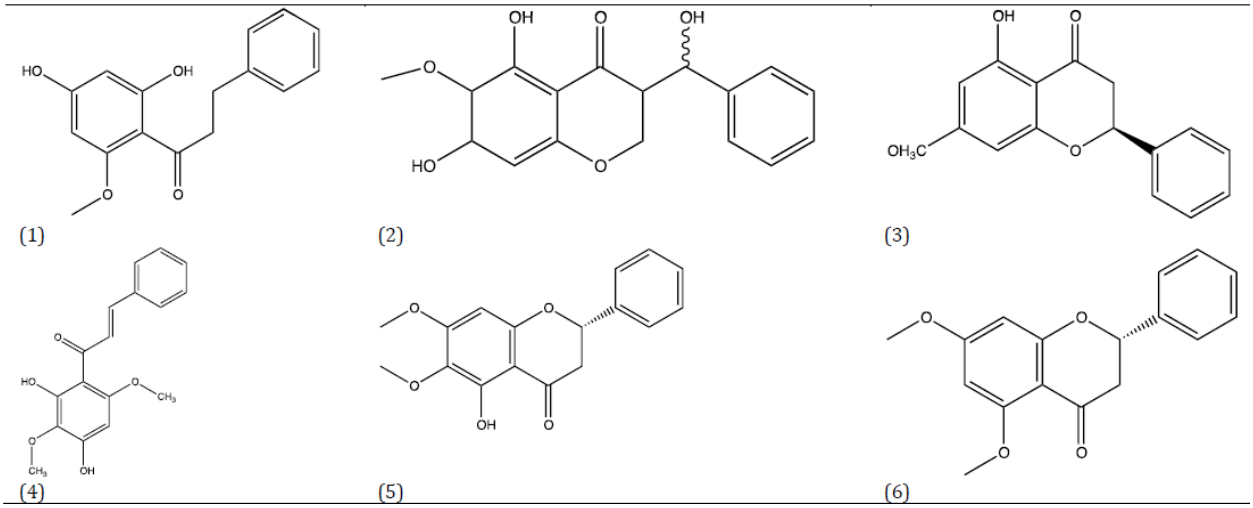


(3)

2.3.11 *Polygonum limbatum*

Cardamomin (1), (±)-polygohomoisoflavanone (2), (S)-(−)-pinostrobin (3), 2′,4′-dihydroxy-3′,6′-dimethoxychalcone (4), (2S)-(-)-5-hydroxy-6,7-dimethoxyflavanone (5), (2S)-(-)-5,7-dimethoxyflavanone (6). (Tamilselvan et al,2011)

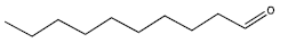


2.3.12 *Polygonum minus*

Decanal (1), dodecanal (2), decanol (3), 1-dodecanol, (4), undecanal (5), tetradecanal (6), 1-undecanol (7), nonanal (8), 1-nonanol (9), β- Caryophyllene (10), rutin (11), catechin (12), quercetin (13), isorhamnetin (14), hexanal (15), 1-hexanol (16), α-Pinene (17), kaempferol (18), undecane (19), nonanal (20), 1-nonanol (21), isobornyl acetate (22), n-decanoic acid (23), α-cubebene (24), xanthorrhizol (25), (E)-caryophyllene (26), trans-α-bergamotene (27), α-bisabolol (28), farnesene (29), β-himachalene (30), α- selinene (31), valencene (32), δ-cadinine (33), alloaromadendrene (34), α-curcumene (35), (-)-α-panasinsene (36), cis –lanceol (37), farnesol (38), humulene (39), nerolidol (40), dodecanoic acid (41), β-caryophyllene oxide (42), trans-α-(Z)-Bergamotol (43),

tetradecanal (44), alloaromadendrene oxide-(1) (45), translongipinocarveol (46), neoisolongifolene,8-bromo- (47) isocaryophyllene (48),drimenol (49), drimenin (50), phytol (51), 6,7-methylenedioxy- 5,3‟‟,4‟‟,5‟‟ tetramethoxyflavone (52), 7-4‟‟,5‟‟-dimethylenedioxy-3,5,3‟‟-trimethoxyflavone (53). (Pattanayak and Sunita, 2009)

(1)



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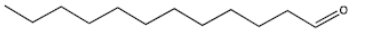

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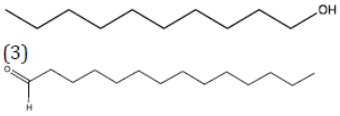

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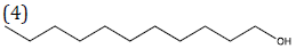

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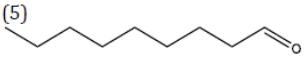

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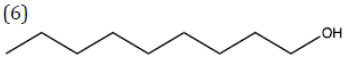

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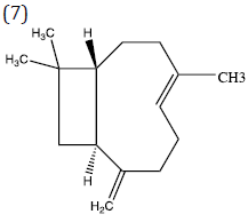

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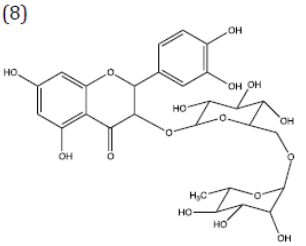

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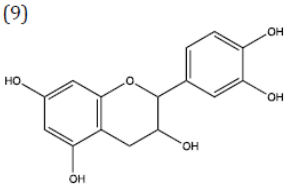

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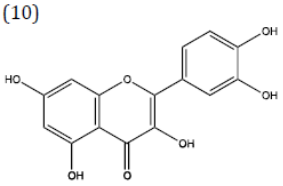

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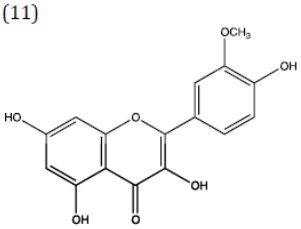

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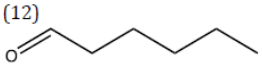

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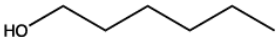

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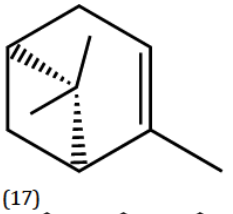

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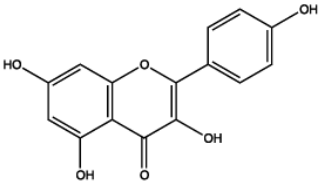

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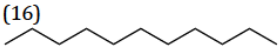

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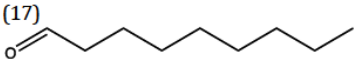

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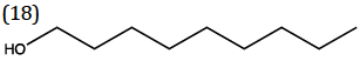

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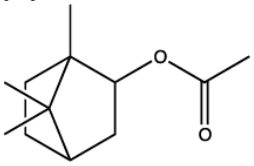

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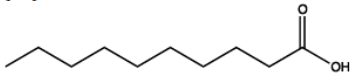

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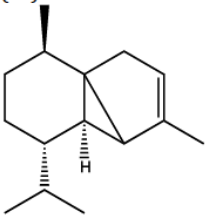

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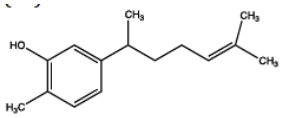

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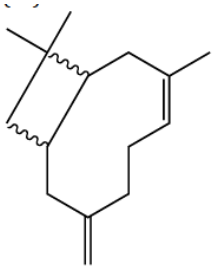

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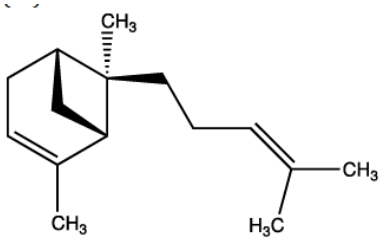

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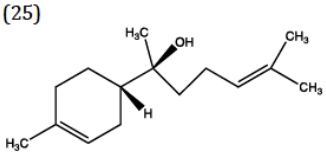

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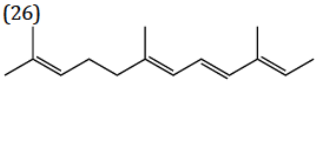

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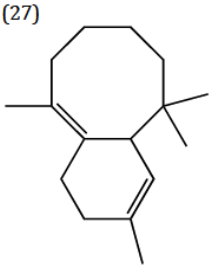

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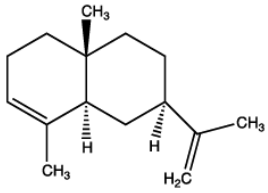

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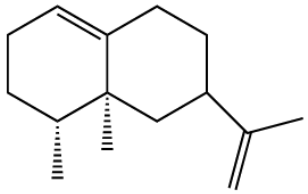

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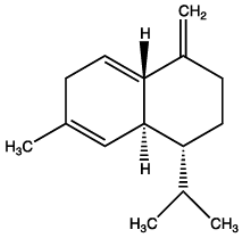

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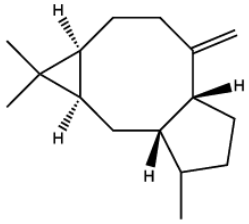

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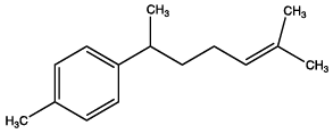

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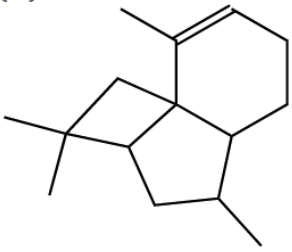

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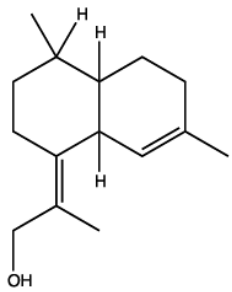

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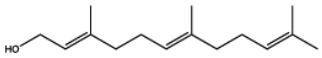

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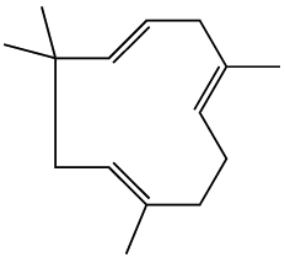

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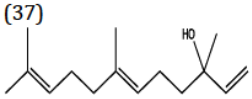

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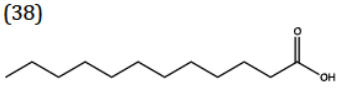

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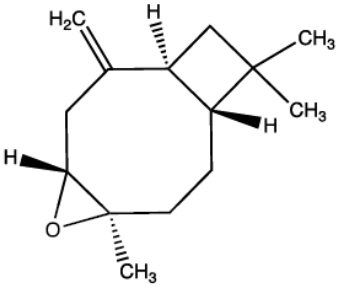

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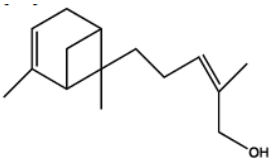

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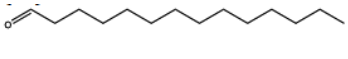

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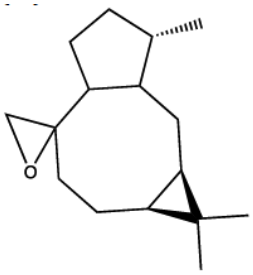

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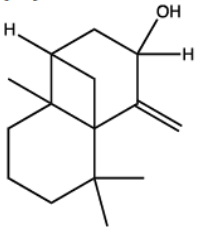

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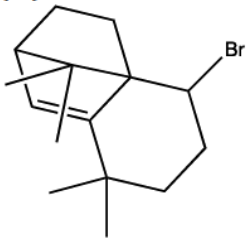

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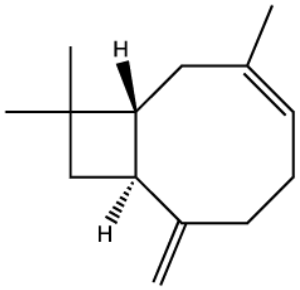

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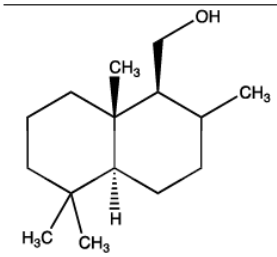

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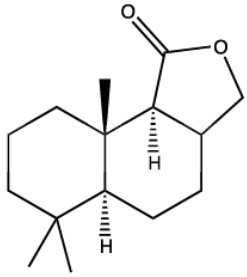

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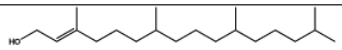

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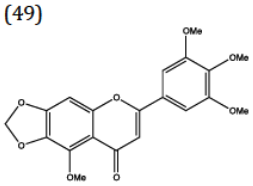

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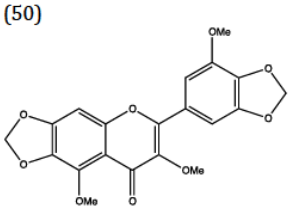
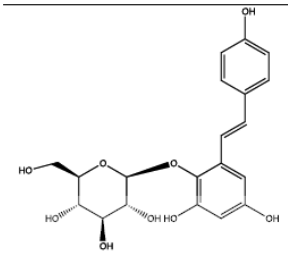


(53)

2.3.14 *Polygonum multifloruma*

Tetrahydroxystilbene-glucoside (2,3,5,4'-tetrahydroxystilbene- 2-O- β-D-glucoside) (1), Chrysophanol (2), physcion (3), emodin (4), aloeemodin (5), rhein (6), physcion-8-O- β-Dglucoside (7), emodin-8-O- β-D-glucoside (8), noreugenin (9), apigenin (10), daucosterol (11), β -sitosterol (12), stearic acid (13),5-methyl-2-(1-methylethyl) phenyl-β-Dglucopyranoside(14) , hyperoside (15), rutin (16), vitexin (17), β -amyrin (18) [110], gallic acid (19), hypaphorine (20), catechin (21), proanthocyanidin B1(22), proanthocyanidin B2 (23), epicatechin (24). (Devi *et al*, 2010)



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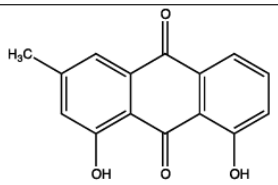

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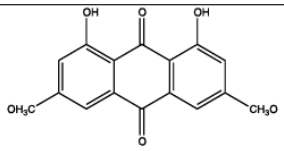

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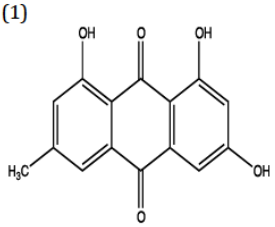

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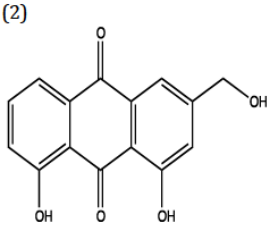

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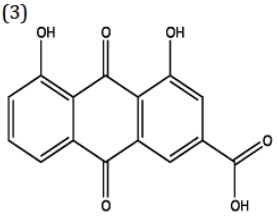

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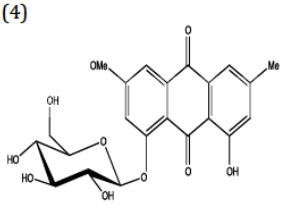

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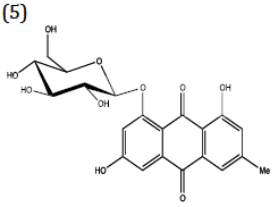

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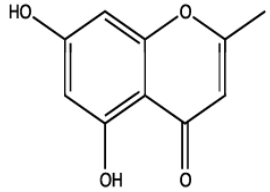

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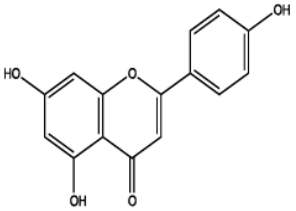

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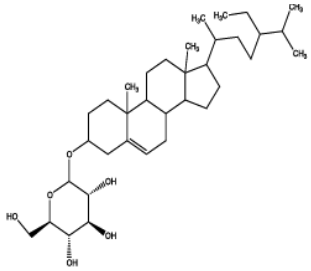

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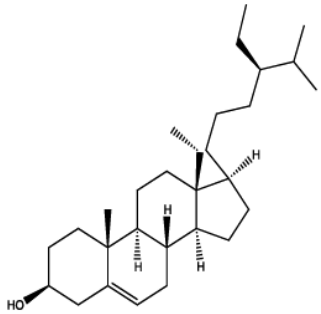

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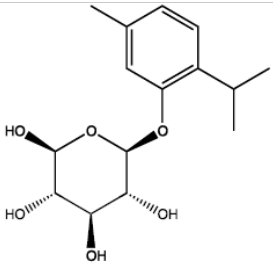

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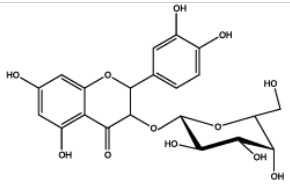

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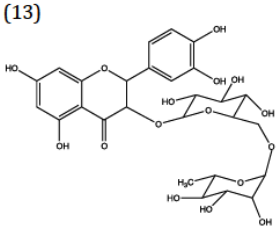

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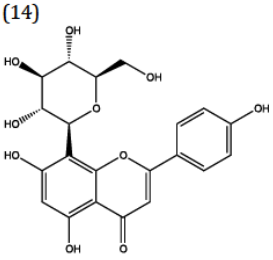

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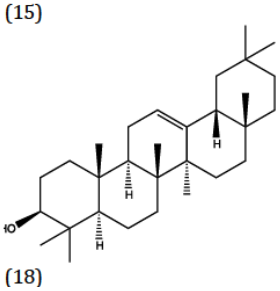

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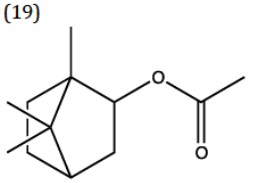

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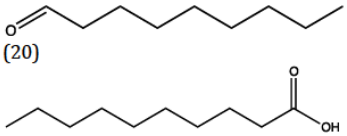

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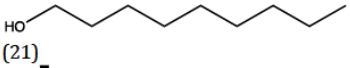

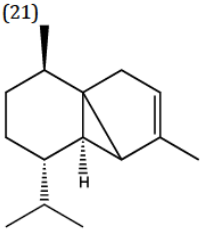

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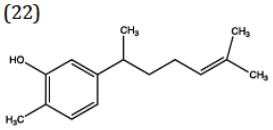

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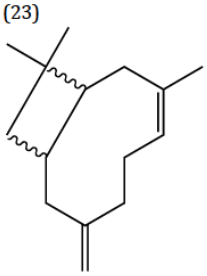

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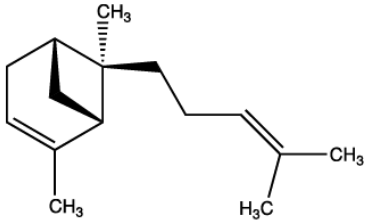

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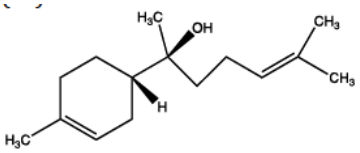

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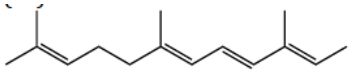

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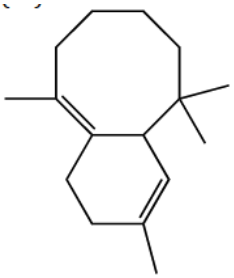

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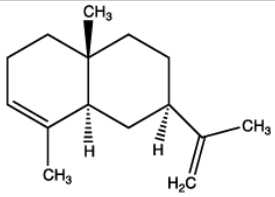

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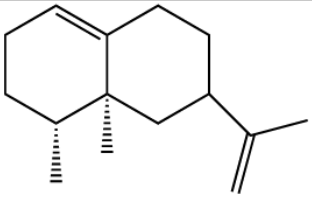

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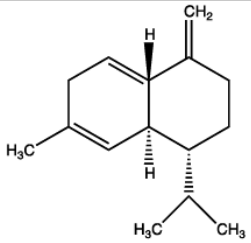

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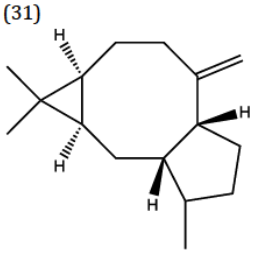

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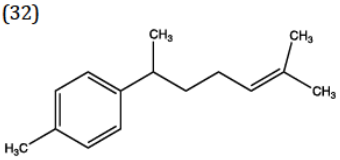

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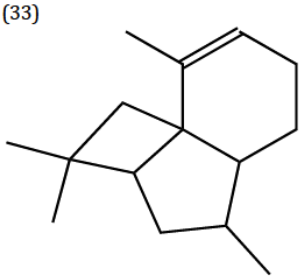

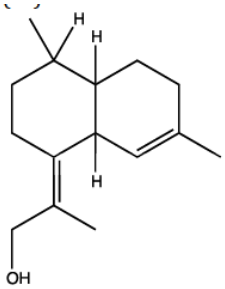

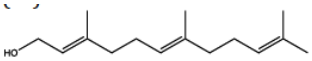

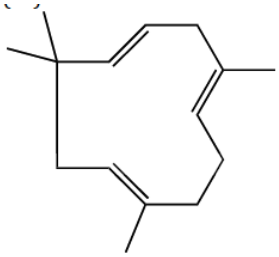

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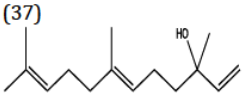

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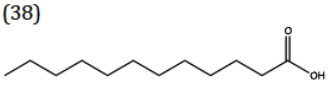

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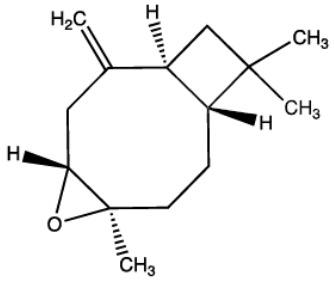

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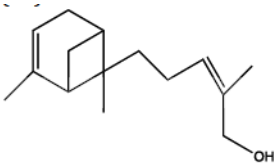

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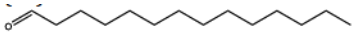

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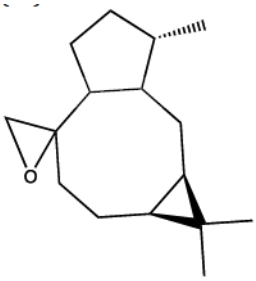

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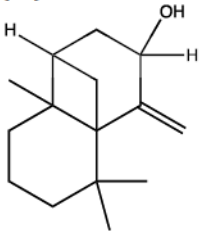

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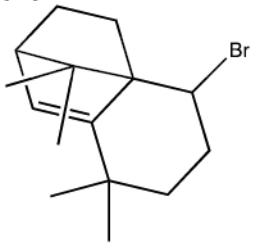

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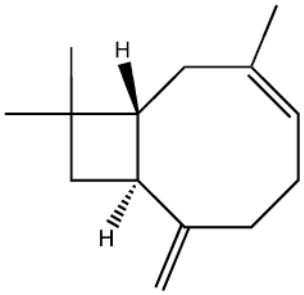

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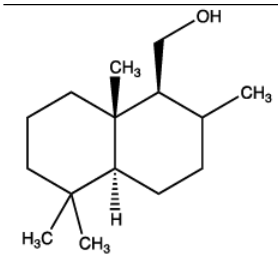

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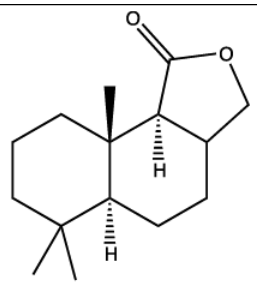

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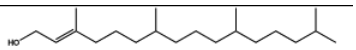

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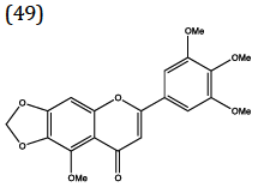

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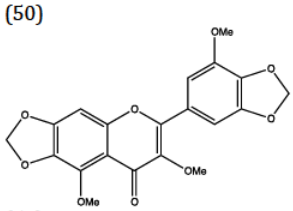
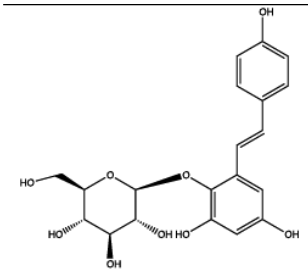


(53)

2.3.15 *Polygonum multifloruma*

Tetrahydroxystilbene-glucoside (2,3,5,4'-tetrahydroxystilbene- 2-O- β-D-glucoside) (1), Chrysophanol (2), physcion (3), emodin (4), aloeemodin (5), rhein (6), physcion-8-O- β-Dglucoside (7), emodin-8-O- β-D-glucoside (8), noreugenin (9), apigenin (10), daucosterol (11), β -sitosterol (12), stearic acid (13),5-methyl-2-(1-methylethyl) phenyl-β-Dglucopyranoside(14), hyperoside (15), rutin (16), vitexin (17), β -amyrin (18), gallic acid (19), hypaphorine (20), catechin (21), proanthocyanidin B1(22), proanthocyanidin B2 (23), epicatechin (24). (Tamilselvan et al,2011)



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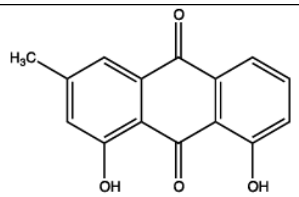

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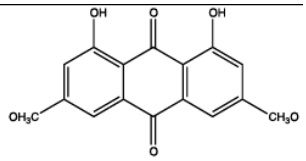

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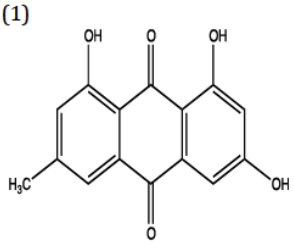

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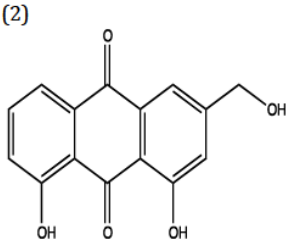

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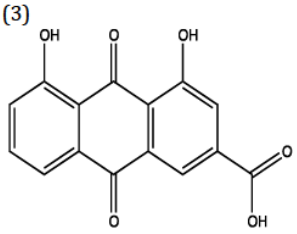

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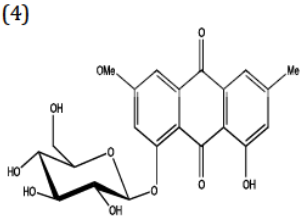

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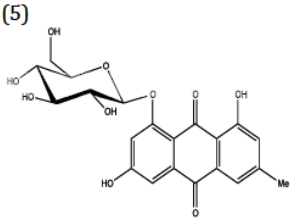

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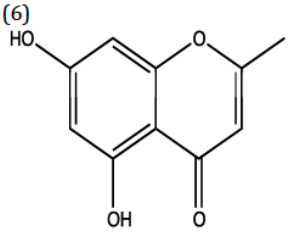

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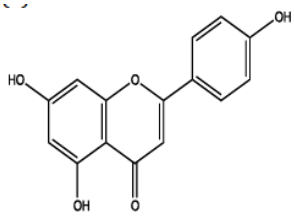

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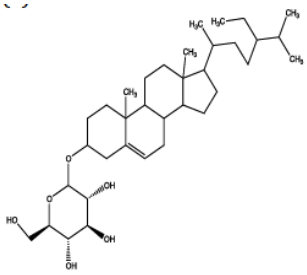

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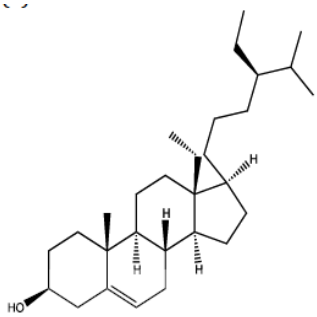

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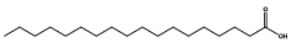

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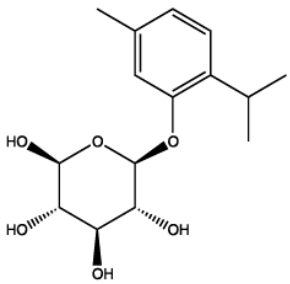

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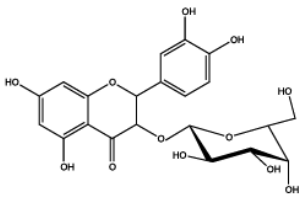

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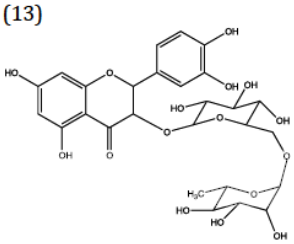

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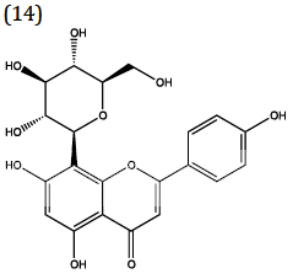

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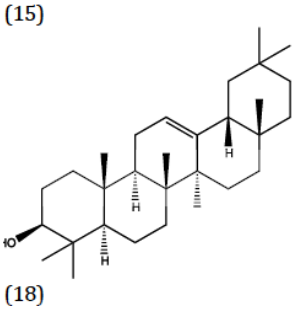

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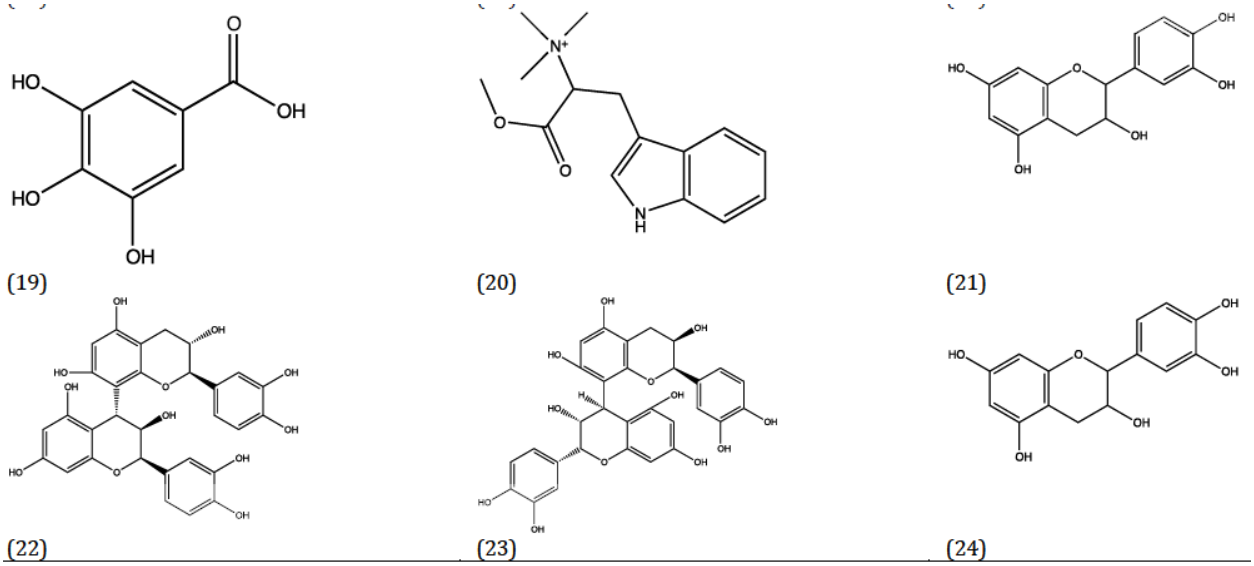
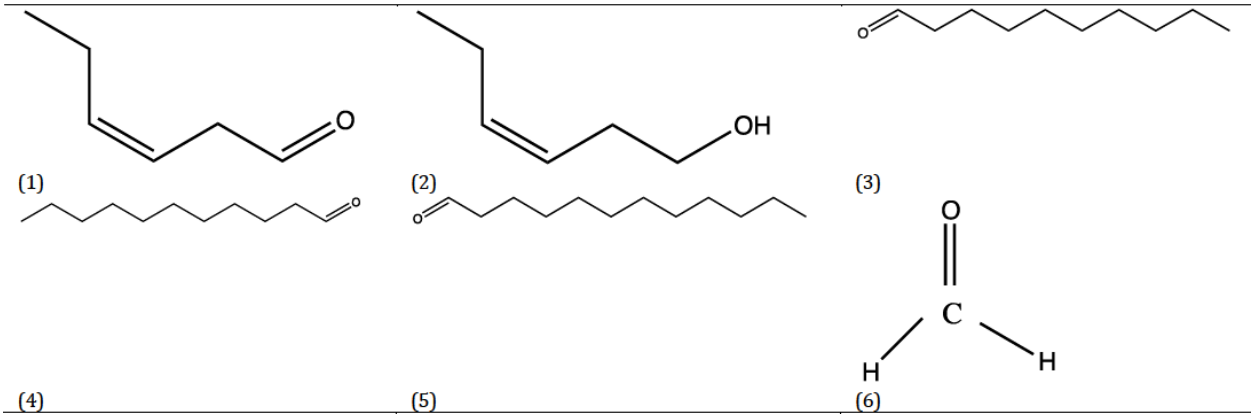
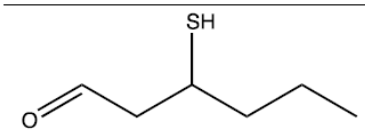


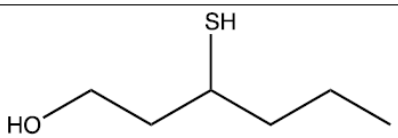
2.3.16 *Polygonum odoratum*

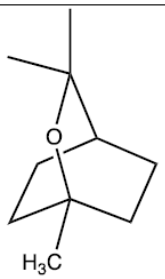
(Z)-3-hexenal (1), (Z)-3-hexenol (2) decanal (3), undecanal (4), dodecanal (5), aldehydes (6), 3-sulfanyl-hexanal (7), 3-sulfanylhexan- 1-ol (8), eucalyptol (9), undecane (10), 1-nonanol, (11) decanol (12), n-decanoic acid (13), 1-nonene (14), β-elemene (15), β-caryophyllene (16), allo-aromadendren (17),α-caryophyllene

(18), eremophillene (19), 7-epi-alpha-selinene (20), ledol (21), nerolidol (22), globulol (23), caryophyllene oxide (24), cubenol (25), eupatoriochromene (26), drimenol (27), hexahydro farnesyl acetone (28), isophytol (29), n-hexadecanoic acid (30) saponin (31), flavonoid (32) (Pattanayak and Sunita, 2009)









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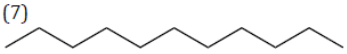

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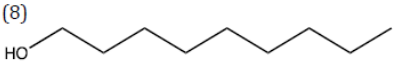

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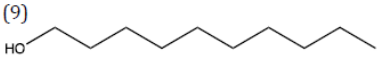

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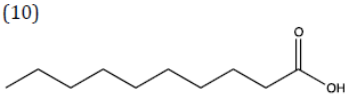

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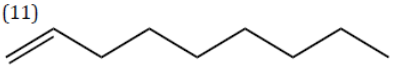

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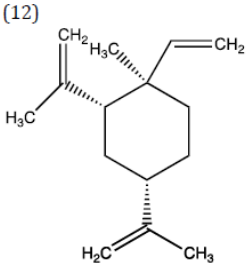

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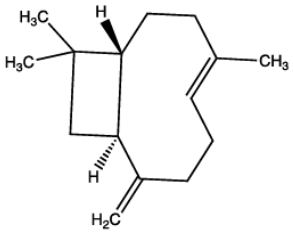

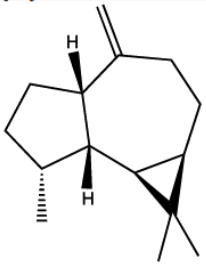

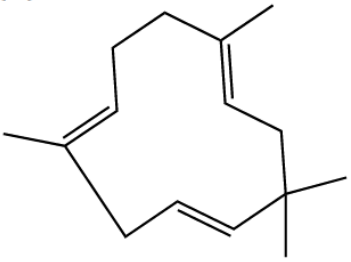

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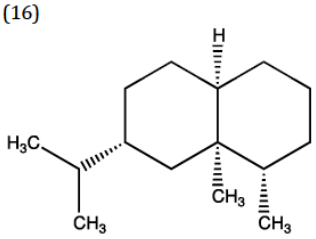

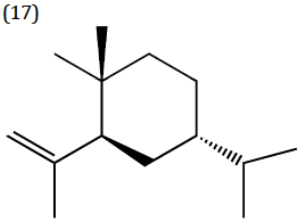

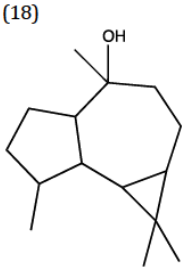

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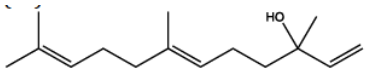

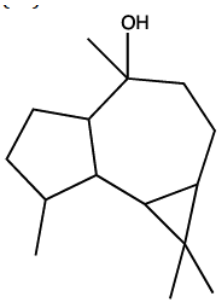

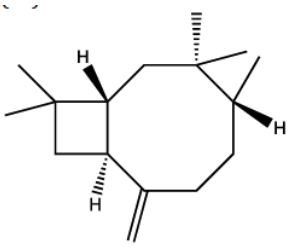

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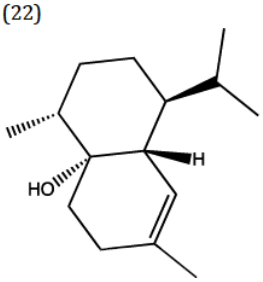

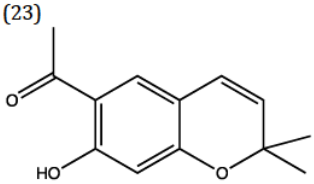

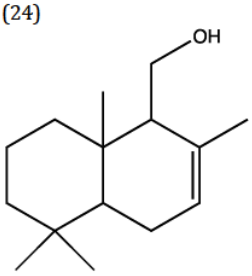

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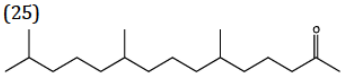

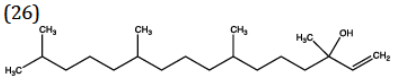

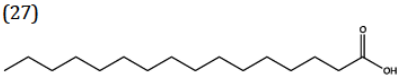

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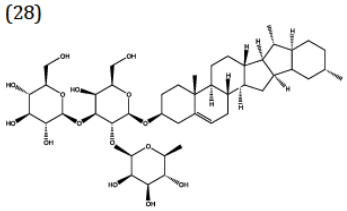
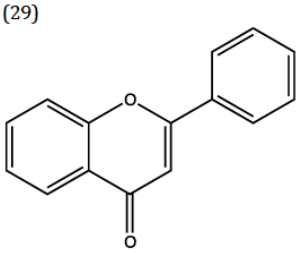




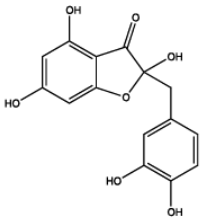
(31)

(32)

2.3.17 *Polygonum orientale*

alphitonin (1), methyl 3, 4-dihydroxybenzoate (2), apocynin (3), kaempferol-3-O-β-D-glucoside (4), 1,3,5-trihydroxybenzene (5), 3,3'-dimethoxyellagic-acid-4-O- β -D-glucoside (6), kaempferol-3-O- α-L-rhamnoside (7), quercetin-3-O-α-L-rhamnoside (8), kaempferol (9), 3,5,7-trihydrochromone (10), 5,7,4'- trihydroxydihydroflavonol (11), dihydroquercetin (12), quercetin (13), p-hydroxyphenylethanol ferulate (14), phydroxyphenylethanol- p-coumaric (15) catechin (16), isoorientin (17), orientin (18), quercetin-3-O-(2"-O- α-rhamnopyranosyl) -β- glucarono-pyranoside (19), taxifoliol (20), luteolin (21), ombuine-3-O-β-D-galactopyranoside (22), ombuine-3-O-rutinoside (23), tryptophan (24), quercetin-3-O-methyl ether (25), kaempferol-3-O-(2"-O- α-L-

rhamnopyranosyl) -β-Dglucuronopyranoside (26), quercetin-3-O-β-D-glucuronide (27), gallic acid (28), alphitonin (29), taxifolin (30), kaempferol-3- O-β-D-glucoside(31). (Tamilselvan et al, 2011)



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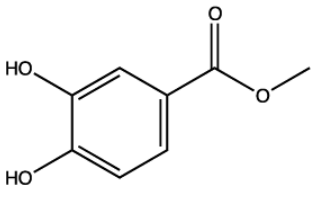

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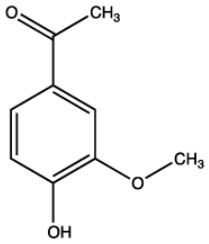

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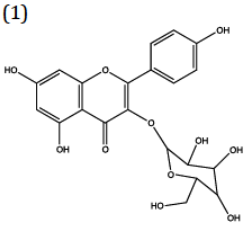

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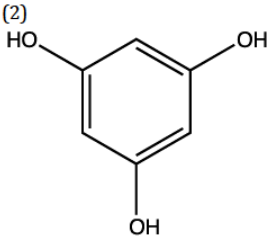

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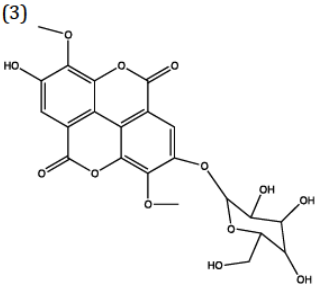

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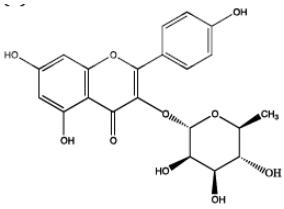

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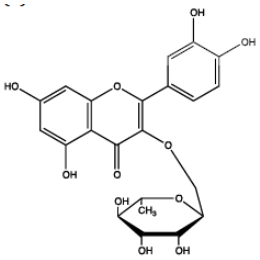

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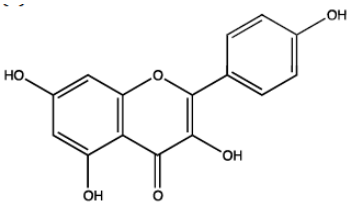

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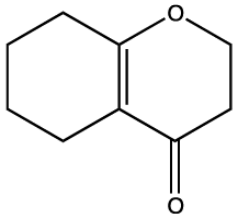

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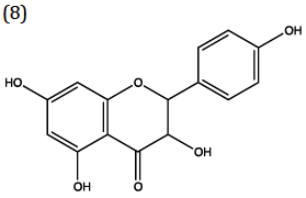

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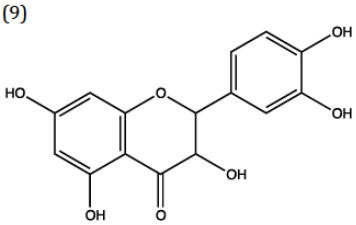

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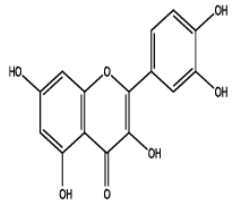

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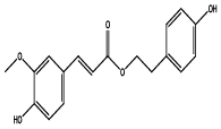

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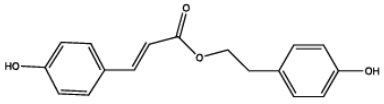

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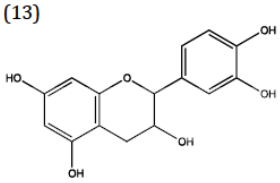

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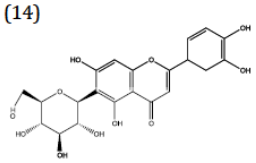

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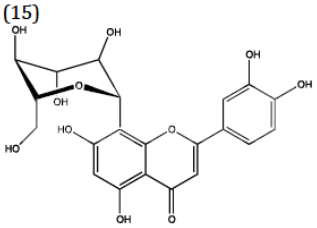

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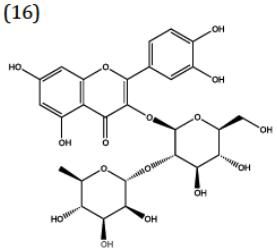

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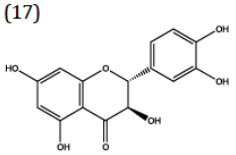

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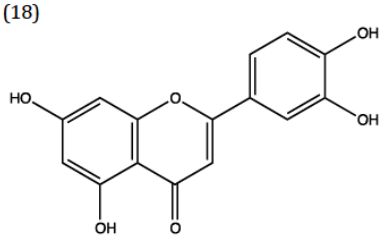

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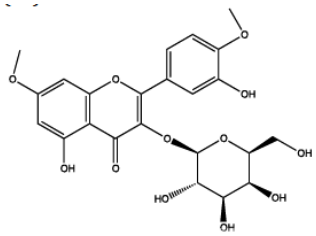

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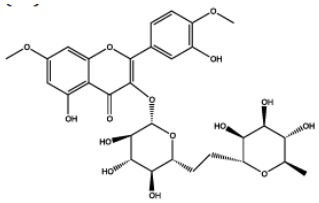

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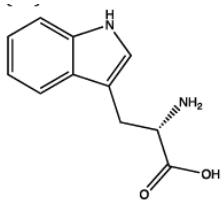

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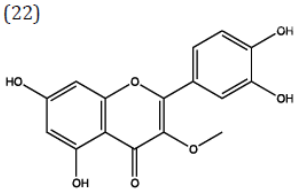

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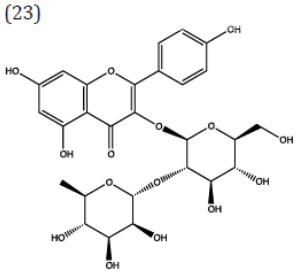

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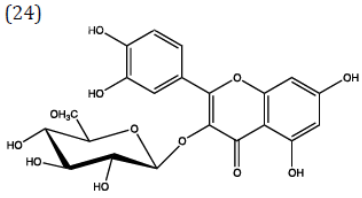

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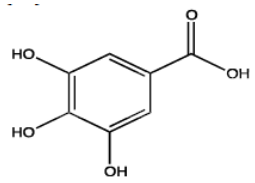

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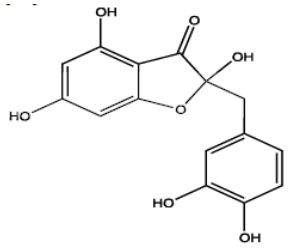
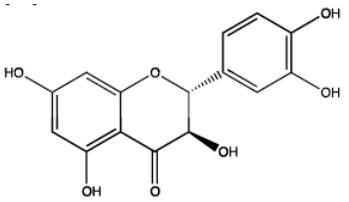


(29)



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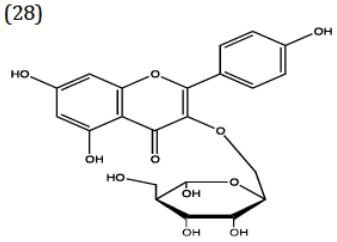
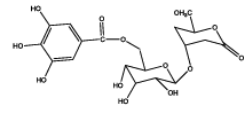


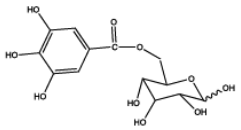
(31)

2.3.18 *Polygonum paleaceum*

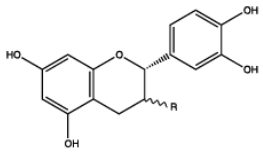
Paleaceolactoside (1), 6-O-galloyl-glucose (2), (+)-catechin (3), (−)-epicatechin (4), procyanidin B1 (5), procyanidin B2 (6), procyanidin C1 (7), chlorogenic acid (8), methyl 5-Ocaffeoylquinate (9), 3-O-caffeoyl quinic acid (10), rutin (11), quercetin-3-O-β-d-glucuronide (12), caffeic acid (13), gallic acid (14) (Devi *et al*, 2010)



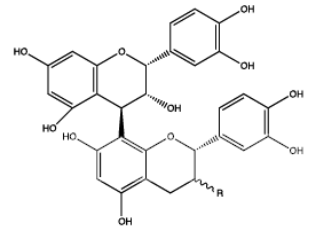
(1)



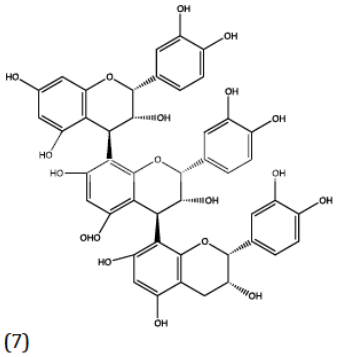
(2)



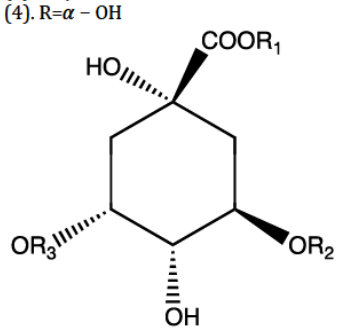
(3). R= β −OH,
(4). R=α − OH



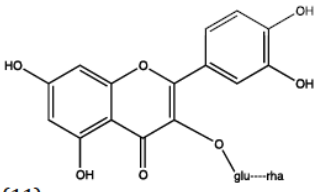
(5). R=α − OH,
(6). R=β − OH



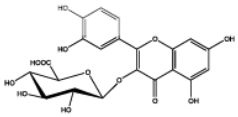
(7)



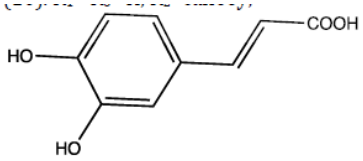
(8). R_1= R_3 =H, R_2=caffeoy,
(9). R_1=CH_3, R_2=caffeoy
(10). R_1= R_3 =H, R_2=caffeoy,



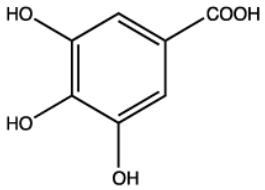
(11)



(12)

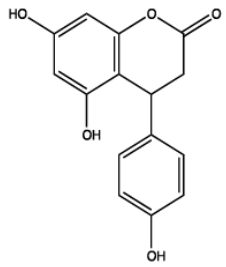


(13)

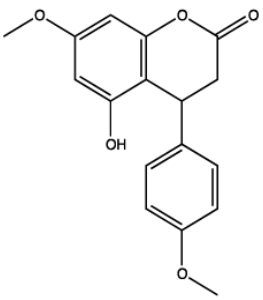


2.3.19 *Polygonum perfoliatum*

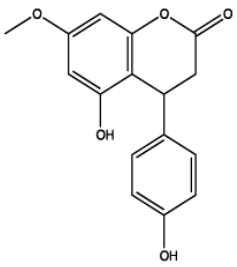
4-dihydro-4-(4’-hydroxyphenyl)-5,7-dihydroxycoumarin (1), 3,4-dihydro-5-hydroxy-7-methoxy-4-(4’-methoxyphenyl) coumarin (2), 3,4-dihydro-5-hydroxy-4-(4’-hydroxyphenyl)-7- methoxycoumarin (3), 3,4-dihydro-5,7-dihydroxy-4-(4’-methoxyphenyl) coumarin (4)[11], 6’-acetyl-3,6- diferuloylsucrose (helonioside B) (5), 2’4’6’-triacetyl-3,6- diferuloylsucrose (6), 1’2’4’6’-tetraacetyl-3,6-diferuloylsucrose (7), 1’2’6’-triacetyl-3,6-diferuloylsucrose (8), 2’6’-diacetyl-3,6- diferuloylsucrose (9),1,3,6-tri-p-coumaroyl-6’- feruloyl sucroses (10), vanicoside A (11), vanicoside B (12), quercetin-3-O- β-D-glucuronide (13) , 5-hydroxymethyl-2-furaldehyde (14), methyl caffeoate (15), protocatechuic aldehyde (16), quercetin (17), pinocernbrin (18), catechin (19), taxifolin (20), taxifolin-3- O- *β*-D-xylopyranoside (21), 13-epitorulosal (22), coumarin-7-O-*β*-D-glucose glycosidic (23), 8-oxo-pinoresinol (24), 3’,5- dihydroxy-3,4’,5’,7-tetramethoxy-flavone (25), rutin (26). (Pattanayak and Sunita, 2009)



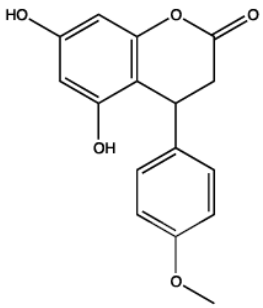
(1)



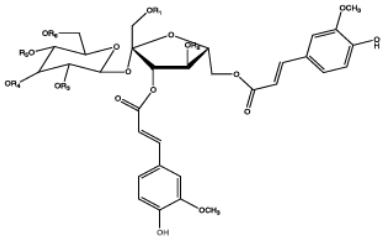
(2)



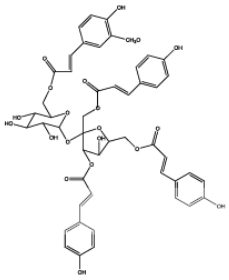
(3)



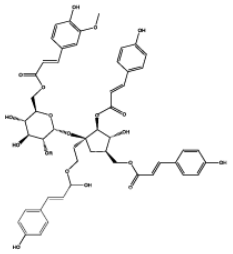
(4)



	R_1	R_2	R_3	R_4	R_5	R_6
(5)	H	H	H	H	H	$COCH_3$
(6)	H	H	$COCH_3$	H	$COCH_3$	$COCH_3$
(7)	$COCH_3$	H	$COCH_3$	H	$COCH_3$	$COCH_3$
(8)	$COCH_3$	H	$COCH_3$	H	H	$COCH_3$
(9)	H	H	$COCH_3$	H	H	$COCH_3$

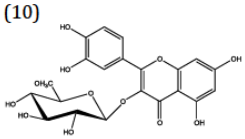


(10)

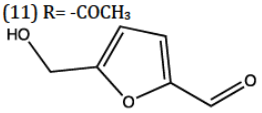


(11) R= -$COCH_3$

(12) R=H



(13)



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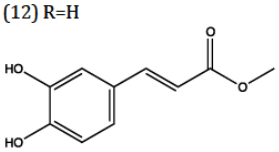
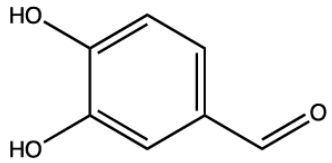
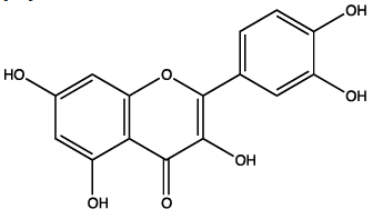


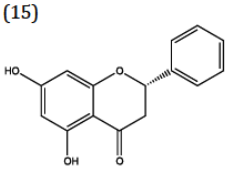
(15)



(16)



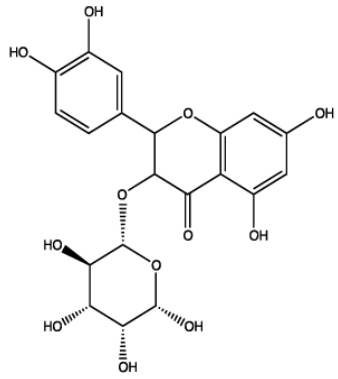
(17)



(18)

2.3.20 *Polygonum sachalinensis*

Quercetin-3-O-β-D-galactopyranoside (1), quercetin-3-Oarabinopyranoside (2), lapathoside D (3), N-transferuloyltyramine (4), lapathoside C (5), hydropiperoside (6), vanicoside B (7), phenylacetonitrile (8), (E)-β-ocimene (9), linalool (10), (E)-4,8-dimethyl-1,3,7-nonatriene (11), (E,E)-α- farnesene (10).



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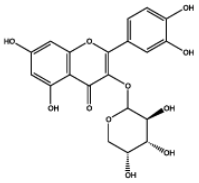

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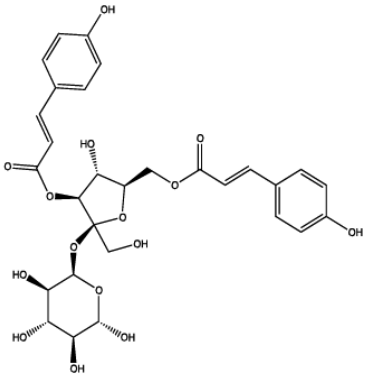

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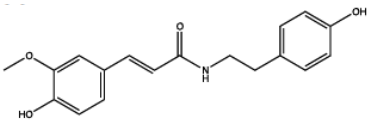

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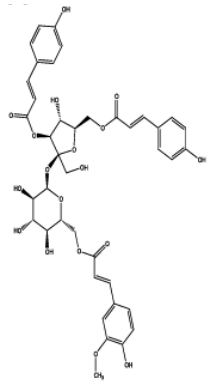

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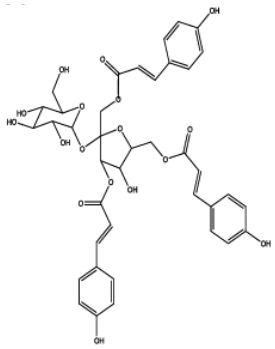

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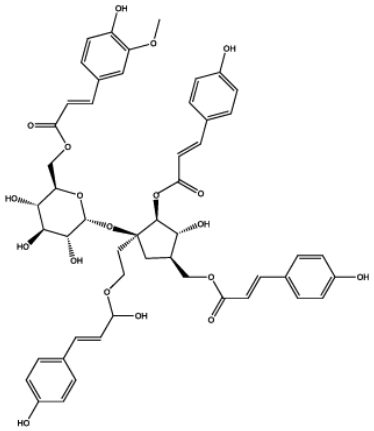

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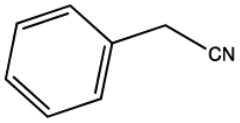

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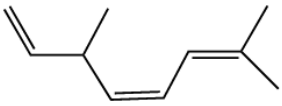

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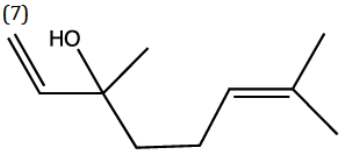

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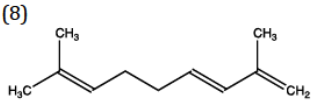

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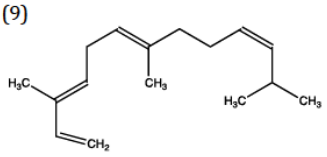

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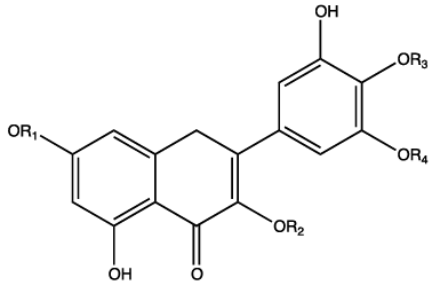
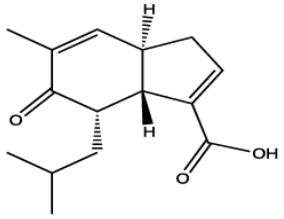
2.3.21 *Polygonum viscosum*

3',5-dihydroxy-3,4',5 ',7-tet- ramethoxyfavone (1), quercetin 3-O-(6-feruloyl)-β-D-galactopyranoside (2), 4-isobutyl-6-methyl-5-oxo-3a,4,5,7atetrahydro- 1H-inden-13-oic acid (3). (Devi *et al*, 2010)





(3)

	R1	R2	R3
(1)	Me	Me	Me
(2)	H	6-feruloyl- β -D-galactopyranosyl	H
(4)	H	6-caffeoyl-β –D-galactopyranosyl	H

2.4 Pharmacological Review:

2.4.1 Antimicrobial activity: The disc diffusion method (Bauer et al., 1966) was used to test antimicrobial activity against thirteen bacteria and three fungi (Table 1). Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were then kept at low temperature (4^{o}C) for 24 h to allow maximum diffusion. There is a gradual change of test materials concentration in the media surrounding the discs. The plates were then incubated at 37^{o}C for 24 h to allow maximum growth of the organisms.

The test material having antimicrobial activity, inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out three times and the mean of the readings is required (Bayer et al., 1966).

Standard disc of kanamycin (30 µg/disc) was used for comparison purpose.

Table 1. Antimicrobial activity of extractives of *P. lanatum* and Kanamycin

Test bacteria and fungi	HEX	DCM	ME	Compound 1	Kanamycin
Bacteria					
Bacillus cereus	13	13	-	ND	39
B. megaterium	12	11	-	ND	32
B. subtilis	-	-	-	ND	20
Staphylococcus aureus	7	7	-	ND	22
Sarcina lutea	10	8	-	13	20
Escherichia coli	7	8	ND	7	23
Pseudomonas aeruginosa	11	10	8	ND	26
Salmonella paratyphi	12	13	10	9	30
S. typhi	8	10	ND	ND	20
Shigella boydii	12	11	9	ND	26
S. dysenteriae	8	7	ND	8	24
Vibrio mimicus	7	8	ND	ND	24
V. parahemolyticus	15	11	-	ND	38
Fungi					
Candida albicans	9	10	ND	8	24
Aspergillus niger	14	12	7	ND	32
Sacharomyces cerevacae	10	10	-	ND	30

"-" Indicates 'no activity' and "ND" indicates 'not done'.

The n-hexane (HEX) and dichloromethane (DCM) soluble fractions (500 µg/disc) and pure compound 1 (300 µg/disc) were screened against 13 test bacteria and 3 fungi and exhibited mild to moderate antimicrobial activity against most of the test organisms whereas, the methanolic (500 µg/disc) crude extract showed poor antimicrobial activity in most cases (Table). The zone of inhibition produced by n-hexane, dichloromethane and methanol extracts were found to be 07 - 15 mm, 07 - 13 mm and 07 – 10 mm respectively. The n-hexane soluble fraction showed mild activity against Bacillus megaterium, Pseudomonas aeruginosa, Salmonella paratyphi & Shigella boydii and

moderate activity against Bacillus cereus, Vibrio parahemolyticus and Aspergillus niger. The dichloromethane soluble fraction showed mild activity against Bacillus megaterium, Shigella boydii, Vibrio parahemolyticus & Aspergillus niger and moderate activity against Bacillus cereus & Salmonella paratyphi. B. subtilis was found to be resistant to the test samples.

2.4.2 In the **cytotoxicity study**, the n-hexane and dichloromethane soluble fractions were found to be highly lethal to brine shrimp nauplii. As a result, LC50 values could not be determined. The LC50 of methanol extract and the purified compound 1 were found to be 3.35 μg/ml and 114.88 μg/ml respectively. Although all the test samples were lethal to brine shrimp nauplii, the hexane and dichloromethane extracts were comparatively more active than the methanol extract and compound 1. This suggests that the hexane and dichloromethane extract may contain additional cytotoxic agents.

Although the extractives showed strong cytotoxicity against brine shrimp nauplii, none of them demonstrated significant inhibition of growth of the test microorganisms. This was probably due to the development of partial or complete resistance of the microorganisms against the test samples, which might be the result of the indiscriminate use of antibacterial agents.

2.4.2.1 *Polygonum lanatum*

Oral administration of either hexane, and ethyl acetate extracts at a dose of 300 mg/kg body weight showed statistically significant ($p < 0.001$) inhibition of rat paw edema by 41.09% and 30.15%, respectively, which was comparable to that of standard drug phenylbutazone (42.15%). Compared to the inhibition of acetic acid-induced writhing by aminopyrine (69.94%, $p < 0.001$), treatment with either hexane, or ethyl acetate extracts or methanol extracts elicited significant inhibition of acetic acid-induced writhing reflex by 44.80% ($p < 0.001$), 33.87% ($p < 0.01$) and 62.29% ($p < 0.001$), respectively. In

addition, mild to potent diuretic activity was observed after oral administration of these extracts in swiss albino mice.

2.5 Ethnobotanical Review of plants of this genus:

2.5.1.1 ***Polygonum hydropiper (L.)*** root extract on chloroform against both bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram-positive (Bacillus subtilis, Bacillus megaterium, Stapphylococcus aureus and Enterobacter aerogenes) and four gram negative (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Shigella sonnei) bacteria. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 16 - 64 μg/ml. The antifungal activities were found strong against six fungi (Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Rizopus oryzae and Tricophyton rubrum). It can be used in the folk medicine at different parts of the world to treat many diseases including bacterial and fungal infections. (Hasan, 2009)

2.5.1.2 In vitro and in vivo anti-inflammatory activities of *Polygonum hydropiper*

methanol extract *Polygonum hydropiper L*. (Polygonaceae) has been traditionally used to treat various inflammatory diseases such as rheumatoid arthritis. However, no systematic studies on the anti-inflammatory actions of *Polygonum hydropiper* and its inhibitory mechanisms have been reported. This study is therefore aimed at exploring the anti-inflammatory effects of 99% methanol extracts (Ph-ME) of this plant. The effects of Ph-ME on the production of inflammatory mediators in RAW264.7 cells and peritoneal macrophages were investigated. Molecular mechanisms underlying the effects, especially inhibitory effects, were elucidated by analyzing the activation of transcription factors and their upstream signalling, and by evaluating the kinase activities of target enzymes.

Additionally, a dextran sulphate sodium (DSS)-induced colitis model was employed to see whether this extract can be used as an orally available drug. (Yang, 2012)

2.5.1.3 Screening of antibacterial, antifungal and cytotoxic activities of *Polygonum hydropiper L.* stem extracts.

The aim of the study was to investigate the antibacterial, antifungal and cytotoxic activities of ethanol extract of *Polygonum hydropiper* stem. Disc diffusion method measuring minimum inhibitory concentration (MIC) was used to demonstrate antibacterial and antifungal activities. Stem extract showed significant antibacterial activities against three gram-positive (*Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus*) and four gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Shigella sonnei*) bacteria resulting 16 to 64 μg/ml MIC values against these bacteria.

Further, the antifungal activities of stem extract were not highly remarked but still to be considered as inhibitory to tested fungi. LC50 of the extract against brine shrimp nauplii was 35.46μg/ml and indicates the cytotoxic potentiality of *Polygonum hydropiper* stem. Results obtained in this study suggest that

polygonum hydropiper stem can also be used as a source of antimicrobial and cytotoxic substances for possible for plant protections. (Hasan, 2011)

2.5.2 *Polygonum acuminatum*

Aerial parts of *P. acuminatum*, are used to heal infected wounds and for other disorders related to fungal infections. Recently (Derita et al., 2009) reported that evaluate the antifungal properties of aerial parts of *P. acuminatum*.

2.5.3 *Polygonum amplexicaule*

Emodin-8-O-β-D-glucoside of *P. amplexicaule* significantly stimulated cell proliferation at 0.1-100 μg/mL and the proportion of cells in S-phase amplified from 16.33 to 27.29%

in osteoblastic MC3T3-E1 cells. Moreover, ethanol extracts of P. amplexicaule improved alkaline phosphatase (ALP) expression in MC3T3-E1 cells at the concentration from 0.1 to 100 μg/mL and inhibited PGE (2) production induced by TNF-α in osteoblasts at the concentrations ranging from 10 to 100 μg/mL in MC3T3-E1 osteoblasts.

2.5.4 *Polygonum aviculare*

The supplementation of *P. aviculare* ethanol extract to high-fat diet-induced obese mice significantly decreased body weight gain, adipose tissue weight, adipocyte size, and lipogenic gene expression as well as serum triglyceride, leptin, and malondialdehyde (MDA) levels.

2.5.5 Polygonum bistorta

Ethanolic extract of *P. bistorta* showed strong antiinflammatory effect. Nikawa et al, reported that the aqueous extract strongly inhibits the mutagenicity of Trp-P-1. The hexane and chloroform fractions and their sub-fractions were evaluated for their cytotoxic activity against P338 (Murine lymphocytic leukaemia), HepG2 (Hepatocellular carcinoma), J82 (Bladder transitional carcinoma), HL60 (Human leukaemia), MCF7 (Human breast cancer) and LL2 (Lewis lung carcinoma) cancer cell lines in culture.

2.5.6 *Polygonum capitatum*

Ethanolic extracts of *P. capitatum* possessed antibacterial, analgesic, antiinflammatory, hypothermia, diuretic and antioxidative activities .

2.5.7. *Polygonum chinense*

The chloroform and ethanol extract of whole plant of *P. chinense* demonstrated a strong activity against Bacillus subtilis, Stapphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger.

2.5.8 *Polygonum Cillinerve*

The administration of *P. Cillinerve* with gavage was able to overcome the cyclophosphamide-induced immunosuppression and significantly to raise the total oxidant capacity (TOC), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) level. It also raised the liver, spleen, thymus indices and decreased the malondialdehyde (MDA) level in mice.

2.5.9 *Polygonum cuspidatum*

Extract of *P. cuspidatum* has antiinflammatory activities such as inhibition of NF-kB have been reported. Polydatin of *P. cuspidatum* has favorable potency to develop a hypolipemic and hepatoprotective agent in clinic. Ethanol and ethyl acetate extracts of *P. cuspidatum* root exhibit an antiproliferative effect on human lung cancer cells. Piceid of *P. cuspidatum* represents a safe and new candidate for a skin-lightening agent. 2-methoxy-6-acetyl-7-methyljuglone may act as a potent anti-oxidant, which significantly interferes with the Mitogen-activated protein kinases apoptotic cascades, probably rescuing cells by inhibiting the death pathways. (Han et al., 2005) reported that ethyl acetate extract of *P. cuspidatum* can be a potent candidate for rheumatoid arthritis treatment. Extract of *P. cuspidatum* shown that possesses wound-healing activity. Resveratrol and emodin have shown anticancer potential in various cancer cells, including hepatocarcinoma cells.

2.5.10 *Polygonum ferrugineum*

Lopez et al., reported that *P. ferrugineum* extracts as used to heal infected wounds and as antiseptic, antibiotic antifungal agent in traditional medicine.

2.5.11 *Polygonum flaccidum*

Mazid et al., reported that alpha-santalone, of *P. flaccidum* could be a potent candidate for analgesic and the diuretic.

2.5.12 *Polygonum glabrum*

P. glabrum extract is clinically effective as antiinflammatory drug and works by the mechanism of action similar to that of nonsteroidal antiinflammatory drugs (NSAIDs), also has been researched for anthelmintic activity. Which showed activity against Hymenolepsis. (nana var. fraternal. Kiron et al.,) reported that analgesic activity of aqueous extract of *P. glabrum* has performed by formalin-induced paw licking in rats.

2.5.13 *Polygonum hypoleucum*

P. hypoleucum Ohwi is a Chinese herbs that has been used for the treatment of arthritis, rheumartitis, cough, influenzaand nephritis . The previous studies found that *P. hypoleucum* is a growth modulator for tumor cells and human mesangial cells.

2.5.14 *Polygonum hyrcanicum*

Aerial parts of the *Polygonum hyrcanicum* are used for the treatment of liver problems, anemia, hemorrhoids and kidney stones. Methanolic extract from aerial parts of *P. hyrcanicum* showed high activity against Trypanosoma brucei rhodesiense (IC50 = 3.7 microg/mL).

2.5.15 *Polygonum lapathifolium*

Phenylpropanoid esters of sucrose, vanicoside B and lapathoside A, of *P. lapathifolium* exhibited significant antitumor-promoting effects on mouse two-stage skin carcinogenesis induced by 7,12-dimethylbenz anthracene and tetradecanoyl phorbol

acetate (TPA) as a promoter. Methanolic extract of *P. lapathifolium* stems have potential anthelmintic and anti-emetic properties.

2.5.16 *Polygonum limbatum*

Methanol extract and compounds of *P. limbatum* have showed cytotoxicity and antimicrobial activity.

2.5.17 *Polygonum minus*:

Various researchers reported the different biological and pharmacological activities of *Polygonum minus* in both in vitro and in vivo experimental models. It has been found to exhibit antiulcer, antimicrobial , antioxidant, anticytotoxicity and antigenotoxicity activities .

2.5.18 *Polygonum multiflorum*

This herb possesses many effects, such as lipid lowering, antioxidation, toxin detoxification, anti-tumor, and lubricating intestine, to treat cardiovascular disorders, neurological disorders, and other diseases commonly associated with aging. Modern chromatographic separation studies have demonstrated that many bioactive compounds in *P. multiflorum*, e.g. stilbene glycosides, are responsible for its medicinal activities. 2,3,5,4'-Tetrahydroxystilbene-2-O-beta-d-glucosidel, a major active stilbene glycoside in *P. multiflorum*, has been reported to possess antioxidative, antiinflammatory, endothelial protective, and oncogenic enzyme inhibitory activities. Hexane extract of *P. multiflorum* neuroprotective effect against glutamate-induced neurotoxicity via inhibition of apoptosis.

2.5.19 *Polygonum odoratum*

The n-Butanol fraction from the methanol extract of this herb exhibited dramatic hypoglycemic effects in STZ-induced diabetic mice. Chia et al. reported that the water

extract of rhizome of *P. odoratum* decreased the blood glucose level in starch-loaded mice.

2.5.20 *Polygonum orientale*

Water extract of *P. orientale* comprising flavonoids as its principles was of potential use in the treatment of cardiovascular and cerebrovascular diseases and an injection prepared from the flavonoid-enriched water extract had demonstrated protective effects on myocardial ischaemia. Leaves and flowers of *P. orientale* showed a protective effect on H9c2 myocardial cells oxidative injury. A flavonoid-enriched extract of *P. orientale* was reported to show cardioprotective effect.

2.5.21 *Polygonum paleaceum*

Recently, a few pharmacological studies showed that extract of *P. paleaceum* has antiinflammatory effect on inflammatory rates and antitumor activity to K562, HL-60 (Human promyelocytic leukemia cells) in vitro and S180, Hep A in vivo, which are related to oxygen free radical scavenging and antilipid peroxidation activity of *P. paleaceum*.

2.5.22 *Polygonum perfoliatum*

Recently, contemporary studies have shown that *P. perfoliatum* has a variety of pharmacological functions including antiinflammatory, antibacterium and antitumor effects. Pharmacological studies indicated that the flavonoids in *P. perfoliatum* have antiviral, anti-oxidative andantitumor activities.

2.5.23 *Polygonum punctatum*

Previous pharmacological reports with the ethanol/water extract of the entire *P. punctatum* disclosed antifungal, antihistaminic, antiinflammatory, antipyretic and hypotensive activities

2.5.24 *Polygonum sachalinensis*

Previous work showed that leaves extracts of *P. sachalinensis* can be used as a plant fungicide against powdery mildew that flavonones and anthraquinones of flower extracts are good antioxidant compounds and that phenylpropanoid glycosides from the rhizomes exhibit β-glucosidase inhibitory activity.

2.5.25 *Polygonum spectabile*

Geraldo et al., reports on the investigation of the antimicrobial and antiviral activity of different extracts and compounds isolated from the aerial parts of *P. spectabile.*

2.5.26 *Polygonum stagninum*

The n-hexane, ethyl acetate and methanol extracts of the aerial parts of *P. stagninum* were assessed for analgesic and anti-inflammatory properties in experimental mice and/or rat models.

2.5.27 *Polygonum tinctorium*

Antifungal, cancer chemopreventive and antibacterial activities. Yu et al., 1998 reported that the potent anti-HIV-1 and HSV-1 activity of an aqueous extract from the fermented leaves of *P. tinctorium* while seeds and leaves of *P. tinctorium* have an antioxidant and anticancer properties.

2.5.28 *Polygonum viscosum*

Ethanolic extract of young shoots of *P. viscosum* was found to possess antibacterial activity.

Chapter 3: Materials

The following materials were used during the course of phytochemical study:

3.1 Solvents:

- Petroleum ether
- Methanol
- Chloroform
- Acetic acid
- Ethyl acetate
- Toluene
- Acetone
- Benzene
- Dichloromethane

3.2 Glassware:

- Thin layer chromatography (TLC) tank
- TLC plates, size in cm (20 x 20), (20 x 5)
- Precoated TLC plates
- Quick fit flasks
- Capillary tube
- Micropipette 1000 microliter

3.3 Equipment:

- Rotary evaporator
- Grinding Machine
- Electronic balance
- Distill water maker
- UV Light

3.4 Silica gel:

- TLC grade (PF-254)
- Column grade (60-120 mesh)

3.5 Spray Reagent:

- Vanillin-H_2SO_4

3.6 Filter aids:

- Filter paper (Whatman no: 1)
- Cloth
- Cotton pad
- 1000 ml Beakers

3.7 Figures of Equipment:

3.7.1 Rotary Evaporator

3.7.2 Grinding Machine:

3.7.3 Electronic balance

3.7.4 Distill water maker

Chapter 4: Methods

4.1 Selection of plants

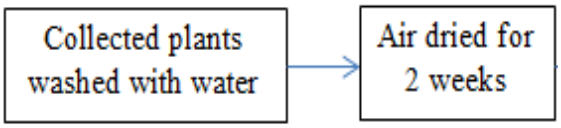
Fresh plants of *Polygonum Lanatam* (family Polygonaceae) were selected for biological investigation.

4.2 Collection of the plant part

For this present investigation the plants of *Polygonum Lanatam* was collected from Manikganj on October 2013. The specimen of plant was taxonomically identified at the Bangladesh National Herbarium, Mirpur, Dhaka.

4.3 Drying of the plant part

The collected plants were washed with water and unwanted materials were discarded. Collected plants were air and sun dried for 14 days.



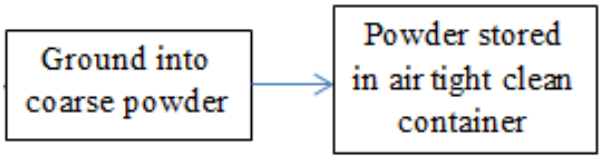
4.4 Storage and Preservation of plant part

Most plant parts from desired plants have undergone a period of storage before they were finally used for research purpose in the laboratory. During this period many undesirable changes may occur in the plant parts if they were not properly stored and preserved against the reabsorption of moisture, oxidation, excessive heat, humidity, direct sunlight, growth of molds and bacteria and infestation by insects and rodents. Proper storage and preservation of plant parts are thus are very important factors in maintaining a high

degree of quality in them. All efforts towards proper storage should be geared to protect the drugs from all the above deteriorating factors and agents.

4.5 Grinding of the plant parts

The dried small pieces of plant parts were grinded into small fine particles by a grinder machine from. The powder was stored in an air tight container and kept in a cool dark and place until analysis commenced.



4.6 Cold extraction

On 27th November, 2014, 500gm of coarse plant powder was soaked in 1500ml distilled methanol at room temperature. The solvent was added in powder in such a way so that the solvent front remains at least three inches above the powder. The mixture was then kept in an air tight container for 72 hours. The container was occasionally stirred to soak the powder homogenously.

On 2nd December 2014, 177.05gm of coarse plant powder was again soaked in 750ml distilled methanol at room temperature. The solvent was added in powder in such a way so that the solvent front remains at least three inches above the powder. The mixture was then kept in an air tight container for 72 hours. The container was occasionally stirred to soak the powder homogenously.

On 17th January, 2015, 500gm of coarse plant powder was again soaked in 1500ml distilled methanol at room temperature. The solvent was added in powder in such a way so that the solvent front remains at least three inches above the powder. The mixture was

then kept in an air tight container for 72 hours. The container was occasionally stirred to soak the powder homogenously.

4.7 Filtration of extract

After three days of cold extraction, the solvent was decanted and filtered through two-folded fine cotton cloth and 1480ml of filtrate was obtained.

4.8 Drying of extract

Using rotary evaporator, the methanolic extract of plant was evaporated at 55-60 degree Celsius temperature and a rotation speed of 160-180 rpm for 1 month. After this drying process, a slurry concentration were obtained, which were kept in small 50 ml beakers for further drying. During transfer to the beaker the extracts were rinsed by acetone.

4.9 Separation of oil part

The crude extract was kept untouched for several days. And after this, an oil layer was formed on the upper surface of the extract. The oil was then separated from the upper surface of the extract through decantation. The oil portion separated from extract was 27ml. the extract portion remained after decantation is termed as crude extract by which further analysis was commenced.

4.10.1 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an

analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots.

They all have a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates.

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

As the solvent moves past the spot that was applied, equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere except where an organic compound is on the plate.).

4.10.2 Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent.

The Rf formula is

Rf = distance traveled by sample / distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

Rf values and reproducibility can be affected by a number of different factors such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the TLC plate, sample size, and solvent parameters. These effects normally cause an increase in Rf values. However, in the case of layer thickness, the Rf value would decrease because the mobile phase moves slower up the plate.
If it is desired to express positions relative to the position of another substance, x, the Rx (relative retention value) can be calculated:

Rx=distance of compound from origin / distance of compound x from origin
Rx can be greater than 1.

4.10.3 Materials & reagents

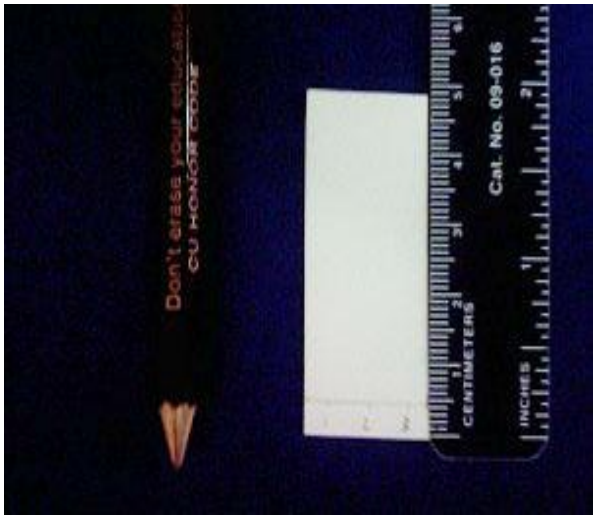
1. TLC plate
2. TLC tank
3. Cutter
4. Scale
5. Pencil
6. Solvents & reagents
   - ✓ Methanol

- ✓ Ethanol
- ✓ Ethyl acetate
- ✓ Benzene
- ✓ Diluted sulfuric acid

7. Hot plate

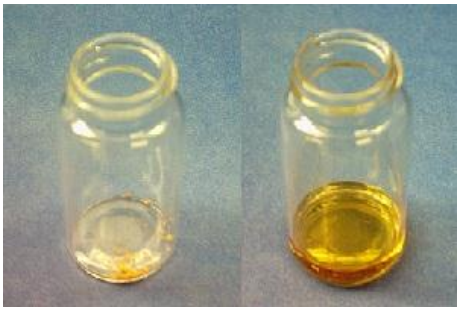
4.10.4 Test TLC procedure

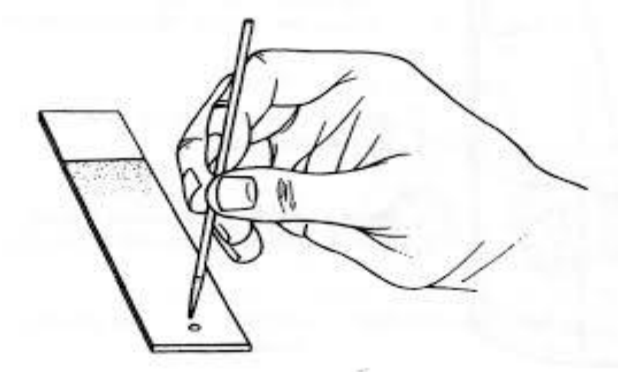
1. Each large TLC sheet (made up of metal & coated with silica) was cut horizontally into plates which are 5 cm tall by 1.5cm in widths. Handle the plate was carefully handled so that the coating of adsorbent was not damaged.



2. 0.5 cm from the bottom of the plate was measured. Using a pencil, draw a line was drawn across the plate at the 0.5 cm mark. This is the origin: on which the extract was spotted. The name of the samples spotted on the plate was lightly marked under the line.

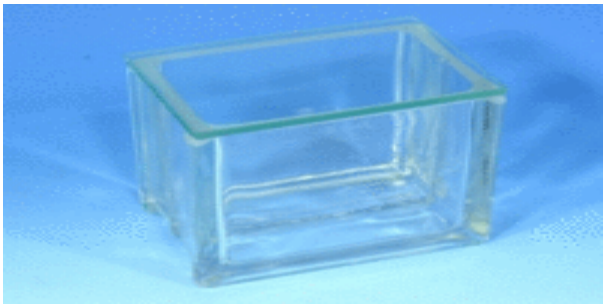
3. ~1 mg of the hard extract was dissolved in 1 ml of methanol in a watch glass to make the solution of the extract.

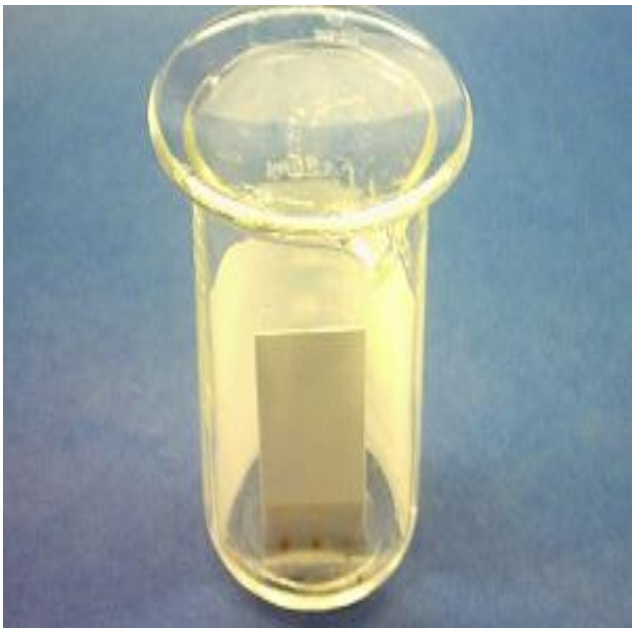




4. The prepared extract solution was then spotted on TLC plate

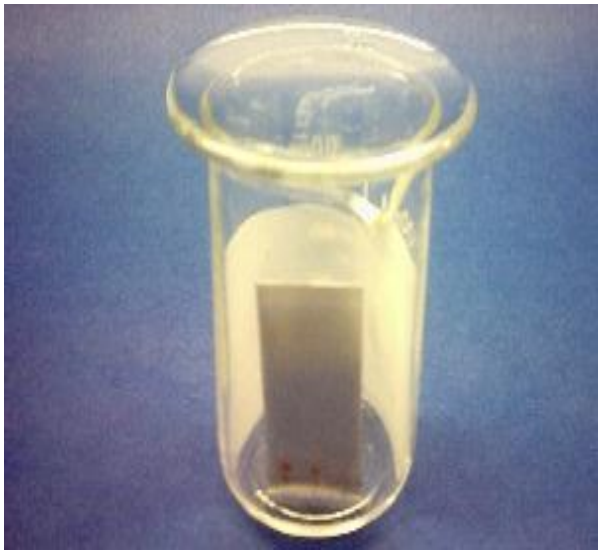
5. Measured solvents were taken in TLC tank

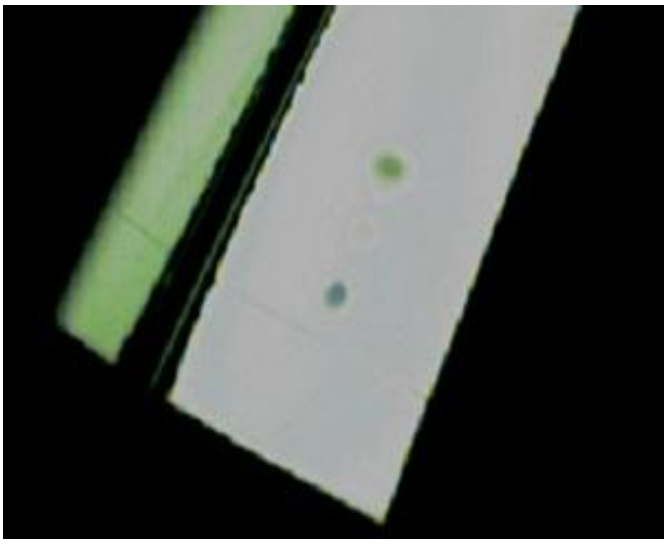




6. The prepared TLC plate was placed in the tank in such a way so that the solvent front remains under the drawn line, the tank was covered with the lid, and left undisturbed on the bench top. The solvent rose up the TLC plate by capillary action. Make sure the solvent does not cover the spot

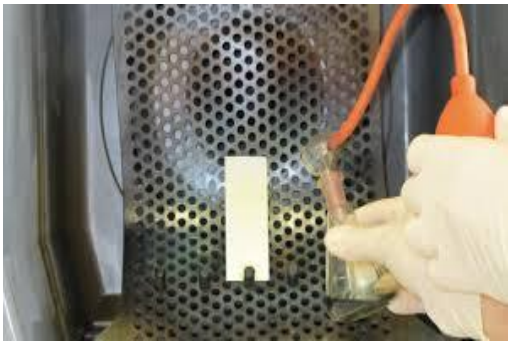
7. The plate was developed until the solvent is about half a centimeter below the top of the plate. The plate was removed from the beaker and the solvent front was immediately marked with a pencil. The plate was allowed to dry.





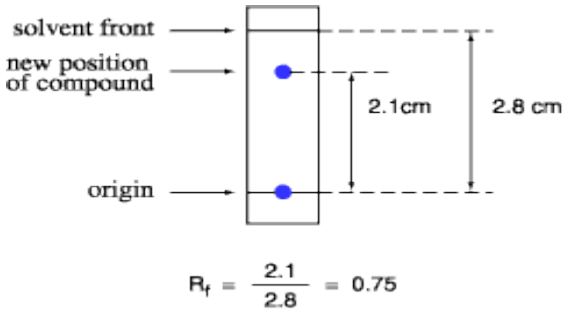
8. The plate was then kept under UV lamp to observe any band if appeared.

9. The TLC plate was then sprayed by sulfuric acid in fume hood.



10. The plate was then heated on hot plate so that the band color can be appeared clearly.

11. Then Rf valued by measuring the distance traveled by the sample and the distance travled by the solvent in the TLC plate.



$$R_f = \frac{2.1}{2.8} = 0.75$$

Test TLC was performed for the crude extract of *Polygonum lanatam* on the basis of trial & error for several times by changing the ratio of solvents used as mobile phase.

Different solvents used as mobile phase and results based on this in test TLC

4.10.6 Using polar solvents

1. **Set-A:**

Reagent	**Amount**
Ethyl acetate	8 ml
Ethanol	2 ml

❖ **Result:**

An orange band was observed under UV lamp.

Distance traveled by the sample = 5.3

Distance travled by the solvent = 5.6

Therefore Rf value = 5.5/5.6 = 0.87

1. **Set-B:**

Reagent	**Amount**
Ethanol	10 ml

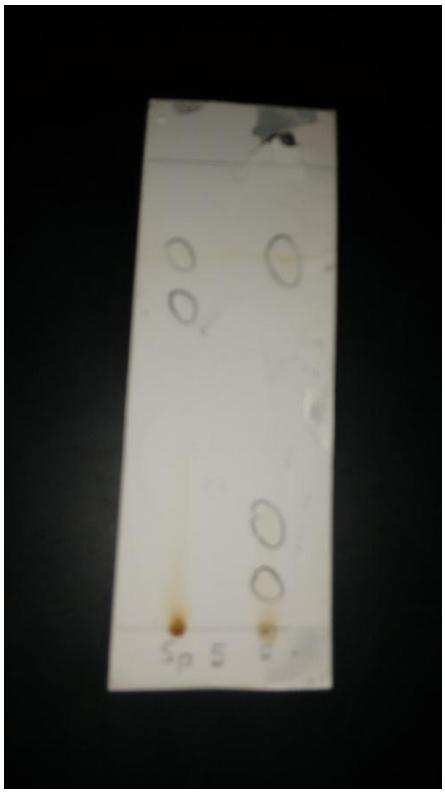
❖ **Result:**

- Two orange bands were observed under UV lamp.
- Distance traveled by the sample for band (1) = 1.3
- Distance traveled by the sample for band (2) = 5.4
- Distance travled by the solvent = 5.6
- Therefore Rf value for band (1) = 1.3/5.6 = 0.232

  Rf value for band (2) = 5.4/5.6= 0.964

2. Set-C:

Reagent	Amount
Ethyl acetate	10 ml



❖ **Result:**

- Three orange bands were observed under UV lamp.
- Distance traveled by the sample for band (1) = 3.6
- Distance traveled by the sample for band (2) = 4.3
- Distance travled by the solvent = 5.
- Therefore Rf value for band (1) = 3.6/5 =0.72

  Rf value for band (2) = 4.3/5 = 0.86

4.10.7 Using non polar solvents

1. **Set A:**

Reagent	**Amount**
Benzene	9 ml
Ethanol	1 ml

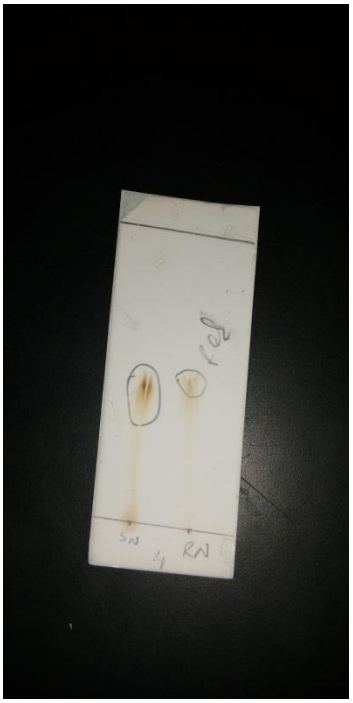
❖ **Result:**

- Two orange bands were observed under UV lamp.
- Distance traveled by the sample for band (1) = 1.2
- Distance traveled by the sample for band (2) = 2.4
- Distance traveled by the solvent = 7.3
- Therefore Rf value for band (1) = 1.2/7.3 = 0.164

  Rf value for band (2) = 2.4/7.3= 0.329

2. **Set B:**

Reagent	**Amount**
Benzene	6 ml
Ethanol	4 ml



❖ **Result:**

- An orange band was observed under UV lamp.
- Distance traveled by the sample for band = 2.6
- Distance traveled by the solvent = 5.2
- Therefore Rf value for band = 2.6/5.2 = 0.5

3. **Set B:**

Reagent	**Amount**
Benzene	5 ml
Ethanol	5 ml

❖ **Result:**

- An orange band was observed under UV lamp.
- Distance traveled by the sample for band = 4.8
- Distance traveled by the solvent = 5.2
- Therefore Rf value for band = 4.8/5.2 = 0.96

4.11 Preparative Thin Layer Chromatography:

4.11.1 Apparatus and reagents:

- Ethanol
- Ethyl acetate
- Chloroform
- Spatula
- Silica gel
- Distilled Water
- Glass rod
- Preparative TLC tank
- Test TLC plates
- Test TLC tank
- Sulfuric acid
- Heater
- Forcep
- UV spectrometer

4.11.2 TLC plate preparation:

TLC plates had prepared by Silica gel and distilled water. 500 gm of silica gel was mixed with distilled water of a suitable amount to make a suitable paste that could be made as a layer on a square shaped glass plate. The paste was made and its viscosity was checked by making a sample plate. Then the paste was smoothly applied on the glass plates to make a suitable TLC plate. Then the plates were kept for 24 hour to let them dry.

Fig: Preparative TLC plate

4.11.3 Sample Introduction:

When the TLC plates were completely dried, sample was introduced on side the plate by using micropipette. A few microgram of the sample extract was diluted by ethanol in a small beaker then the diluted sample was introduced by the micropipette. After this, the plate was dried for few minutes.

4.11.4 Tank preparation:

Fig: preparative TLC tank

The Tank for Preparative TLC is a larger one then the test TLC tank. The tank was cleaned well first then dried by electrical drier. The TLC reagents Ethanol 350 ml was introduced into the tank and the closed it for few moments to saturate the internal environment by the reagent.

4.11.5 Operation:

The prepared TLC plate was introduced to the tank very carefully that must not touch the wall of the tank and the reagent must not cross the sample line. Then the tank closed by the closure and waited to run the reagent through the plate. When the reagent reaches the top, it came out from the tank and let it for drying.

4.11.6 Observation:

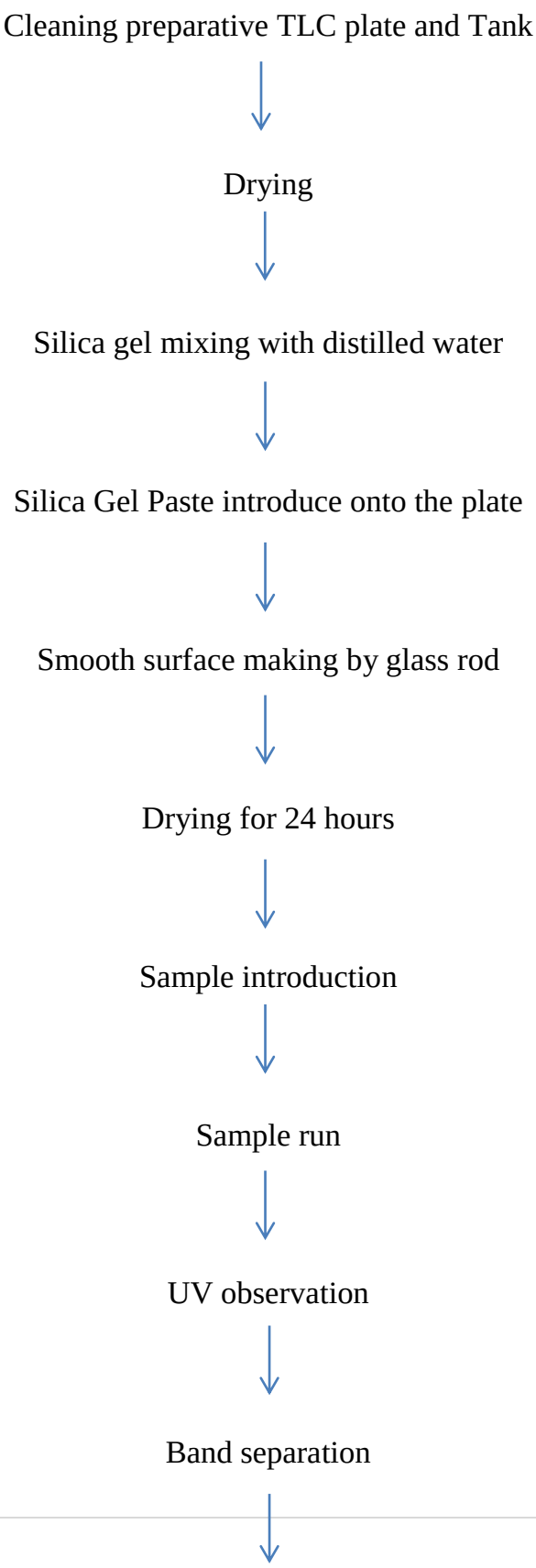
After drying the plate it was observed under UV light at Wavelength 257nm and three bands were observed. After that the plates were placed into the Fume hood for charring with H_2SO_4. Then the three bands became clearer.

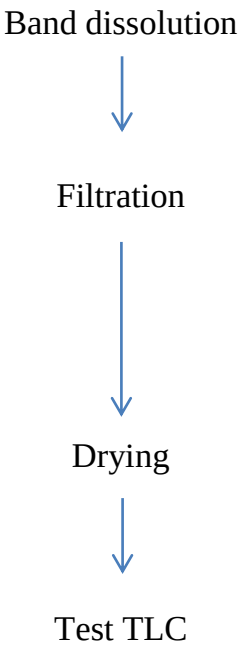
4.11.7 Band collection:

The bands were collected by a clean Spatula into a small beaker. Then the contents of the beaker were dissolved in chloroform and ethyl acetate and kept for one day. Then the contents were filtered by filter paper and kept the filtrate for 3 days to make that contents dried and to remove solvents. Then the dried content was dissolved by acetic acid and chloroform for test TLC purpose. Then the sample was introduced onto test TLC plate and a test TLC was done with Ethanol.

Result: The test TLC showed a single band for each band in preparative TLC.

Flow Chart:


Cleaning preparative TLC plate and Tank
Drying
Silica gel mixing with distilled water
Silica Gel Paste introduce onto the plate
Smooth surface making by glass rod
Drying for 24 hours
Sample introduction
Sample run
UV observation
Band separation



4.11.8 Discussion:

Result of preparative TLC said that the sample contain some different compounds which gave the bands. The bands were for a single compound for each band as the test TLC for each band showed only a single band.

4.12 Crystal Separation:

Crystals were found in both the main methanolic extract and the oil extract of the plant. Both extracts were vigorously ringed by methanol. The extract was dissolved in methanol except the crystals. The extract was come out form the crystal in the beaker by continuous ringing with pure methanol. The crystals from the oil extract were slightly larger than those from main extract.

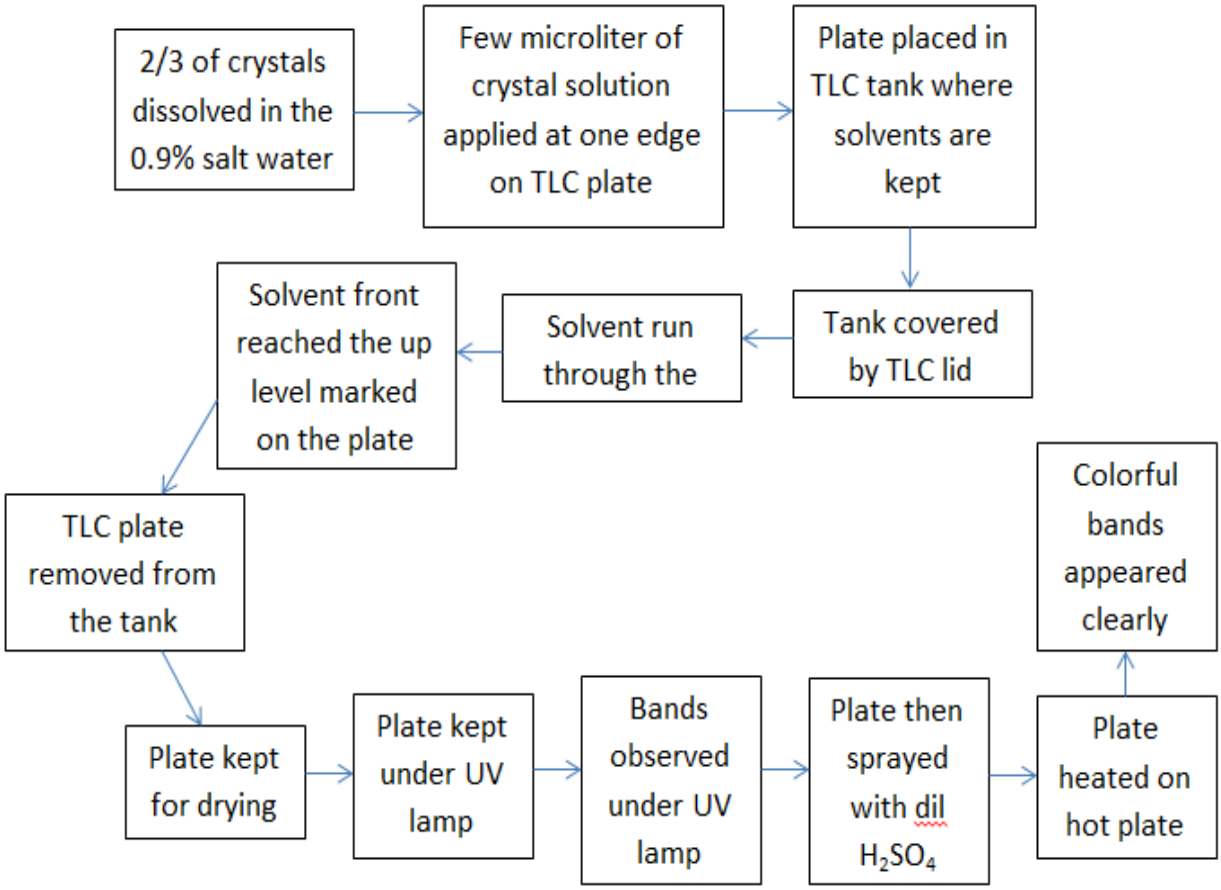
Fig: Crystal form methanolic extract

Fig: Crystal from the oil portion

4.13 Thin Layer Chromatography for crystals:

Two methods of TLC were used for the crystals.

4.13.1.1 Method 1 by using 0.9% salt water



4.13.1.2 Different solvents used as mobile phase

3. **Set-A (Polar solvent)**

Reagent	**Amount**
Ethyl acetate	8 ml
Ethanol	1.2 ml
Water	0.8 ml

- **Result:**

  No band was found.

4. **Set-B (Semi Polar solvent):**

Reagent	**Amount**
Chloroform	5 ml
Ethyl acetate	4 ml
Acetic acid	1 ml

- **Result:**

  No band was found.

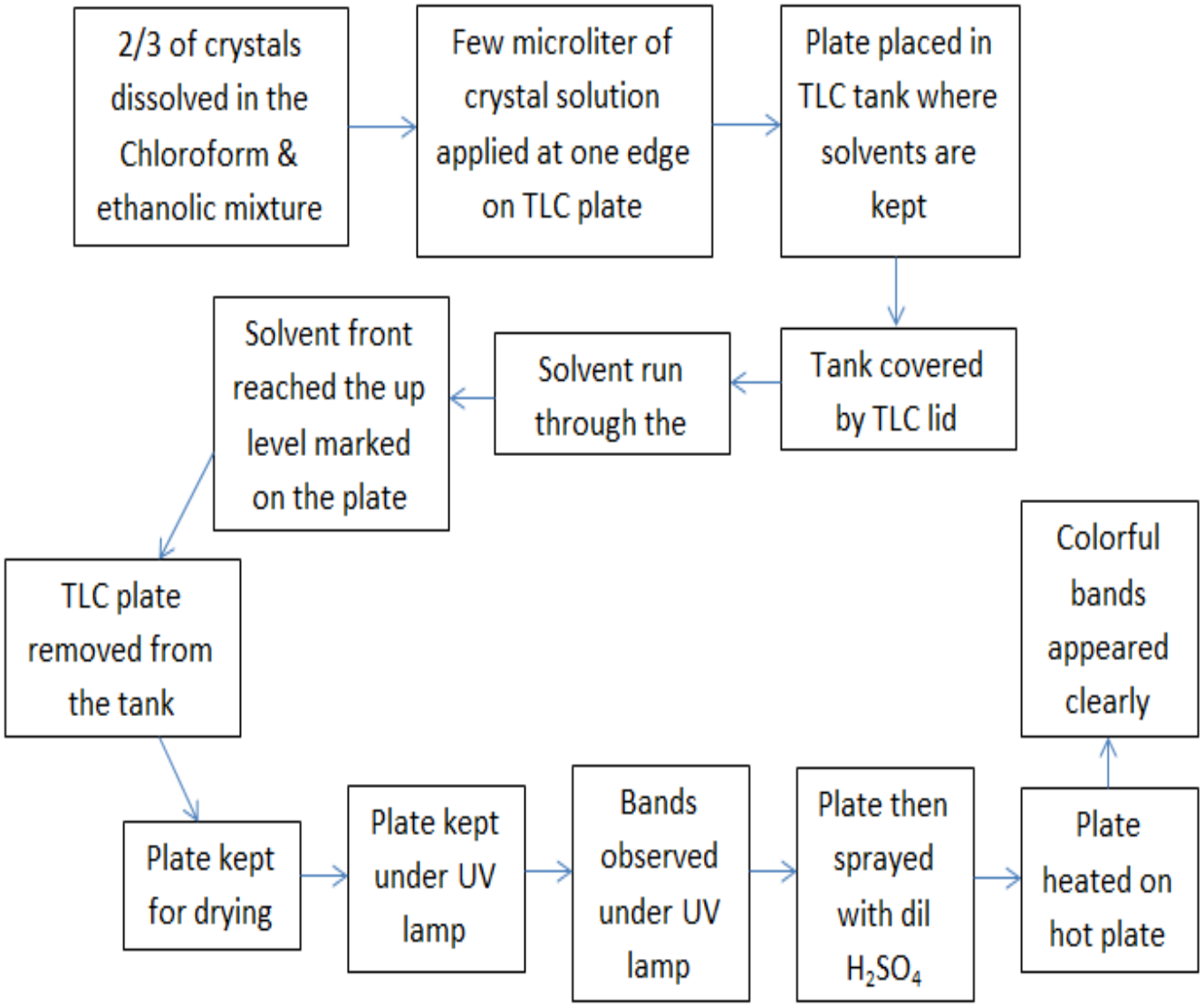
5. Set-C (Non Polar solvent):

Reagent	Amount
Benzene	9 ml
Ethanol	1 ml

❖ **Result:**

- A clear white band was observed under UV lamp.
- Distance traveled by the sample = 4.75
- Distance travled by the solvent = 5
- Therefore Rf value = 4.75/5= 0.95

4.13.2 Method 2 by using Chloroform & ethanolic mixture.



4.13.3 Different solvents used as mobile phase

6. Set-A (Polar solvent)

Reagent	Amount
Ethyl acetate	8 ml
Ethanol	1.2 ml
Water	0.8 ml

- **Result:**
  - A vertical white band was observed under UV lamp.
  - Distance traveled by the sample = 5.1
  - Distance travled by the solvent = 5.6
  - Therefore Rf value = 5.1/5.6 = 0.91

7. **Set-B (Semi Polar solvent):**

Reagent	**Amount**
Chloroform	5 ml
Ethyl acetate	4 ml
Acetic acid	1 ml

- **Result:**

No band was found.

8. **Set-C (Non Polar solvent):**

Reagent	**Amount**
Benzene	9 ml
Ethanol	1 ml

- **Result:**

No band was found

4.14 Infrared Spectroscopy of the crystals:

4.14.1 Infrared spectroscopy (IR):

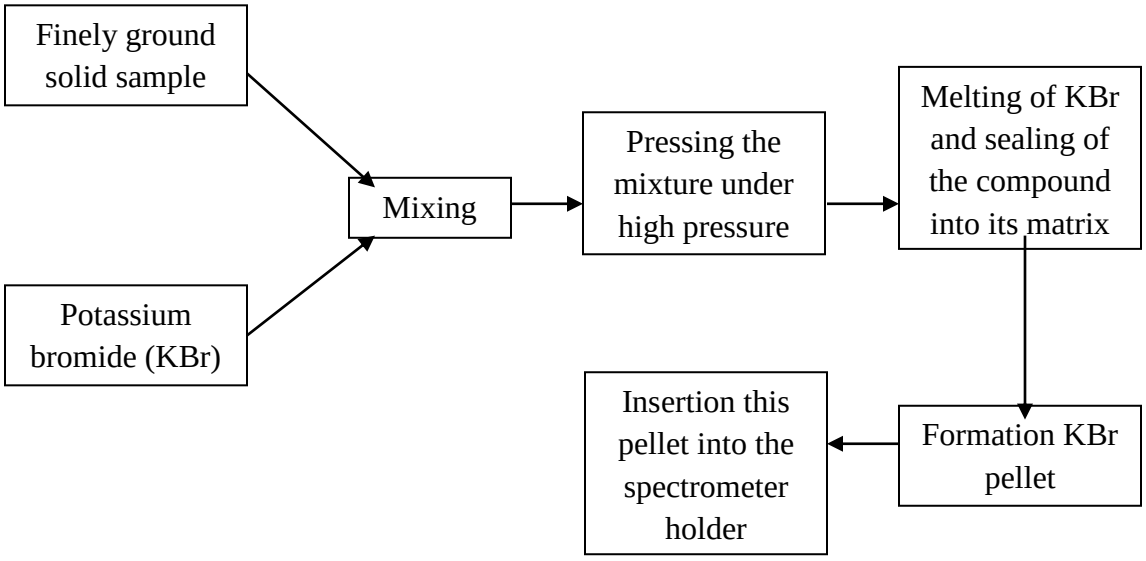
IR is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantify some components of an unknown mixture and for the analysis of solids, liquids, and gases. The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a development in the manner in which the data is collected and converted from an interference pattern to a spectrum. It is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum.

Fig: IR spectroscopy

4.14.2 Principle

Molecular bonds vibrate at various frequencies depending on the elements and the type of bonds. For any given bond, there are several specific frequencies at which it can vibrate. According to quantum mechanics, these frequencies correspond to the ground state (lowest frequency) and several excited states (higher frequencies). One way to cause the frequency of a molecular vibration to increase is to excite the bond by having it absorb light energy. For any given transition between two states the light energy (determined by the wavelength) must exactly equal the difference in the energy between the ground state and the first excited state.

4.14.3 Preparation of samples for FTIR



4.14.4 IR instrumentation & process

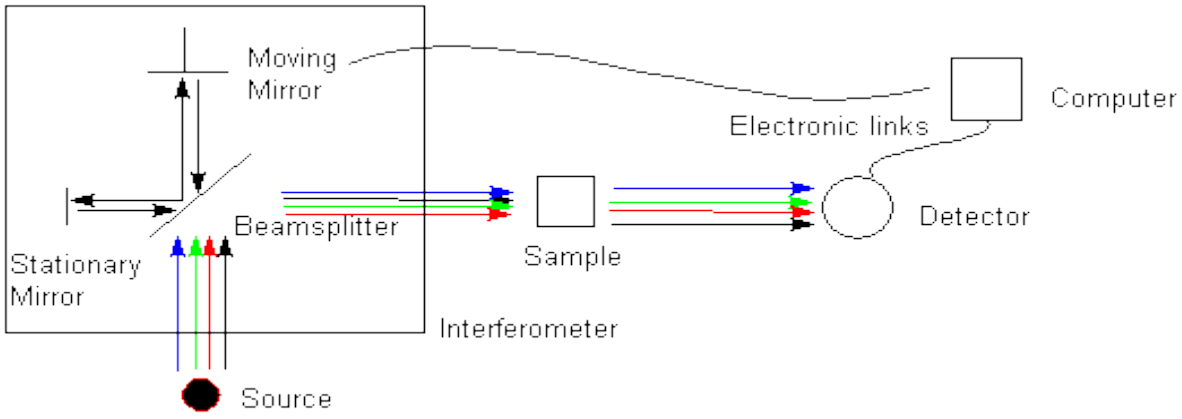


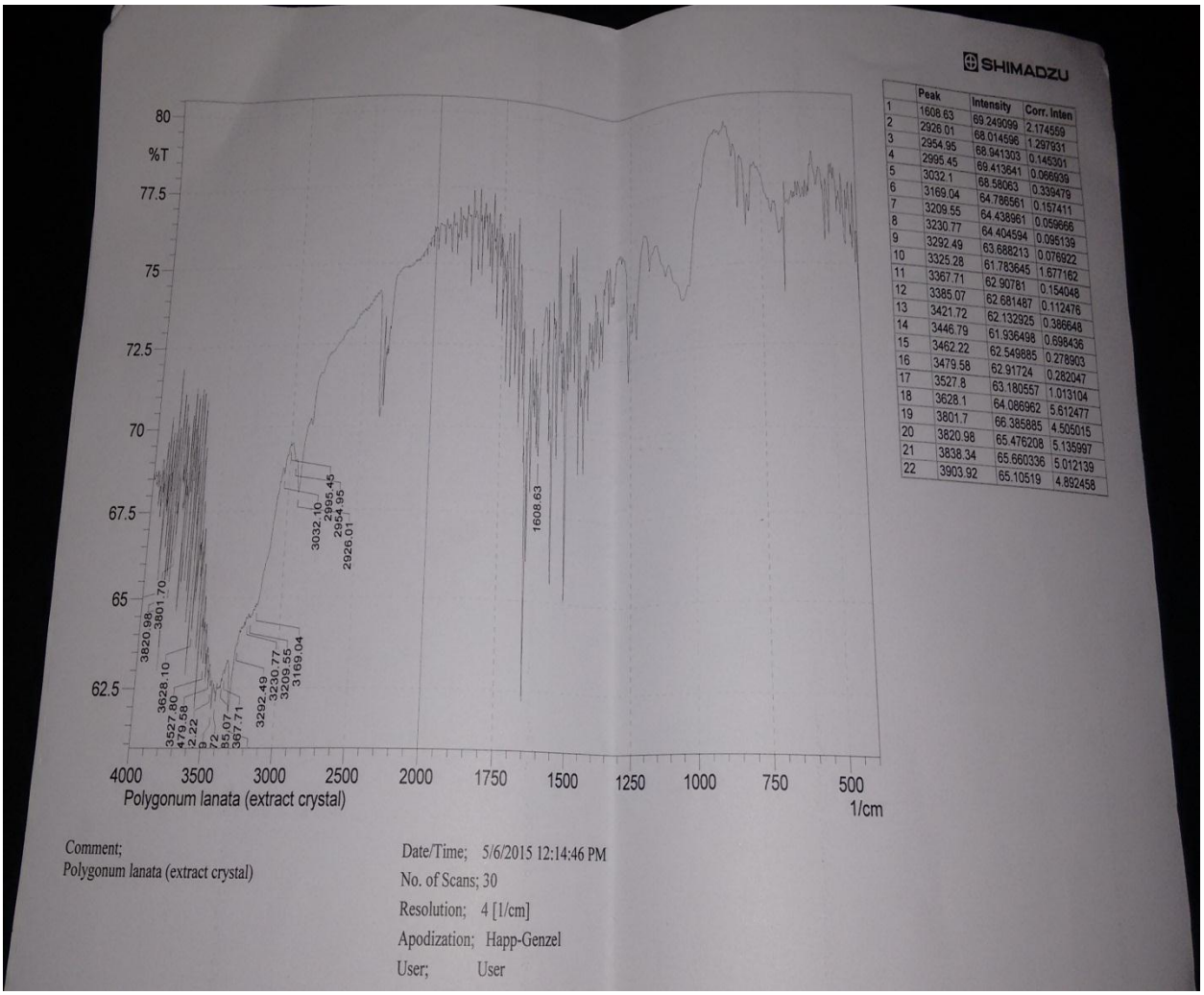
Figure: FTIR instrument and process

- The FTIR uses an inferometer to process the energy sent to the sample
- In the inferometer, the source energy passes through a beam splitter, a mirror placed at 45° angle to the incoming radiation. This allows the incoming radiation to pass through but separates it into perpendicular beams, one undeflected, the other oriented at 90° angle.
- One beam, the one oriented at 90° goes to a stationary or fixed mirror, and returned to the beam splitter.
- The undeflected beam goes to a moving mirror and also returned to the beam splitter.
- When the beams meet at the beam splitter, they recombine, but the path length differences of the two beams causes both constructive and destructive interferences.
- The combined beam containing these interference patterns is called the interferogram having a wide range of wavelengths.
- The interferogram is oriented towards the sample by the beam splitter.
- As it passes through the sample, the sample simultaneously absorbs all of the wavelengths that are normally found in its infrared spectrum.

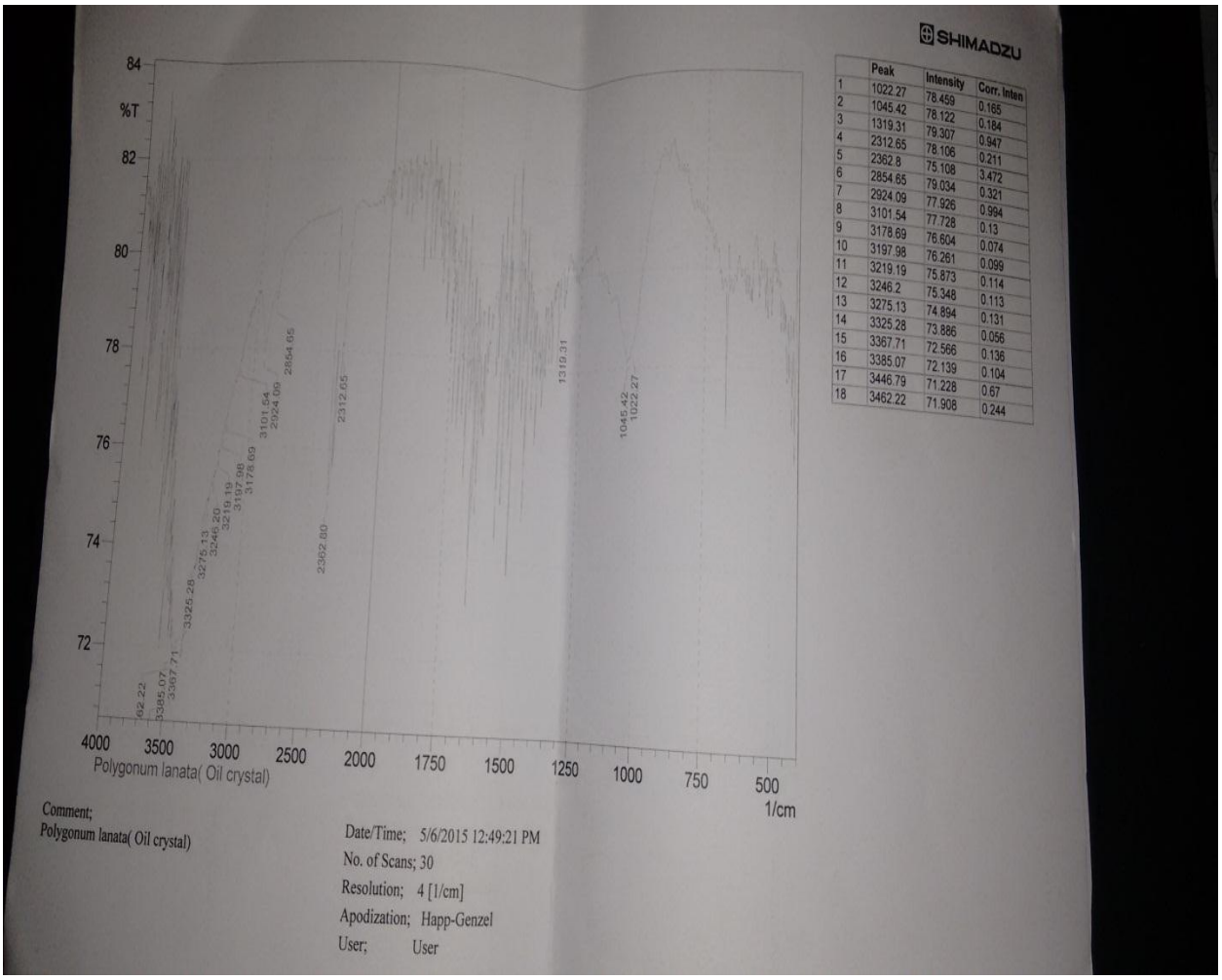
- The modified interferogram signal that reaches the detector contains information about the amount of energy that was absorbed at every wavelength.
- The computer modifies the modified interferogram to a reference laser beam to have a standard of comparison.

4.14.5 Result:

IR spectrum of the methanolic extract:



IR spectrum of the oil extract:



	Peak	Intensity	Corr. Inten
1	1022.27	78.459	0.165
2	1045.42	78.122	0.184
3	1319.31	79.307	0.947
4	2312.65	78.106	0.211
5	2362.8	75.108	3.472
6	2854.65	79.034	0.321
7	2924.09	77.926	0.994
8	3101.54	77.728	0.13
9	3178.69	76.604	0.074
10	3197.98	76.261	0.099
11	3219.19	75.873	0.114
12	3246.2	75.348	0.113
13	3275.13	74.894	0.131
14	3325.28	73.886	0.056
15	3367.71	72.566	0.136
16	3385.07	72.139	0.104
17	3446.79	71.228	0.67
18	3462.22	71.908	0.244

Both the spectrums are showing a lot of overlapping peak which is very difficult to interpret.

Chapter 5: Result and Discussion

5.1 A several numbers of Thin Layer Chromatography (TLC) were performed on the methanolic extract of *Polygonum lanatam, whose results are given below:*

5.1.1 Using Polar solvent

Reagent	**Amount**
Ethyl acetate	8 ml
Ethanol	2 ml

- **Result:**

An orange band was observed under UV lamp.

Distance traveled by the sample = 5.3

Distance travled by the solvent = 5.6

Therefore Rf value = 5.3/5.6 = 0.87

Reagent	**Amount**
Ethanol	11 ml

- **Result:**
  - Two orange bands were observed under UV lamp.
  - Distance traveled by the sample for band (1) = 1.3
  - Distance traveled by the sample for band (2) = 5.4
  - Distance travled by the solvent = 5.6
  - Therefore Rf value for band (1) = 1.3/5.6 = 0.232

Rf value for band (2) = 5.4/5.6= 0.964

Reagent	Amount
Ethyl acetate	11 ml

- **Result:**
  - Three orange bands were observed under UV lamp.
  - Distance traveled by the sample for band (1) = 0.7
  - Distance traveled by the sample for band (2) = 1.5
  - Distance travled by the solvent = 5.
  - Therefore Rf value for band (1) = 0.7/5 = 0.14

    Rf value for band (2) = 1.5/5 = 0.3

5.1.2 Using non polar solvents

Reagent	Amount
Benzene	9 ml
Ethanol	1 ml

- **Result:**
  - Two orange bands were observed under UV lamp.
  - Distance traveled by the sample for band (1) = 1.2
  - Distance traveled by the sample for band (2) = 2.4
  - Distance traveled by the solvent = 7.3
  - Therefore Rf value for band (1) = 1.2/7.3 = 0.164

    Rf value for band (2) = 2.4/7.3= 0.329

Reagent	Amount
Benzene	6 ml
Ethanol	4 ml

- **Result:**
  - An orange band was observed under UV lamp.
  - Distance traveled by the sample for band = 2.6
  - Distance traveled by the solvent = 5.2
  - Therefore Rf value for band = 2.6/5.2 = 0.5

Reagent	Amount
Benzene	5 ml
Ethanol	5 ml

- **Result:**
  - An orange band was observed under UV lamp.
  - Distance traveled by the sample for band = 4.8
  - Distance traveled by the solvent = 5.2
  - Therefore Rf value for band = 4.8/5.2 = 0.96

5.1.3 Discussion:

Some of the tests showed bands and some didn't. The reason behind, may be either there are no such compound to show band by those solvents or there were some technical error during the test period or there may be something error in the procedure. We got Rf values from the experiment of Thin Layer Chromatography where bands were observed. The Rf values were calculated by dividing the distance travelled by the sample from the distance travelled by the solvent.

We studied many compounds of this plant from the literature reviews of many journals and websites. Then we collected their Rf values to match with our calculated Rf values which we got from our Thin Layer Chromatography experiment and we observed that Rf values of most of our Thin Layer Chromatography results nearly matches with the exact Rf values of those compounds isolated previously from this plant.

But the Rf values were not exactly matched with the Rf values of pure compound previously isolated from this plant. These errors may be occurred owing to our carelessness or technical error occurred during the experiment process or may be the extract was contaminated or partially impured by any impurities or due to the contamination of the stationary phase which was silica plate. Due to all of these reasons, we couldn't come at exact decision for a particular compound identification.

5.2.1 Preparative TLC:

The test of preparative TCL shown 3 bands of different colors when the test was done with the solvent system of ethanol.

5.2.2 Discussion:

When we performed preparative TLC, three clearly seperated bands were observed. When we separated the bands by using spatula and dissolved in chloroform and ethyl acetate and then performed test TLC with the same solvent system we got single band in each test TLC each of which was done for each band in preparative TLC. This means that, all the bands in the preparative TLC plate was for each single compound. The Rf values from the test TLC were calculated. . The Rf values were calculated by dividing the distance travelled by the sample from the distance travelled by the solvent.

We studied many compounds of this plant from the literature reviews of many journals and websites. Then we collected their Rf values to match with our calculated Rf values which we got from our Thin Layer Chromatography experiment and we observed that Rf values of most of our Thin Layer Chromatography results nearly matches with the exact Rf values of those compounds isolated previously from this plant.

But the Rf values were not exactly matched with the Rf values of pure compound previously isolated from this plant. These errors may be occurred owing to our carelessness or technical error occurred during the experiment process or may be the extract was contaminated or partially impured by any impurities or due to the contamination of the stationary phase which was silica plate. Due to all of these reasons, we couldn't come at exact decision for a particular compound identification.

5.3 Infrared Spectroscopy Result Discussion:

After IR spectroscopy of the crystal of *P. lanatam* extract crystals, The IR spectrum showed lots of peaks which are clustered together and these can't be properly read out. The reason of this problem could be that, the crystals were not pure crystal or there may be an error in operating the machine or may be some error in procedure.

Chapter 6: Conclusion

The plant on which this research was done is an important medicinal plant. We have collected the extract and perform some very important tests of which test TLC and Preparative TLC were carefully done. We got a suitable amount of crystals from the extract of the plant and performed TLC and Infrared spectroscopy of some of those crystals and we tried to determine the chemical constituents of the plant extract

However, further studies are necessary to elucidate new compounds from this plant. This report may serve as a footstep to use this plant as a new source of medication.

Chapter 7: Reference

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