East West University

"Identification of VILDAGLIPTIN (Anti-diabetic drug) in Methanolic extract of *Artocarpus heterophyllus* seeds"

By

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A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelors of Pharmacy

Dedicated To

My Family and Friends

……

DECLARATION BY THE CANDIDATE

I, Joynab Akhter Joly hereby declare that this dissertation, entitled **"Identification of VILDAGLIPTIN (Anti-diabetic drug) in Methanolic extract of *Artocarpus heterophyllus* seeds"** submitted by me to the Department of Pharmcay, East West University,in the partial fulfillment of the recquirement for the degree of Bachelors of Pharmacy is a genuine & authentic research work carried out by me during Spring 2013-Fall 2013 under the guidance of Dr. Repon Kumar Saha, Assistant Professor, Department of pharmacy, East West University, Dhaka.

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CERTIFICATION BY THE INVIGILATOR

This is to certify that the dissertion, entitled **"Identification of VILDAGLIPTIN (Anti-diabetic drug) in Methanolic extract of *Artocarpus heterophyllus* seeds"**is a bonafide research work done,under our guidance and supervision,by Joynab Akhter (ID : 2010-1-70-016), in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowlwdged.

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ABSTRACT

Artocarpus heterophyllus or jackfruit is the national fruit of Bangladesh. It is the most widespread source of the genus. The objective of this study is to characterize the different compounds that were extracted and separated from seeds of *Artocarpus heterophyllus* and were carried out using different methods using High-Performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, Thin Layer Chromatography (TLC) to identify an anti-diabetic drug(Vildagliptin) in the methanolic extract of Artocarpus heterophyllus seeds. Standard in vitro procedures were used to screen possible phytochemical, antibacterial,cytotoxic and anti oxidant activities. Thin layer chromatography, ulra-violet spectroscopy and HPLC were used to detect the presence of various types of compound in methanolic extract and also for identification of a standard drug(Vildagliptin).Antioxidant effects of sseds were measured by DPPH scavenging assay.Antioxidant effects of mathanolic extract were measured by DPPH scavenging assay. Disc diffusion assay of the methanolic extract was performed to show the antibacterial effect using gram positive,gram negative strains of bacteria and fungi.Then experiment continued again with Thin layer chromatographic analysis of these fractions against two anti-diabetic drugs Vildagliptin and Meropenem which were used as a standard. Thin layer chromatographic analysis of Chloroform fraction indicated to the presence of bioactive compound in this fraction wherein Vildagliptin was a standard. Further investigation is needed to ensure the anti diabetic activity of this chloform fraction.[1]

Also cytotxic activity of all seven fractions were done in brian shimp lethality assay. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities in plant extracts. Different

concentration of all the fractions were used in the experiment and the ethyl acetate fraction is found to be severe cytotxic than other fractions, The butanol fraction is least cytotxic among them.The methanolic extract showed stronger antioxidant,antidiabetic and slight antibacterial activity. Further investigation is needed for the proper identification and isolation of these bioactive compounds to produce safer drugs for treatment of harmful diseases.[1]

KEYWORDS:

Artocarpus heterophyllus. Antibacterial, antioxidant, brian-shrimp lethality assay, High performance liquid chromatography, thin layer chromatography.

RATIONALE AND OBJECTIVE OF THE WORK

Topical fruits are important constituents in the daily diets of billions of people; and many such fruits either harvested from a wide range of minor species-from wild trees or locally cultivated ones. Bangladesh is gifted by extraordinary natural resources which continuously help us in many ways. One of the most beneficial natural resources is the plant resource which provides us with food, shelter and medicine. Now a days, scientists are isolating bioactive phytochemical from plant and converting them to effective medicines against diseases. These medicines often have less side effects and less bacterial resistance.Many such fruit trees have multi –purpose uses and their plant products satisfy a variety of local non-food purposes ranging from timber to forest.According to the World Health Organization, more than 80% of the world population in developing countries depends on plant-based medicines for basic healthcare needs.The most important of these bioactive compounds of plants are lectins,alkaloids,flavonoids,tannins and phenolics.Therefore, research is focusing on the search for new types of natural chemotherapeutic agents derived from plants.which are proving to be excellent sources of new compounds. *Artocarpus heterophyllus* is a well known Bangladeshi medicinal plant which has potential pharmacological as well as medicinal properties. *Artocarpus heterophyllus* has several medicinally important phytochemical constituents such as several flavones colorings, morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, glycosides, lipids, protein and cellulose. The jackfruit also contains free sugar (sucrose), fatty acids, ellagic acid, and amino acids like arginine, cystine, histidine,lycine, leucine. Methionine, theonine**.** It also contains several minerals such as Na, K, Ca, P, Fe, Zn, Mn which are healthy and beneficial for health. All the parts of this plant are useful. For example, the pulp contains two important groups of

phytoestrogens; isoflavones and lignans.The fresh seed contains crudeproteins (606 g), fat (0.4 g), carbohydrates (38.4 g), fiber (1.5 g), ash (1.25 to 1.50 g) and moisture (51.6 to 57.77g).Jackfruit seed extract was found to inhibit the proteolytic activities of different animal pancreatic preparations effectively.[2]

The aim of this research project was to carry out the characterization of the various Compounds present in the methanolic extract of seed of the *Artocarpus heterophyllus.*

The objective of this work is also to explore the possibility of developing new drug candidates from this plant for the treatment of various diseases.[2]

CHAPTER ONE

INTRODUCTION

1. BOTANICAL DESCRIPTION OF PLANT PARTS OF *ARTOCARPUS HETEROPHYLUS*

Artocarpus heterophyllus reaches 8-25 m in height; straight stemmed, branching near the base at an angle of 32-88 deg; canopy dense, dome shaped or rarely pyramidal; diameter varies with age, in 5-year-old trees it ranges from 3.5 to 6.7 m; trunk rarely buttressed, with a girth of 30-80 cm and a circumference of 42-96 cm; bark greyish-brown, rough, uneven, somewhat scaly; inner bark thick, ochre; all parts smooth, having either no hairs or minute, white hairs up to 0.5 mm long with tips easily broken, giving twigs and leaves a slightly rough feel; trees produce a long taproot; when injured, all living parts of the tree exude a copious, white gummy latex. [2]Leaves 4-25 x 2-12 cm, coriaceous, glossy, usually glabrous; top dark green, underside pale green; may be flat, wrinkled or with upcurled sides; arranged alternately on horizontal branches, and spirally on ascending branches with 2/5 phyllotaxis; broadest at or above the mid-portion; pinnately nerved, with 5-12 pairs of veins; those on flower-bearing branches obovate or oblong, those on young shoots oblong, narrow; entire when mature, 2 or 3 lobed when young; apex blunt, short and pointed; base cuneate or pointed; midrib and main veins greenish-white to pale greenish-yellow; at the nodes, stipules fused around stem, leaving an encircling scar after they fall off.[4] Individual flowers borne on an elongated axis and forming a racemoid inflorescence; male spikes produced singly, elongated, whitish-green or dark green with smooth skin, becoming yellowish and rough when mature, oblong, cylindrical, clavate, ellipsoidal or barrel shaped, distal end with a 1.5-2.5 mm wide annular ring, 3-10 x 1-5 cm, slightly hairy. Hanging or drooping peduncle 1.5-3.5 cm long and 4-5 mm thick, many densely crowded sterile or fertile

flowers; sterile flower has a solid perianth and the fertile one is tubular and bi-lobed. Female spikes either solitary or paired, oblong or cylindrical with rough, light to dark green skin, 5-15 cm, peduncle 8-9 mm thick; base with 3-4 mm wide and green annulus.[3] A multiple fruit consisting of several achenes (syncarp), each of which is indehiscent and 1-seeded, cauliflorus, 20-100 x 15-50 cm, the entire fruit weighing 4.5-50 kg; oval, oblong or ellipsoid, pale or dark green when young, greenish-yellow, yellow or brownish when mature; peduncle green, 2-10 cm long, 1-3.5 cm thick, covered by a rubbery rind and hard pyramidal, pointed or blunt spines. Inside are the fruitlets, which are the true fruits, 4-11 x 2-4 cm, 6-53g, composed of fleshy aril and the seed; aril waxy, firm or soft, yellow, golden yellow to yellow-orange, sweet, aromatic, 2-6.5 x 0.1-0.7 mm, 5-42 g. Fruits contain more than 500 firm or waxy seeds, oval-oblong or oblong-ellipsoid, thickened at the hilum, flattened in a plane parallel with the sagittal, 2-4.5 x 1-3.7 cm, 2.5-14 g. The generic name comes from the Greek words ‘artos’ (bread) and ‘karpos’ (fruit); the fruits are eaten and are commonly called breadfruit. The specific name, ‘heterophyllus’, is Latin for various leaved, or with leaves of different sizes and shapes; it is from the Greek word ‘heteros’ (different).[2]

1.1 History of cultivation

A. heterophyllus reportedly originated in the rainforests of India and Malaysia. The species then spread to neighbouring Sri Lanka, southern China, Southeast Asia, and further to tropical Africa, including Kenya, Uganda, Tanzania, Mauritius and Madagascar. A. heterophyllus was probably introduced in the Philippines in the 12th century, and domestication of the crop started thereafter.

It is commonly planted on smallholder Indian cane farms, in home gardens in Fiji, and occasionally in rural gardens and home gardens in other areas of the Pacific.[3]

1.2 Natural Habitat

A. heterophyllus grows in tropical, near tropical and subtropical regions. The species extends into much drier and cooler climates than do other Artocarpus species. It can also withstand lower temperatures and frost; it bears fruit at latitudes up to 30 deg. north and south, with good crops at 25 deg. north and south. The tree will not tolerate drought or flooding, and for optimum production it requires a warm, humid climate and evenly distributed rainfall.[3]

1.2.1 Geographic distribution

Native : Bangladesh, India, Malaysia[2]

Exotic : Algeria, Angola, Australia, Benin, Botswana, Brazil, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, China, Comoros, Congo, Cote d'Ivoire, Democratic Republic of Congo, Djibouti, Egypt, Equatorial Guinea, Eritrea, Ethiopia, Fiji, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Indonesia, Kenya, Lesotho, Liberia, Libyan Arab Jamahiriya, Madagascar, Malawi, Mali, Mauritania, Mauritius, Morocco, Mozambique, Myanmar, Namibia, Niger, Nigeria, Philippines, Rwanda, Sao Tome et Principe, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Sri Lanka, Sudan, Surinam, Swaziland, Tanzania, Togo, Tunisia, Uganda, Zambia, Zimbabwe [3]

1.2.2 Varieties

In South India, jackfruits are classified as of two general types: 1) *Koozha chakka*, the fruits of which have small, fibrous, soft, mushy, but very sweet carpels; 2) *Koozha pazham*, more important commercially, with crisp carpers of high quality known as *Varika*. These types are apparently known in different areas by other names such as *Barka*, or *Berka* (soft, sweet and broken open with the hands), and *Kapa* or *Kapiya* (crisp and cut open with a knife). The equivalent types are known as *Kha-nun nang* (firm; best) and *Kha-nun lamoud* (soft) in Thailand; and as *Vela* (soft) and *Varaka*, or *Waraka* (firm) in Ceylon. *The Peniwaraka*, or honey jak, has sweet pulp, and some have claimed it the best of all. The *Kuruwaraka* has small, rounded fruits. Dr. David Fairchild, writing of the honey jak in Ceylon, describes the rind as dark-green in contrast to the golden yellow pulp when cut open for eating, but the fruits of his own tree in Coconut Grove and those of the Matheson tree which he maintained were honey jaks are definitely yellow when ripe. The *Vela* type predominates in the West Indies.

Firminger described two types: the *Khuja* (green, hard and smooth, with juicy pulp and small seeds); the *Ghila* (rough, soft, with thin pulp, not very juicy, and large seeds). Dutta says *Khujja*, or *Karcha*, has pale-brown or occcasionally pale-green rind, and pulp as hard as an apple; *Ghila*, or *Ghula*, is usually light-green, occasionally brownish, and has soft pulp, sweet or acidulously sweet. He describes 8 varieties, only one with a name. This is *Hazari*; similar to *Rudrakshi*; which has a relatively smooth rind and flesh of inferior quality.[3]

The **'Singapore'**, or **'Ceylon'**, jack, a remarkably early bearer producing fruit in 18 months to 2 1/2 years from transplanting, was introduced into India from Ceylon and planted extensively in

1949. The fruit is of medium size with small, fibrous carpers which are very sweet. In addition to the summer crop (June and July), there is a second crop from October to December. In 1961, the Horticultural Research Institute at Saharanpur, India, reported the acquisition of air-layered plants of the excellent varieties, **'Safeda'**, **'Khaja'**, **'Bhusila'**, **'Bhadaiyan'** and **'Handia'** and others. The Fruit Experimental Station at Burliar, established a collection of 54 jackfruit clones from all producing countries, and ultimately selected **'T Nagar Jack'** as the best in quality and yield. The Fruit Experimental Station at Kallar, began breeding work in 1952 with a view to developing short, compact, many-branched trees, precocious and productive, bearing large, yellow, high quality fruits, 1/2 in the main season, 1/2 late. 'Singapore Jack' was chosen as the female parent because of its early and late crops; and, as the male parent, **'Velipala'**, a local selection from the forest having large fruits with large carpers of superior quality, and borne regularly in the main summer season. After 25 years of testing, one hybrid was rated as outstanding for precocity, fruit size, off-season as well as main season production, and yield excelling its parents. [3]

(A)

(B)

(C)

(D)

(E)

(F)

Figure 1 : Jackfruit (A) ; Jackfruit leaves (B) ; Jackfruit flower(C) ; Jackfruit tree (D); Jackfruit seeds (E) & (F)[4]

1.2.3 Biophysical limits

Altitude: 0-1600 m,

Mean annual temperature: 16-22 deg. C,

Mean annual rainfall: 1000-2400 mm

Soil type: A. heterophyllus thrives in deep, alluvial, sandy-loam or clay loam soils of medium fertility, good drainage and a pH of 5-7.5. It flourishes in rich soils of medium or open texture and grows even in the poorest soils, including gravelly or lateritic soils, shallow limestone, shallow light soils, and sandy or stony soils. It exhibits moderate tolerance to saline soils.[5]

1.2.4 TAXONOMY

Kingdom-	Plantae
Subkingdom-	Tracheophyta
Superdivision-	Spermatophyta
Division-	Maqnoliopsida
Class-	Equisetopsida
Subclass-	Magnoliidae
Order-	Rosales
Family-	Moraceae
Genus-	*Artocarpus*
Species-	*Artocarpus heterophyllus*

Table 1.1: Different names of jackfruit [5]

Scientific names	Common names
***Artocarpus heterophyllus* Lam.**	Lanka (Ilk.)
***Artocarpus jaca* Lam.**	Langka (Ilk., Tag., Bis.)
***Artocarpus philippensis* Lam.**	Nangka (Bis. Tag., Ibn.)
***Polyphema jaca* Lour.**	Nanka (Bis., Sul.)
***Artocarpus maxima* Blanco**	Jack fruit (Engl.)
***Saccus elasticus* OK.**	Mu bo luo (Chin.)
Saccus integer OK.	
***Saccus heterophyllus* OK.**	
***Radermachia integer* Thunb.**	
***Artocarpus integer* Merr.**	
***Bo luo mi* (Chin.)**	

Table 1.2: Other vernacular names [5]

Other vernacular names
ASSAMESE: Konthal, Konto phol, Kontok phol, Kontoki.
BENGALI: Kathal.
CHINESE: Shu bo luo, Niu du zi guo
DANISH: Jackfrugttrae.
DUTCH: Nangka.
FRENCH: Jacquier.
GERMAN: Indischer Brotfruchtbaum, Jackfrucht, Jackfruchtbaum.
GUJARATI: Phannasa.
HINDI: Cakki, Katahal, Kathal, Kanthal.
ITALIAN: Falso albero del pane.
JAPANESE: Nagami pannoki, Paramitsu.

KANNADA: Halasina hannu, Halasu, Panasero.
KHMER: Khnor.
KOREAN: Baramil
LAOTIAN: Mai mi, Mak mi, Mi.
MALAY: Nangka (Indonesia, Bali), Nangka bubor, Keledang (Timor).
MALAYALAM: Chakka.
MARATHI: Phanas.
NEPALESE: Rukh kutaherr.
ORIYA: Panasa.
PERSIAN: Derakhte nan.
PORTUGUESE: Jaca.
SANSKRIT: Panasah, Panasam.
SINHALESE: Jak, Kos.
SPANISH: Arbol del pan, Fruta del pobre, Jaca, Jaka, Jaqueiro.
SWAHILI: Fenesi.
SWEDISH: Jacktrad.
TAMIL: Palaa, Palavu.
THAI: Khanun, Makmee, Maak laang.
VIETNAMESE: Mit.

Table 1.3 : Food Value Per 100 g of Edible Portion [6]

	Pulp (ripefresh)	Seeds (fresh)	Seeds (dried)
Calories	98		
Moisture	72.0-77.2 g	51.6-57.77 g	
Protein	1.3-1.9 g	6.6 g	
Fat	0.1-0.3 g	0.4 g	
Carbohydrates	18.9-25.4 g	38.4 g	

Fiber	1.0-1.1 g	1.5 g	
Ash	0.8-1.0 g	1.25-1.50 g	2.96%
Calcium	22 mg	0.05-0.55 mg	0.13%
Phosphorus	38 mg	0.13-0.23 mg	0.54%
Iron	0.5 mg	0.002-1.2 mg	0.005%
Sodium	2 mg		
Potassium	407 mg		
Vitamin A	540 I.U.		
Thiamine	0.03 mg		
Niacin	4 mg		
Ascorbic Acid	8-10 mg		

The pulp constitutes 25-40% of the fruit's weight.

1.2.5 Health benefits of Jackfruit

1. Excellent for bone health

Jackfruit is rich in magnesium which plays a vital role in calcium absorption. This fruit has bone strengthening properties and also helps prevent osteoporosis, a bone related disorder that is common as you age. Jackfruit is known to slow down degeneration of bone and tissues surrounding it.

2. Plays a crucial role in preventing cancer

Though health benefits of jackfruit in regard with cancer are being studied, the fact remains that this fruit is a rich source of phytonutrients isoflavones, saponins and lignans which have anti-cancer properties and play a vital role in preventing harmful free radicals that cause cancer and

other life threatening diseases. Phytonutrients present in this fruit prevent formation of cancer cells and also battle stomach ulcers effectively.

3. Beneficial for persons with high blood pressure and other cardiovascular problems

Persons with high blood pressure and other cardiovascular problems should include jackfruit in their diet. This fruit provides a healthy amount of potassium which plays a key role lowering high blood pressure and thereby minimizing risk of clogged arteries, a stroke, or heart-attack.

4. Home remedy for Asthma sufferers

Jackfruit is used as a home remedy for Asthma sufferers. Aside from consuming the ripe fruit, in many cultures, extract of jackfruit root is given to asthma sufferers for relief. Root of jackfruit is boiled and tenderized. Extract of this tenderized root is known to provide relief to persons suffering from asthma.

5. Provides improved immunity

Jackfruit has essential minerals and vitamins that help improve immunity. This fruit has powerful nutrients that play an important role in protection against bacterial and viral infections. Regular consumption of jackfruit strengthens immune system functions while boosting functions of white blood cells.

6. Helps in prevention of Anemia

Jackfruit contains iron which is known to play a vital role in prevention of Anemia. Intake of the fruit is also aids in healthy blood circulation.

7. Energy Booster

Like many other sweet fruits, jackfruit too is refreshing and energizing. Sugars, sucrose and fructose present in this fruit boost energy. Furthermore the absence of cholesterol and saturated fats makes it a popular health snack with all ages.

8. Excellent for Thyroid Metabolism

Jackfruit contains copper which is known to play an important role in thyroid metabolism, hormone production in particular. The composition of minerals in this fruit is essential for healthy thyroid metabolism.

9. Excellent digestive fruit

In many cultures jackfruit is consumed because of its health benefits in regard with digestion. The fruit offers high quality fiber content which is essential for good bowel movement. Furthermore, fiber helps prevent constipation and protects against colon mucous membrane by keeping away carcinogenic chemicals that take refuge in the large intestine.

10. Essential for healthy eye functions

Jackfruit provides vitamin A which promotes healthy eye functions. A person that includes jackfruit is less likely to develop macular degeneration with age.[6]

1.2.6 Nutritional Value of Jackfruit

Aside from being tasty, jackfruit is also a powerhouse when it comes to balanced nutrition. The mineral and vitamin composition found in jackfruit is healthy for body and mind. This fruit is a rich source of nutrients which include niacin, zinc, thiamin, potassium, sodium, calcium, iron, vitamin C and vitamin A amongst others. Because of its low calorie content jackfruit is popular with health conscious individuals. A serving of jackfruit (100g) contains just 95 calories. Jackfruit seeds are rich in protein. The nutrition packed in jackfruit offers a wide range of health benefits.[6]

1.2.7 Jackfruit Nutritional Value (Per 100g)

(Source- USDA Nutrient Database)

Vitamins

- Vitamin E- 0.34 mg
- Vitamin A- 110 IU
- Vitamin C- 13.7 mg
- Folates- 24 mcg
- Riboflavin- 0.055 mg
- Niacin- 0.920 mg
- Thiamin- 0.105 mg
- Pyridoxine- 0.329 mg

Minerals

- Zinc- 0.42 mg
- Manganese- 0.197 mg
- Calcium- 34 mg

• Selenium- 0.6 mg

• Iron- 0.60 mg

• Phosphorous- 21 mg

• Magnesium- 37 mg

Nutrients

• Dietary fiber- 1.5 mg

• Energy- 95 kacl

• Total Fat- 0.64 g

• Carbohydrates- 23.5 g

• Total Fat- 0.64 g

• Cholesterol- 0 mg

Phyto-Nutrirnts

• Lutein- zeaxanthin- 157 mcg

• Carotene- B – 61 mcg

• Crypto-xanthin-B- 5 mcg

Electrolytes

• Sodium- 3 mg

• Potassium- 303 mg[7]

CHAPTER TWO

LITERATURE REVIEW

2.1Phytochemical review

2.1.1 Nutritive compositions:

The moisture content, dry matter and ash content of three types of jackfruits namely Khaja(green, hard and smooth with juicy pulp and small seeds), Gala (rough,soft,with thin pulp,not very juicy,and large seeds) and Durosha (rough,soft,intermediate size of pulps and seeds) was found around 21.10-42.25%, 57.75-78.90% and 2.13-4.07% respectively among the varieties, A good amount of proteins content was found in all varieties of jackfruit seeds, it ranged from 13-18%.Crude fibre content of seed varied from 1.56-2.60%. Jackfruit is a good source of many mineral contents like N,P,K,Ca,Mg,S,Zn,Cu etc. Starch content in seed was found from 12.86-17.90%[].Analysis of the emission spectrum of the jackfruit seed in powdered form confirms the presence of two hitherto undetected elements, manganese and magnesium. The Fourier transform infrared spectrum reveals the presence of some specific functionl groups, attributed to the different bands present in the spectrum[].A jackfruit meal comprising of flesh and seeds contain 80% and 20% available carbohydrate respectively[]. The moisture content of theboiled jackfruit flesh is high (82% FW). Jack seeds contain 4.7% protein (FW),11.1% total dietary fibre(FW) and 8% resistant starch(FW).Jackfruit meal elicited a GI of 75.The Glycaemic Load(GL) of the normal serving size of the meal is medium.The slowly available glucose(SAG) percentage of jackfruit meal (30%) is twice that of the standard. The boiled jackfruit flesh contain disintegrated starch granules while seeds contain intact swollen and disintegrated granules.The jackfruit seeds are a good source of starch(22%) and dietary fibre.The meal is categorized as a low GI meal due to the collective contributions from dietary fibre, slowly available glucose and un-gelatinised (intact) starch granules in the seeds. In another work, they have investigated nutritional, phytochemical content and antioxidant activity of seeds of the

jackfruit (*Artocarpus heterophyllus* Lam.), one of the most ancient fruit indigenous to Western Ghats of India. The antioxidant properties were evaluated using free radical scavenging, metal chelating, ferric reducing antioxidant power and reducing power assays.[8]

2.1.2 Binding properties:

Starch obtained from Artocarpus heterophyllus fruit seeds posseses comparable binding properties.It was found that an increase in binder concentration led to decrease in friability and increase in disintegration time of the tables[8].

2.2 Pharmacology Review

2.2.1 Anti-diabetic activity

A dynamic study was carried out to determine several pharmacognostic parameters and examine traditional claims for the anti-diabetic activity of the plant. Aqueous extract of *Artocarpus heterophyllus Lam*. leaves was prepared and their anti-diabetic activity was investigated in rats. Glimepiride prepared in Tween 80 solution was used as a standard drug; plant extract, in CMC (1 %) solution was used as a test drug. The animals were fasted for 16 hour prior to the induction of diabetes. STZ freshly prepared in citrate buffer (pH 4.5) was administered i.p. at a single dose of 50 mg/kg. [8]Development of diabetes was confirmed by polydipsia, polyurea and by measuring blood glucose concentrations 72 hour after injection of STZ. Rats with blood glucose level of 250 mg/dl or higher were considered to be diabetic and selected for experiment.[8]

Extracts of *A.heterophyllus* leaves decreases the blood glucose levels statistically significantly ($P<0.01$). Treatment with extracts of *A. heterophyllus* leaves decreases total cholesterol (TC) and increases high density lipid cholesterol (HDL) significantly ($P<0.01$)[8]

The aqueous extract of *A. heterophyllus* supplementation for 21 days showed antidiabetic and antihyperlipidemic potential as shown by restoration of blood glucose level and biochemical profiles. We can further explore *A. heterophyllus* for the important leads for antidiabetic drugs. Detail biological studies are further required to establish the MOA(Mechanism of action).[8]

Another study showed about antidiabetic activity of *Feronia limonia* Fruit and *Artocarpus heterophyllus* Bark extracts used as edible medicine by local tribal population of Vellore districts were analyzed in streptozotocin induced diabetic rats**.** Preliminary phytochemical screening revealed the presence of high contents of flavonoid in methanolic extract of *Feronia limonia* and ethyl acetate extract of *Artocarpus heterophyllus* compared to other extracts. Diabetes was induced by single intraperitoneal injection of streptozotocin (45 mg kg^{-1}). Diabetic rats were treated with these extracts at a dose of 200 and 400 mg kg^{-1} for 30 days. Hypoglycemic activities of extract treated diabetic rats were assessed by the percentage reduction in fasting blood glucose level[9]

2.2.2 Antioxidant activity

The four different seed extracts (ethanol, acetone, ethyl acetate ,and water) of *Artocarpus heterophyllus* showed effective flavonoids content ,reducing potential and antioxidant activity due to the presence of high amounts of phenolic compounds.[9]

Secondary metabolites including alkaloids, saponins, flavanoids and phenolics content were determined in the jackfruit seeds. Nutritional properties including moisture, fats, carbohydrates, proteins, ash content and metal content in the seeds were also estimated. Polyphenolic content and antioxidant properties of dichloromethane: methanol (1:1) extract of jackfruit seeds was found to be high and well correlated. Results indicated jackfruit seeds to be a good source of

nutritional and antioxidant components and hold their potential for value addition and nutraceutical development.[9]

From another study, Jackfruit also has been reported to contain antioxidant prenylflavones. Recently, antioxidant capacity of fruit pulp has been evaluated. However, jackfruit seeds are less popular as vegetable and are eaten when boiled or roasted.

From another study[], polyphenolic content and antioxidant properties of dichloromethane: methanol (1:1) extract of jackfruit seeds was found to be high and well correlated. Results indicated jackfruit seeds to be a good sourse of nutritional and antioxidant components and hold their potential for value addition and nutraceutical development.[9]

2.2.3 Antifungal effect:

The novel chitin-binding lectin from seeds of *A. integrifolia*(jackfruit).denoted jackin, is 14 kDa proteins, made up of 3 chains linked by disulfide bonds. It inhibited the growth of *Fusarium moniliforme* and *Saccharemyces cerevisia*, and presented hemagglutination activity against human and rabbit erythrocytes.[]

2.2.4 Cytotoxic effect:

In in –vitro cytotoxic assays like MTT and SRB against different cell lines like HEK293, A549, HeLa and MCF-7 the IC50 values of methanolic extract of *Artocarpus heterophyllus* were found 35.26 µg/ml and 35.27 µg/ml against A549 cell line proving that the methanolic extract exhibited significant anti-cancer potential with no toxicity on normal cell line.[9]

I a study, crude extracts from the tegmen of *A. heterophyllus* were tested in vitro for their antitumor activity[]. The methanolic extract showed maximum cytotxicity on HEp2 cells up to 1:4 dillution. Cytotoxic changes observed was cell aggregation, cell rounding and cell death.[9]

2.2.5 Antibacterial Effect:

The crude methanolic extracts of the stem and root barks,leaves,fruits and seeds of *A. heterophyllus* and their subsequent partitioning with petrol,dichloro methane, ethyl acetate and butanol gave fractions that exhibited a broad spectrum of anti bacterial activity. The butanol fractions of the root bark and fruits were found to be the most active. None of the fractions were active against the fungi tested.

In another study, the anti bacterial and antifungal activities were effective in ethanol.ethyl acetate and aqueous fractions of seed extract than ethyl acetate fraction in *A. heterophyllus* seed extracts.The MIC reported that ethanol, acetone and aqueous extract posseses minimum inhibition in both extract than ethyl acetate.

Studies[9] showed that the jack fruit partially purified lectin had a potent anti-bacterial activity against *S. aureus, B. subtilis*.

2.2.6 Immunomodulatory effect:

A major protein, Jacalin has been isolated from jackfruit seeds and possessed immunological properties. Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens. Several plant products have been reported for immunomodulatory activity and many formulations of these plant products are

available to enhance the immune system. Plants are the essential and integral part in complementary and

alternative medicine. Plants have the ability of the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases .

Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. There are many plants, which are having immunostimulatory where as other have

immunosuppressant activity .*Artocarpus heterophyllus* Lam. is an important source of compounds like morin, dihydromorin, cynomacurin, artocarpin, isoartocarpin, cyloartocarpin, cycloheterophyllin (C30H30O7), artocarpesin, oxydihydroartocarpesin, artocarpetin, norartocarpetin, cycloartinone, betulinic acid, artocarpanone and heterophylol which are useful in fever, boils, wounds, skin diseases, convulsions, diuretic, constipation,ophthalmic disorders and snake bite etc. The leaves are useful in fever, ulcers, boils wounds, skin diseases, antidiarrhoeal, analgesic and as immunomodulator.[10]

The ripe fruits are sweet, cooling, laxative, aphrodisiac, and tonic. The seeds are sweet, diuretic, aphrodisiac and constipating. The plant is reported to possess antibacterial, anti-inflammatory, antidiabetic ,antioxidant, antifungal [11] and immunomodulatory properties[12]. *Artocapus heterophyllus* Lam. is used as a traditional medicine as analgesic and immunomodulator. Immunomodulator activity has been scientifically proved in fruits whereas analgesic activity is not scientifically proved. [11]

2.2.7 Aphrodisiac activity

A lectin specific for N-acetylgalactosamine was isolated from seed extract of jack fruit by ammonium sulfate precipitation, followed by affinity chromatography on a Affigel-galactosamine-agarose column. The lectin possessed agglutinating activities for human and rat sperm as well as human red blood cells. It was found to have Mr = 62,000 consisting of two dissimilar subunits of Mr = 18,000 and 13,000.It also cross –reacted with an antibody against the lectin of Osage Orange.[11]

CHAPTER THREE METHODS AND MATERIALS

3.MATERIALS AND METHODS

3.1 CHEMICAL REAGENTS

Analytical grade n-hexane (Merck, Germany), dichloromethane (Merck, Germany), chloroform (ERMSURE®, Germany), diethylether (Lab Scan, Germany), Benzene (Merck, Germany), methanol (G.R.GRADE, Bangladesh) , Ethyl acetate (E. Merck, Germany), Formic Acid (UNI - CHEM®, China), pure sulphuric acid (Merck, Germany), Butanol (England) Double ring filter paper (China) ,distilled water & DPPH (2,2-diphenyl-1-picrylhydrazyl) was used for the overall research purpose.[3]

3.2 INSTRUMENTS USED

1. Rotary vacuum evaporator

2. Autoclave machine(HIRAYAMA, Japan)

3. Hot air oven (NUVE)

4. Incubator (EHRET)

5. Electric Weighing Balance

6. Hot plate

8. UV-visible Spectroscopy

9. Digital camera

10. Vortex machine

11. Centrifuge machine

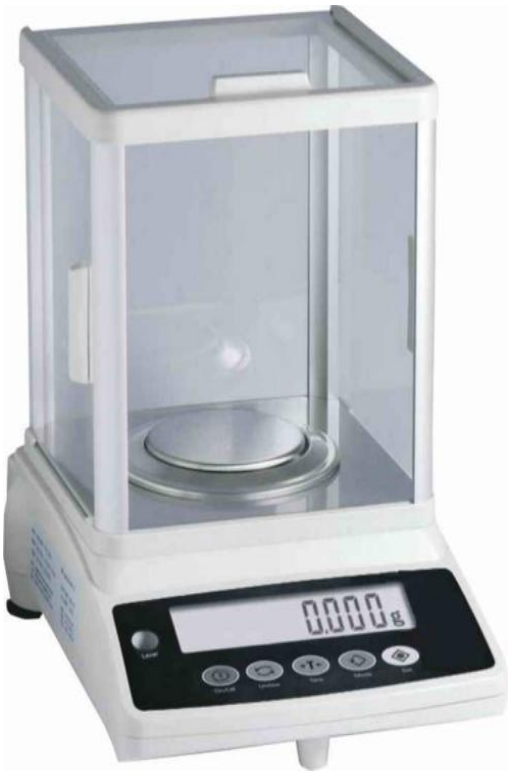
12. HPLC machine (High performance liquid chromatography.

13. Laminar flow cabinet (ESCO®, US federal standard)

(A) (B)

Figure 1.2.1 : Vacuum Rotary Evaporator (A)[21]; Hot air oven (B)[14]

(C)



(D)

Figure 1.2.2 : Laminar Air-flow (C)[13] & Electronic Weighing Balance (D)[14]

(E)

(F) (G)

Figure 1.2.3 : Digital camera (E)[15] ; Laboratory water bath (F);[13]Autoclave machine (G)[13]

(H)

(I) (J)

Figure 1.2.4 : Hot plate (H)[13] ; UV-visible lamp (I) & Vortex machine (J)[13]

Table 1.4: List of Apparatus[14]

Serial No	Apparatus
1.	Beaker (1000 ml,50 ml.100 ml)
2.	Pencil,eraser
3.	Cotton burd
4.	Aluminium foil paper
5.	Agar Bottle (200 ml)
6.	Gloves
7.	Chromatographic jar (TLC jar) , TLC plate
8.	Microneedle, Forcep
9.	Micro pipette, 2-20 µl & 100 – 1000 µl
10.	Plastic funnel & test tubes
11.	Conical flask (250 ml)
12.	Measuring cylinder (100 ml)
13.	Separating funnel
14.	Volumetric flask (500 ml)
15.	Pipette & Pipette pumper
16.	Labeling tape & scissor
17.	Filter paper
18.	Petridishes
19.	Apendroffs

3.3 COLLECTION OF PLANT MATERIALS

After the selection of plant the plant parts for the research purpose (seeds and the white covering of seeds) were collected. Through out Bangladesh the plant *Atrocarpus heterophyllus* is available. From a local market of Dhaka, the dried seeds of this plant have been collected.[14]

3.3.1 PLANT IDENTIFICATION

It is advisable to attempt field identification of sample collected. To aid taxonomic experts in confirming the field identification and to got a permanent record (accession No.: 38308) a voucher specimen. On voucher specimen the dried seeds of sampleplant were attached and some information like local name, medicinal use, location of the sample plant were also written on that voucher specimen.[14]

3.3.2 DRYING OF PLANT SAMPLE

After the collection of sample it needs to be dried to make the sample extract.However the seeds were collected in dried form, and it was stored in a dry plac and at temperature below 300 degree C to prevent microbial growth and consequent degradation of the sample.[14]

3.3.3 GRINDING OF DRIED SAMPLE

Small amount of plant material can be milled using grinder or blender. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent recquired of solvent recquired for the extraction. The dried samples were ground to coarse powder with a electric grinder and powered samples were kept in clean closed glass containers pending extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination .[14]

3.4 PREPARATION OF METHANOLIC EXTRACT OF *Atrocarpus heterophyllus*

● **Initial Calculation :**

Seeds before crush = 2 kg 100 gm

Seeds after crush = 2 kg 100 gm

Covering of seeds Before crush = 150 gm

Covering of seeds after crush = 200 gm

1) **Extract preparation :**

Extract is prepared by soaking crushed seeds in methanol step by step :

Step 1 : 500 gm sample

Total methanol added : 800 ml

Step 2 : 250 gm sample

Total methanol added : 306 ml

Step 3 : 500 gm sample

Total methanol added : 755 ml

Step 4 : 834.81 gm sample

Total methanol added : 1190 ml

Total sample : 2084.81 gm

Total methanol added : 3051 ml

Total Extract found : 500 ml

2) **Rotary**

In a 1000 ml flask 500 ml extract is evaporated to find pure sample which does not contain any solvent added.Here vacuum is employed so that in low temperature extract can be evaporated.In high temperature many essential elements can dry out along with the solvent, that's why low temperature along with the employment of vacuum is accepted.[15]

After rotary sample found : 50 ml approximately

3) Column Chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.[15]

Only hexane(55 ml) fraction is found at the time interval 30 minute.As it is time consuming it is not continued.

4)Vacuum Liquid Chromatography (VLC)

Principle:

Chromatographic purification is an integrated part of organic synthesis. The Dry Column Vacuum Chromatography presented here, has excellent resolving power, is easily applied to large scale chromatography (up to 100 g) and is fast. Furthermore, the technique is economical and environmentally friendly due to significant reductions in solvent and the amount of silica used. Therefore, it is an excellent alternative to the commonly used Flash Column Chromatography for purification in organic synthesis.[15]

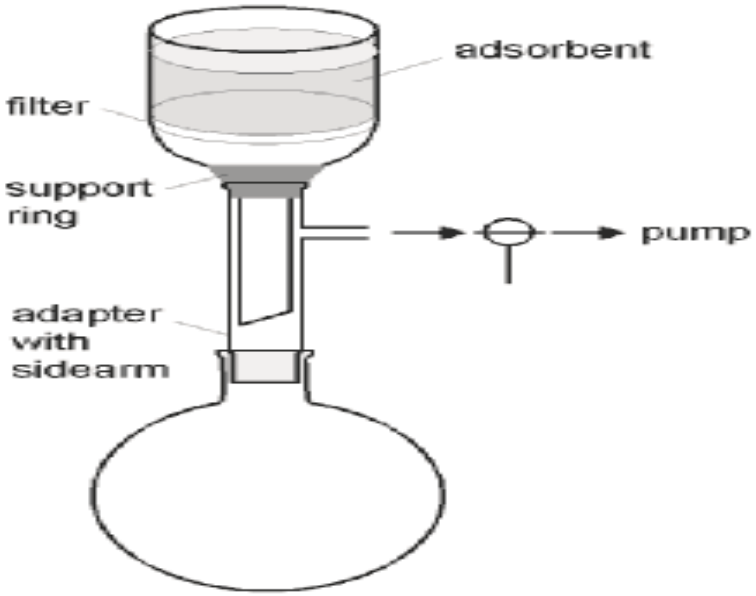


Figure 1.2.5: Vacuum Liquid Chromatography[15]

The experimental procedure can be summarized as follows:

1. At first sample from rotary was mixed with 130 grams silica and filled in a sintered glass funnel of the appropriate size.
2. Vacuum is applied and the surface is pressed firmly to give a completely level, well compacted bed approximately 4.5–5.5 cm high.
3. Then solvents were poured into sintered glass funnel according to their polarity, non polar solvents were poured first then polar solvent poured.
4. The mixture to be separated according to the polarity of solvents.
5. Fractions were collected separately and fractions are monitored by TLC.[15]

7 solvents were used (from non-polar to polar). Each solvent measured = 100 ml

1) N-hexane
2) Di ethyl ether
3) Di chloro methane (DCM)
4) Butanol
5) Chloroform
6) Ethyl acetate
7) Methanol

Each fractionated sample is collected.

4) Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase.[15]

Table 1.5: The compositions of various solvent systems for TLC

[6]

Nonpolar Basic solvent	**Intermediate polar Basic Solvent**	**Polar Basic solvent**
Benzene 9mL Ethanol 1mL Ammonium hydroxide 0.1mL	Chloroform 5Ml Ethyl acetate 4mL Formic acid 1mL	Ethyl acetate 8mL Ethanol 1.2mL Water 0.8mL

Table 1.6: Apparatus[6]

1. TLC tank		5. Spray bottle
2. Pencil		6. Heat gun
3. Scale		7. Petri dish
4. UV-lamp		8. Capillary tube

3.5 THIN LAYER CHROMATOGRAPHIC ANALYSIS USING DIFFERENT FRACTIONS OF AQUEOUS EXTRACT OF *Artocarpus heterophyllus*

PROCEDURE OF THIN LAYER CHROMATOGRAPHIC ANALYSIS

1) At first, the TLC plate was cut to the correct size and using a pencil, a thin line was drawn at the bottom of the plate (approximately 1.5 cm from the bottom).

2) Now, by using TLC pipettes, spots of n-hexane fraction, dichloromethane fraction and chloroform fraction were applied to the line which had been drawn.

3) Then the plate was allowed to be air dried.

4) A suitable solvent system (Chloroform:Ethylacetate:Formic acid= 5:4:1) was taken in the TLC jar (or, chromatographic jar) with a very narrow depth usually 1 cm. Actually, the depth of the solvent was kept such that the sample spot is fairly above it

5) Now, the TLC plate was kept standing with the wall of the beaker in the solvent.

6) The solvent slowly raised due to capillary action and it carried the sample over the TLC plate.

7) Then, the plate was removed until the solvent raised approximately 1 cm from the end and using a pencil a line is drawn across the solvent front.

8) Afterwards, the plate was allowed to dry and it was air dried.

Then, the TLC plate was seen under short-wave UV light.[16]

3.5.1 ACID CHARRING PROCESS OF TLC PLATE

Material Required: Tweezers, Conc. Sulfuric acid, Distilled water, Hot plate and Petri dish.[10]

Procedure

1. 9 ml of distilled water was added to 1 ml of concentrated sulfuric acid t produce a 10% solution of sulfuric acid which was taken in a Petri dish.

2. The TLC plate was dipped in this solution using tweezers with the silica face down.

3. The TLC plate was left in the open air for 10 minutes for air drying.

4. The hot plate was heated to about 90°c and the TLC was heated until the spots developed.[16]

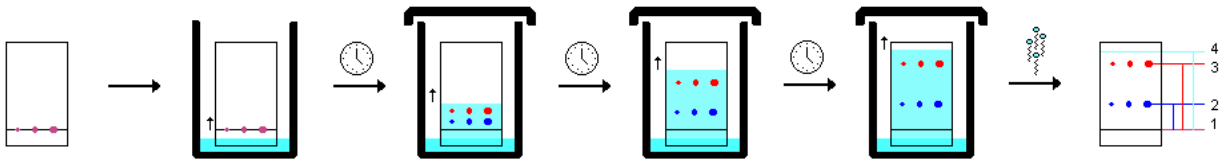


Figure 1.3 : Thin Layer Chromatographic Analysis [17].

3.5.2 ULTRAVIOLET LIGHT – FLUORESCENCE

With a TLC plate containing no fluorescent indicator, the dried chromatogram is visualized under (200-700) nm UV light **[16]**

3.5.3 CHARRING PROCESS OF TLC PLATE

1) 2,2'-Diphenylpicrylhydrazyl : Reagent: 1 ml 0.4% DPPH is added to 9 ml methanol to produce 0.04% DPPH solution. TLC plate is sprayed with this reagent in dark room for 1 minute; then spots are visualized in daylight and immediate picture of TLC plate is captured **[17].**

2)Concentrated H2SO4 (98%): 1 ml concentrated H2SO4 (98%) is added to 9 ml distilled water. And TLC plate is sprayed with this reagent for 1 minute, dried and heated for spots visualization **[16]**

3.5.4 APPLICATION OF TLC TECHNIQUE

1) Separation of components from a mixture based on their relative affinity towards the solvent system (mobile phase) and the stationary phase **[16].**

2) To check compatibility of different excipients in a formulation **[16].**

3) TLC is done on analytical scale as a means of monitoring the progress of a reaction **[16].**

4) TLC is done on the preparative scale to identify and purify small amounts of a compound **[16].**

5)To detect any impurities **[16].**

3.5.5 Disadvantages Of TLC technique

1) TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques **[16].**

2) Only small amount of compound can be identified and purified **[16]**

3.6 DPPH CHARRING PROCESS OF TLC PLATE

Materials Required

4% DPPH stock solution (1%), Methanol (9 ml), Test Tube, Pipette, Pipette filter, Petridish and Tweezers.

Procedure

1. 0.4% solution of DPPH was prepared by adding 9 ml of methanol to 1 ml of 4% DPPH stock solution. The procedure was carried out in a dark room as DPPH is light sensitive. [16]

2. By using tweezers the developed TLC plates would be dipped into this solution on the silica face down. [16]

3. The plates were left in the dark room for 30 minutes for the color to develop after which they were observed for the formation of yellow, golden / brown color on the background of purple. This coloration indicates the presence of compounds that have antioxidant properties. [16]

3.7 ANTI-MICROBIAL ASSAY

Determination of Antimicrobial activity by Disc Diffusion Method

Worldwide, infectious disease is one of main causes of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States **[18].**

Death from infectious disease, ranked 5th in 1981, has become the 3rd leading cause of death in 1992; an increase of 58% .It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the US **[18].** This is alarming given that it was once believed that we would eliminate infectious disease by the end of the millenium. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Furthermore, the most dramatic increases are occurring in the 25–44 year old age group

These negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. It is this last solution that would encompass the development of new antimicrobials

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by disc diffusion method .

But there is no standardized method for expressing the results of antimicrobial screening

Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculum volume, culture medium composition, pH, and incubation temperature can influence the results **[18].**

Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method **[18].**

3.7.1 Principle of Disc Diffusion Method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. [19]

Standard antibiotic discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media **[23].** The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter **[19].** In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required **[2o].**

Table 1.7: Apparatus and Reagents [25]

1. Filter paper discs		2. Autoclave
3. Nutrient Agar Medium		4. Laminar air flow hood
5. Petri dishes		6. Spirit burner
7. Sterile cotton		8. Refrigerator

9. Micropipette		10. Incubator
11. Inoculating loop		12. Ethanol

3.7.2 Test Materials of *Artocarpus heterophyllus*

Methanolic extract of ***Artocarpus heterophyllus*** leaves were taken as test sample.

3.7.3 Test Organisms

The bacterial strains used for the experiment were collected as pure cultures from the East West University microbiology laboratory. Both gram positive and gram-negative organisms were taken for the test and they are listed in the following table

Table 1.8: List of micro-organisms used[21]

Gram (+ve) Bacteria	Gram (-ve) Bacteria	Fungi
1. ***Staphylococcus aureus***	1. ***Escherichia coli***	1. ***Candida albicans***
2. ***Beta-hemolytic streptococcus***	3. ***Salmonella typhi***	

3.7.4 Culture Medium and Their Composition

The Nutrient Agar medium was used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms. Nutrient Agar medium contains following things

Table 1.9: Composition of nutrient agar medium

Ingredient	Amount
Bacto peptone	**0.5gm**
Sodium chloride	**0.5 gm**
Bactoyeast extract	**1.0 gm**
Bacto agar	**2.0 gm**
Distilled water q.s.	**100 ml**

Agar medium having this composition was directly brought from the market.

3.7.5 Preparation of the Medium

To prepare required volume of this medium, calculated amount of agar medium was taken in a bottle with a cap and distilled water was added to it to make the required volume. The contents were then autoclaved to make a clear solution.[20]

3.7.6 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 1210C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.[20]

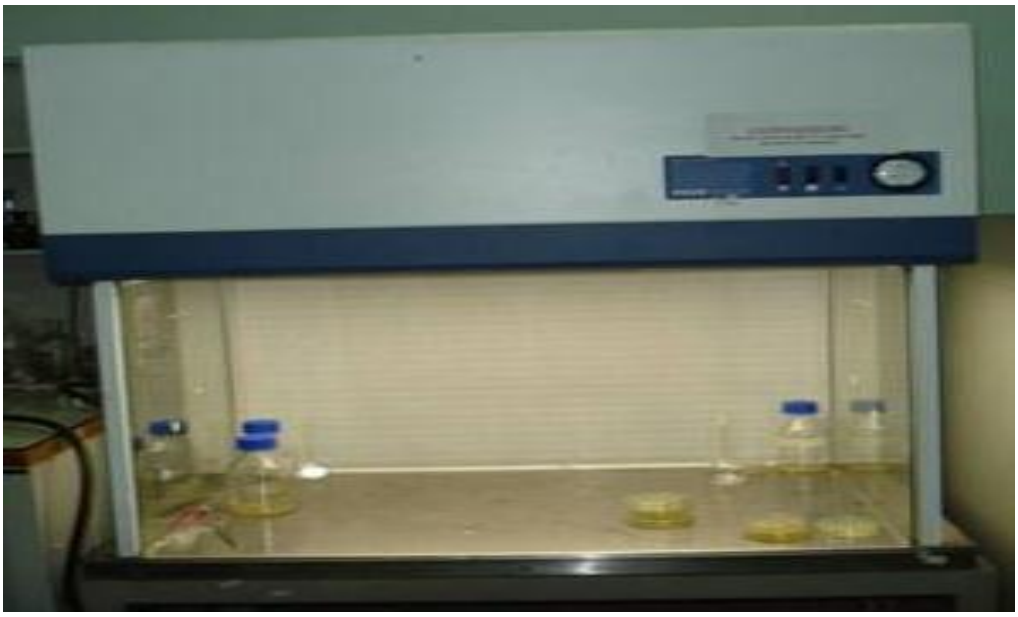


Figure 1.3: Laminar hood

3.7.7 Preparation of Subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37C for their optimum growth. These fresh cultures were used for the sensitivity test.[20]

Figure 1.4 : Incubator

3.7.8 Preparation of the Test Plate

The test organisms were transferred from the subculture to petridish containing about 10 ml of melted and sterilized agar medium.

The bacterial and fungal suspension was taken by a loop a mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the petridish with the help of this cotton bud.[20]

3.7.9 Preparation of Sample Discs with Test Sample

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.[21]

3.8 Diffusion and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 40C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 370C for 24 hours. [21]

3.8.1 Determination of Antimicrobial Activity by Measuring the Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After

incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. [21]

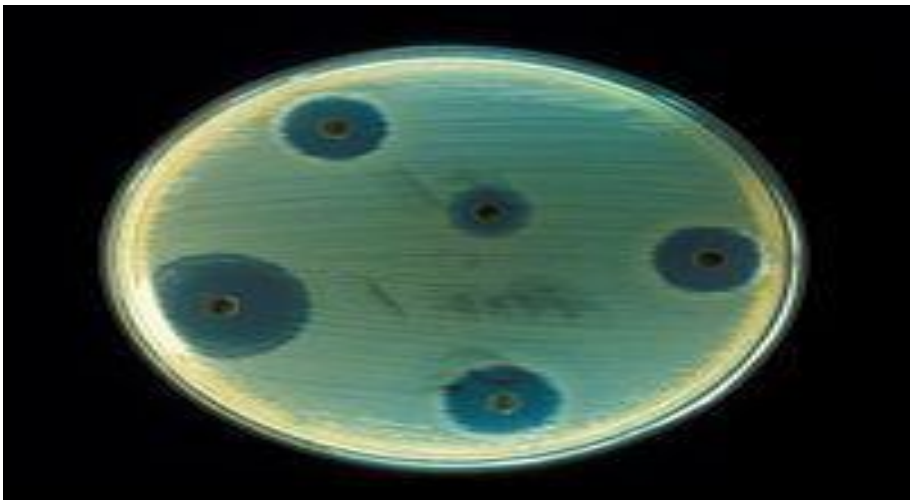


Figure 1.5: Zone of inhibition

Here 7 fractionated samples were used :

1) N- hexane fraction
2) Di chloro methane fraction
3) Di ethyl ether fraction
4) Ethyl acetate fraction
5) Methanol fraction
6) Chloroform fraction
7) Butanol fraction

3.9 ANTI-OXIDANT TESTS

DPPH Test (1, 1-diphenyl-2-picrylhydrazyl radical)

Principle

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding

hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

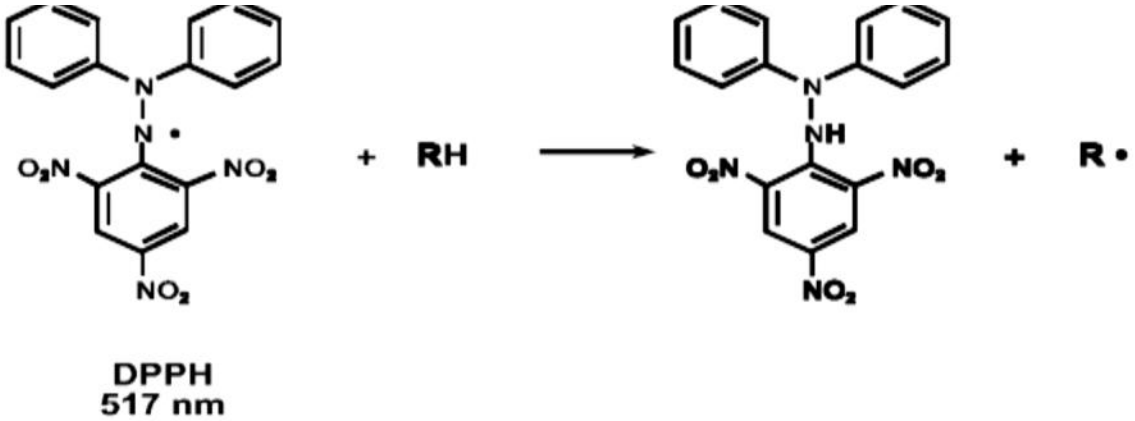


Figure 1.6 : DPPH

Table 2.0: Apparatus[25]

Test tube		Uv-spectrophotometer
Racker		Spatula
Beaker		Analytical balance

Table 2.1: Reagents

DPPH		L-ascorbic acid
Methanol		Water

3.9.1 Procedure

a) The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract (2, 4, 6, 8 & 10 µg/ml, in methanol) were added at an equal volume (10ml) to methanol solution of DPPH (400 µg /ml, 100 µg/m l).

b) Different concentrations of L-Ascorbic acid (2-10 mg/ml) were used as the standard antioxidant.

c) After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the equation, **DPPH antiradical scavenging capacity (%) = [1 – (Abof sample – Abof blank)/Abof control] × 100.**

d) Methanol plus different concentration of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control **[25].**

e) The IC50 values were calculated by the sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging capacity. IC50values denote the concentration of the sample required to scavenge 50% of DPPH radicals **[25].**

3.9.2 Standard Preparation

1) 250 mg Ceevit Tablet (Square) was crushed in mortar and pestle.

2) 20 ml distilled water was added and the solution was filtered.

3) It was then diluted by 10 times (2 ml of the filtered solution was taken and 18 ml water added).

4) The solution was taken in 5 test tubes to prepare 5 different concentrations.

5) 1ml, 2ml, 2ml, 4ml and 5ml solution were taken in 5 different test tubes and the volume adjusted to 5 ml with water in all the test tubes. [25]

3.9.3 Sample Preparation

1) 5gm of Jute leaf powder was soaked in 40 ml MeOH for 3days.

2) Then it was filtered and extract of the Jute leaf was collected.

3) The solutions were taken in 5 test tubes to prepare 5 different concentrations.

4) 10ul, 50ul, 100ul, 200ul, 500ul solution were taken in 5 different test tubes and the volume adjusted to 5 ml with methanol in all the test tubes. [25]

3.9.4 Blank Preparation

Blank was prepared by adding 5 ml methanol in a test tube. In all the 16 test tubes 400 µL DPPH solution was added in dark and left for 2 hours. After that UV absorbance was measured in UV machine at 517 nm.

3.9.5 THIN LAYER CHROMATOGRAPHIC ANALYSIS USING DIFFERENT FRACTIONS OF METHANOLIC EXTRACT AGAINST A STANDARD (VILDAGLIPTIN and MEROPENEM)

Vildagliptin (previously identified as **LAF237**, trade names **Zomelis**, **Galvus**) is an oral anti-hyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor

class of drugs. Vildagliptin inhibits the inactivation of GLP-1[2][3] and GIP[3] by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas.[26]

Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus.

Chemical data	
Formula	$C_{17}H_{25}N_3O_2$
Mol. mass	303.399 g/mol

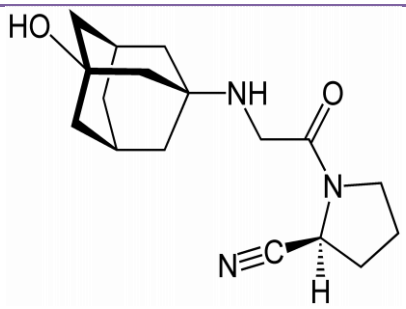


Figure 1.7 : Chemical structure of vildagliptin .

Novartis' Galvus (vildagliptin) is a member of a new class of oral antidiabetic agents known as dipeptidyl peptidase IV inhibitors (DPP-IV) inhibitors or 'incretin enhancers'. Its mode of action is distinct from established antidiabetic medications and appears to include disease-modifying effects in patients with type 2 diabetes.[26]

Galvus (vildagliptin), in advanced-stage development as a treatment for patients with type 2 diabetes, has now received the thumbs up from regulators in Europe. The EMEA has approved

its use in combination with other anti-diabetic medications including metformin, sulfonylureas and thiazolidinediones. Approval has also been granted for Eucreas, a single tablet formulation of Galvus (vildagliptin) and metformin. [26]

3.9.6 MECHANISM OF ACTION

DPP-IV inhibition – diabetes treatment

Glucagon-like peptide-1 (GLP-1) and gastric inhibitor peptide (GIP) are naturally occurring hormones (incretins) that are released from cells in the gut in response to food. They bind to receptors on pancreatic beta cells stimulating the release of the hormone insulin, responsible for the regulation of blood sugar levels. [26]

"Treatment with vildagliptin does not appear associated with weight gain, which is an important benefit for patients with type 2 diabetes."[27]

GLP-1 also reduces the secretion of glugacon, a hormone produced by the pancreas that stimulates the liver to convert glycogen to glucose, thus increasing blood sugar levels. Naturally produced GLP-1 has a very short half-life of less than two minutes.[27]

Patients with type 2 diabetes have impaired incretin function and are thus unable to properly regulate their blood sugar levels, which can lead to adverse clinical sequelae. Novartis' Galvus (vildagliptin) works by inhibiting DPP-IV, an enzyme that breaks down GLP-1. [27]

By delaying the degradation of GLP-1, vildagliptin extends the action of insulin while also suppressing the release of glucagon. This leads to a reduction in elevated blood glucose levels (hyperglycaemia), which is a characteristic feature of type 2 diabetes.[27]

3.9.7 Galvus effective in type 2 diabetics

Administration of Galvus (vildagliptin) to patients with type 2 diabetes suggests it is a safe and effective treatment with the potential to achieve long-term glycaemic control.In phase II trials the addition of vildagliptin 50mg/day to metformin, a standard treatment for type 2 diabetes, resulted in improved glucose control in patients inadequately controlled on metformin alone. Levels of haemoglobin A1c (HbA1c), fasting plasma glucose, mean prandial glucose and peak prandial glucose were reduced to a significantly greater effect after 12 weeks of additional treatment with vildagliptin compared with continued therapy with metformin alone.[27]

Importantly, the results achieved at 12 weeks were sustained over a year, indicating that patients treated with vildagliptin can achieve good long-term glycaemic control.[27]

Long-term treatment with Galvus (vildagliptin) appears well tolerated as reflected by low rates of study discontinuation for adverse events in clinical trials. In the phase II trials, drug-related adverse events occurred in 4.8% of patients receiving vildagliptin in addition to metformin compared with 6.9% of those receiving metformin plus the placebo. [27]

In phase III trials Galvus (vildagliptin) has been evaluated as both monotherapy and in combination with other standard antidiabetic drugs.[27]

3.9.8 Potential to preserve pancreatic beta cell function

Patients with type 2 diabetes experience the progressive loss of pancreatic beta cell function and a concomitant loss of insulin secretion and glycaemic control. In preclinical studies, Galvus (vildagliptin) was found to have a beneficial effect on insulin secretion by increasing beta cell

production and inhibiting programmed cell death (apoptosis).Subsequent clinical studies, in which vildagliptin was administered to patients with type 2 diabetes not previously treated with oral antidiabetic medications, showed that it increased the active forms of GLP-1 and GIP when compared with the placebo. [27]

The drug's ability to improve the functioning of insulin-producing cells in the pancreas, albeit in studies with small numbers of patients, suggests it may have disease-modifying potential in the treatment of type 2 diabetes.[27]

3.9.9 PHARMACOKINETICS

Absorption : Rapidly absorbed

Max plasma concentration achieved 1-2 hr after oral administration.

Oral bioavailability : About 85%

Metabolism : Metabolised by hydrolysis

Excretion : Mainly excreted in the urine [27]

4.0 Adverse drug reaction

Hypoglycemia, delayed gastric emptying , nausea and vomiting flue-like symptoms, headache and dizziness may occur.[28]

4.1 Brine Shrimp lethality Bioassay

4.1.1 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural

products etc. [15] Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism.[29]

(Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discover of new bioactive natural products [16]. Natural product extracts, fractions or pure compounds can be tested for there bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia*, the only genus in the family Artemiidae,[19]

4.1.2 Hatching of shrimp:

Artificial sea water was prepared by dissolving 38 g of NaCl in one liter of distilled water and was filtered to get a clear solution. A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. The eggs of *Artemia salina* were hatched at room temperature (25-30 ºC) for 18-24 h. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. They were then collected by a Pasteur pipette.[29]

Figure 1.8:. *Artemia*: just hatched (left), enriched and 24 hours old (right)

4.1.3 Procedure

1. Solid sample was dissolved in respective solvent and liquid samples were transferred to vials to get concentrations of 10, 5, 2.5, 1.25 and 0.625 in 4 ml artificial sea water with ten nauplii in each vial.
2. Blank contain 4 ml artificial sea water with ten nauplii . After 24 hr incubation at room temperature (25-30 ºC), the number of viable naupliis were counted using a magnifying glass.[19]

5.0 High Performance Liquid Chromatography HPLC

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. HPLC is considered an instrumental technique of analytical chemistry (as opposed to a gravitimetric technique). HPLC has many uses including medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products).

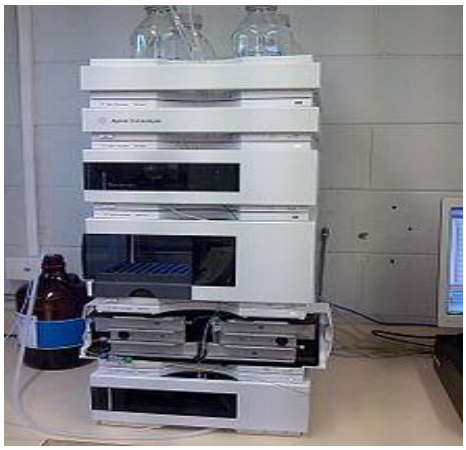


Figure1.9: HPLC instrument

The schematic of an HPLC instrument typically includes a sampler, pumps, and a

detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components.

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.

The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.[29]

5.1.1 Normal phase HPLC

This is essentially just the same as you will already have read about in thin layer chromatography or column chromatography. Although it is described as "normal", it isn't the most commonly used form of HPLC.

The column is filled with tiny silica particles, and the solvent is non-polar – hexane, for example. A typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm.Polar compounds in the mixture being passed through the column will stick longer to the polar silica than non-polar compounds will. The non-polar ones will therefore pass more quickly through the column.[29]

5.1.2 Reversed phase chromatography

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic

solvent to the eluent. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release.[30]

RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces.

The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase.Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C,

and generally non-polar atomic bonds, such as S-S and others) is retained longer because it is non-interacting with the water structure. On the other hand, analytes with higher polar surface area (conferred by the presence of polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ in their structure) are less retained as they are better integrated into water. Such interactions are subject to steric effects in that very large molecules may have only restricted access to the pores of the stationary phase, where the interactions with surface ligands (alkyl chains) take place. Such surface hindrance typically results in less retention.Retention time increases with hydrophobic (non-polar) surface area. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Similarly organic compounds with single C-C-bonds elute later than those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond.

Another important factor is the mobile phase pH since it can change the hydrophobic character of the analyte. For this reason most methods use a buffering agent, such a sodium phosphate, to control the pH. Buffers serve multiple purposes: control of pH, neutralize the charge on the silica surface of the stationary phase and act as ion pairing agents to neutralize analyte charge. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column effluent. Reversed phase columns are quite difficult to damage compared with normal silica columns; however, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases as these will destroy the underlying silica particle.In this case, the column size is the same, but the silica is modified to make it non-polar by attaching long hydrocarbon chains to its surface - typically with either 8 or 18 carbon atoms in them. A polar solvent is used - for example, a mixture of water and an alcohol such as methanol.

In this case, there will be a strong attraction between the polar solvent and polarmolecules in the mixture being passed through the column. There won't be as much attraction between the hydrocarbon chains attached to the silica (the stationary phase) and the polar molecules in the solution. Polar molecules in the mixture will therefore spend most of their time moving with the solvent.

Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of vander Waals dispersion forces. They will also be less soluble in the solvent because of the need to break hydrogen bonds as they squeeze in between the water or methanol molecules, for example. They therefore spend less time in solution in the solvent and this will

slow them down on their way through the column.That means that now it is the polar molecules that will travel through the column more quickly.[30]

Reversed phase HPLC is the most commonly used form of HPLC.

5.1.3 Isocratic and gradient elution

A separation in which the mobile phase composition remains constant throughout the procedure is termed *isocratic* (meaning *constant composition*). The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a *gradient elution*.

One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; *A* is the "weak" solvent which allows the solute to elute only slowly, while *B* is the "strong"solvent which rapidly elutes the solutes from the column. In reversed-phase chromatography, solvent *A* is often water or an aqueous buffer, while *B* is an organic solvent miscible with water, such as acetonitrile, methanol, THF, or isopropanol.In isocratic elution, peak width increases with retention time linearly according to the equation for N, the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks.[30]

Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a

peak forward. This also increases the peak height (the peak looks "sharper"), which is important in trace analysis. The gradient program may include sudden "step" increases in the percentage of the organic component, or different slopes at different times – all according to the desire for optimum separation in minimum time.In isocratic elution, the selectivity does not change if the column dimensions (length and inner diameter) change – that is, the peaks elute in the same order. In gradient elution, the elution order may change as the dimensions or flow rate change.The driving force in reversed phase chromatography originates in the high order of the water structure. The role of the organic component of the mobile phase is to reduce this high order and thus reduce the retarding strength of the aqueous component.[30]

5.1.4 Parameters

5.1.4.1 Theoretical

HPLC separations have theoretical parameters and equations to describe the separation of components into signal peaks when detected by instrumentation such as by a UV detector or mass spectrometer. The parameters are largely derived from two sets of chromatographic theory: plate theory and the rate theory of chromatography.

The HPLC parameters are the:

●Efficiency factor (*N*),

●The retention factor (kappa prime),

● The separation factor (alpha).

Together the factors are variables in a resolution equation, which describes how well two components' peaks separated or overlapped each other. These parameters are mostly only used for describing HPLC reversed phase and HPLC normal phase separations, since those separations tend to be more subtle than other HPLC modes (e.g. ion exchange and size exclusion).

■ Void volume is the amount of space in a column that is occupied by solvent. It is the space within the column that is outside of the column's internal packing material. Void volume is measured on a chromatogram as the first component peak detected, which is usually the solvent that was present in the sample mixture; ideally the sample solvent flows through the column without interacting with the column, but is still detectable as distinct from the HPLC solvent. The void volume is used as a correction factor.

■ Efficiency factor (*N*) practically measures how sharp component peaks on the chromatogram are, as ratio of the component peak's area ("retention time") relative to the width of the peaks at their widest point (at the baseline). Peaks that are tall, sharp, and relatively narrow indicate that separation method efficiently removed a component from a mixture; high efficiency. Efficiency is very dependent upon the HPLC column and the HPLC method used. Efficiency factor is synonymous with plate number, and the 'number of theoretical plates'.

■ Retention factor (*kappa prime*) measures how long a component of the mixture stuck to the column, measured by the area under the curve of its peak in a chromatogram (since HPLC

chromatograms are a function of time). Each chromatogram peak will have its own retention factor (e.g. *kappa*1 for the retention factor of the first peak). This factor may be corrected for by the void volume of the column.

■ Separation factor (*alpha*) is a relative comparison how well two neighboring components of the mixture were separated (i.e. two neighboring bands on a chromatogram). This factor is defined in terms of a ratio of the retention factors of a pair of neighboring chromatogram peaks, and may also be corrected for by the void volume of the column. The greater the separation factor value is over 1.0, the better the separation, until about 2.0 beyond which an HPLC method is probably not needed for separation.[30]

5.1.4.2 Internal diameter

The internal diameter (ID) of an HPLC column is an important parameter that influences the detection sensitivity and separation selectivity in gradient elution. It also determines the quantity of analyte that can be loaded onto the column. Larger columns are usually seen in industrial applications, such as the purification of a drug product for later use.Low-ID columns have improved sensitivity and lower solvent consumption at the expense of loading capacity.[31]

● Larger ID columns (over 10 mm) are used to purify usable amounts of material because of their large loading capacity.

● Analytical scale columns (4.6 mm) have been the most common type of columns, though smaller columns are rapidly gaining in popularity. They are used in traditional quantitative analysis of samples and often use a UV-Vis absorbance detector.

● Narrow-bore columns (1–2 mm) are used for applications when more sensitivity is desired either with special UV-visible detectors, fluorescence detection.[31]

5.1.4.3 Particle size

Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles (very small beads). These particles come in a variety of sizes with 5 µm beads being the most common. Smaller particles generally provide more surface area and better separations, but the pressure required for optimum linear velocity increases by the inverse of the particle diameter squared.[31]

5.1.4.4 Pore size

Many stationary phases are porous to provide greater surface area. Small pores provide greater surface area while larger pore size has better kinetics, especially for larger analytes. For example, a protein which is only slightly smaller than a pore might enter the pore but does not easily leave once inside.[31]

5.1.4.5 Pump pressure

Pumps vary in pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Pressure may reach as high as 40 MPa (6000 lbf/in^2), or about 400 atmospheres. Modern HPLC systems have been improved to work at much higher pressures, and therefore are able to use much smaller particle sizes in the columns (<2 µm). These "Ultra High Performance Liquid Chromatography" systems or RSLC/UHPLCs can work

at up to 100 MPa (15,000 lbf/in^2), or about 1000 atmospheres. The term "UPLC" is a trademark of the Waters Corporation, but is sometimes used to refer to the more general technique.[31]

5.1.5 HPLC General Operating Procedure

Use Gloves and safety glasses for handling buffer/mobile phase – (Mobile phase is toxic and flammable)

Turn on both power strips to apply power to the pump, column heater and detector. Turn on detector power switch located on front of detector 15 – 20 minutes before running samples. Turn on pump power switch; make sure the flow rate on the pump is set to 00.Make sure the attenuator on the detector is set to 8X.

Check that the solvent select knob is set to position 2 and the waste knob is set to waste. These knobs are located on the pump module.

1. Check system set-up

a. Check buffer/mobile phase reservoir (min of 540 ml)

b. Verify LOAD/INJECT switch is set to LOAD, Verify Injection pin is in injection port and that port is locked.

c. Verify reference outlet and column outlet drains are draining into disposal container (buffer / mobile phase is toxic and flammable)

d. Remove reference inlet and reference outlet caps

e. Flush reference cell by filling syringe with mobile phase – use large gas type syringe with metal fitting on the end - with 5 ml buffer/mobile phase (min)

f. Thread syringe into reference inlet located on the left side of the differential refractometer (R401).

g. Inject buffer/mobile phase into reference inlet. Verify complete priming of the reference cell by observing buffer/mobile phase exiting the reference outlet.

h. Replace reference inlet and outlet caps

i. Remove column outlet cap WARNING – Failure to remove cap will cause an over pressure of column and column failure!

j. If many samples need to be analyzed, the reference cell may need to be primed again after several hours of running.

2. Priming the Pump

a. To prime pump inlet – open solvent draw off valve located on lower front panel of pump and drain buffer/mobile phase into a small waste flask. Check mobile phase inlet line to insure no air bubbles are in line. (do not change “waste and solvent” knob)

b. Tighten solvent draw off valve.

c. Verify pressure switch is set to 2000 PSI. (Check range setting knob)

d. Thread syringe into left side bypass valve

e. Flip bypass valve to the right. This will send mobile phase through the reference side.

f. Set flow rate to 0.4 ml/min

g. Turn on pump at pump power switch. (Verify power strip is on)

h. Draw buffer/mobile into syringe for one full cycle of pump (one full cycle is indicated by the pump piston indicator travel) you may not pull liquid into the syringe – the purpose of this is to pull solution into the pump.

i. Set bypass valve to left position, this runs mobile phase through the detectors with the sample.

j. Remove syringe.

k. Empty syringe into disposal container.

l. Set flow rate to 1.8 ml/min

m.Verify pump prime by observing steady pump pressure on gauge. If oscillating pressure is observed – re-prime pump – repeat steps c-m).

5.1.6 Column Description:

Column is a Luna 5 micron NH2100 angstrom

Mobile Phase: 80% Acetonitrile/20% Water

Flow rate: 3 ml/min

Temperature: 40C

Injection Volume: 10 micro Liters.

3. Column Preparation

a. Verify buffer/mobile phase is exiting column outlet into disposal container.

b. Set flow rate to 3 ml/min. If pump stops and reset button lights depress reset button, if Pump stops again contact lab manager.

c. Turn on column heater at plug strip; it has no power switch of its own.

d. Verify temperature is set to 40 C (see bottom of column holder)

e. Wait for column to achieve steady state temperature (about 30 minutes for temperature stabilization and flushing of system).

4. Software Set-up (Done during system flushing)

a. Turn on computer

b. Enter username (analab) and password (Letmein!)

c. After computer boot double click Diamir icon.

d. Enter username (analab) and password (Letmein!)

e. Click on “Systems” tab (lower left hand corner of window)

f. Check the Waters HPLC box

g. Click on Acquisition pull-down menu – select monitoring baseline

h. In the pop-up window select method for analysis (pull down menu). If you are running glucose choose the sugar test. METH method.

i. Verify appearance of baseline on chromatograph chart. If error occurs, close Chromatograph window by clicking on red circle. Repeat steps g-i.

j. Verify baseline is stable (channel 1).

k. Verify scale is set to –200K to 200K. (right click on scale to change)

l. Click (red X circle) to stop software and prepare for sample run.

5. Software Set-up for Sample Run

a. From Acquisition pull-down menu select quick start

b. Choose sample method (same method as step 4.h) – click O.K.

c. Change file name on Injection pop-up window.

d. Edit description

e. Note sample parameters, change if required.

f. Click start button.

g. Verify System Status as" Waiting for Injection".

6. Sample Injection

a. Referring to load / inject valve in step (2e) verify valve handle is in the load position. And flow rate is 3ml/min. If valve handle is not in load position the plug will be forced out upon release by 2000 PSI.

b. Select the Hamilton 10 micro liter syringe.

c. Release the injection port plug by turning the bottom lever down.

d. Remove the round silver port plug and place it in the hole in the valve handle.

e. Draw 10 micro liters of sample into the Hamilton syringe.

f. Insert syringe fully into sample port.

g. Inject sample.

h. Replace round silver port plug into port.

i. Turn bottom lever up until lever is tight.

j. Turn upper switch to “Inject” position. This will automatically start data acquisition.

k. Wait 30 seconds.

l. Turn upper switch to “Load” position.

m. This should start the data acquisition. The display should change from “Waiting For Injection” to “Running”

n. If you cannot see your data, right click on the display and zoom in/out as needed or hold the right mouse button down and the move the cursor vertically over the graph.

7. Clean Syringe

a. Rinse syringe thoroughly with DI water until clean.

8. Chromatograph Data Acquisition

a. Wait until sample clears the column (returns to baseline) as software collects data.

Looking at the whole process

A flow scheme for HPLC

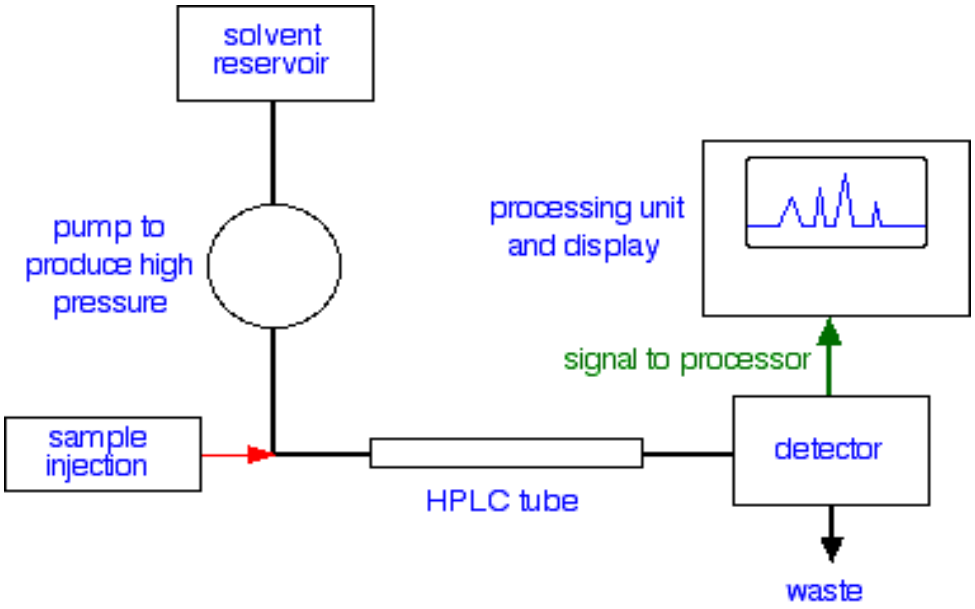


Figure 2.0: Schematic diagram of HPLC[31]

5.1.7 Injection of the sample

Injection of the sample is entirely automated, and you wouldn't be expected to know how this is done at this introductory level. Because of the pressures involved, it is *not* the same as in gas chromatography.

5.1.8 Retention time

The time taken for a particular compound to travel through the column to the detector is known as its *retention time*. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.

Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column

That means that conditions have to be carefully controlled if you are using retention times as a way of identifying compounds.

5.1.9 The detector

There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption.Many organic compounds absorb UV light of various wavelengths. If you have a beam of UV light shining through the stream of liquid coming out of the column,and a UV detector on the opposite side of the stream, you can

get a direct reading of how much of the light is absorbed.The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

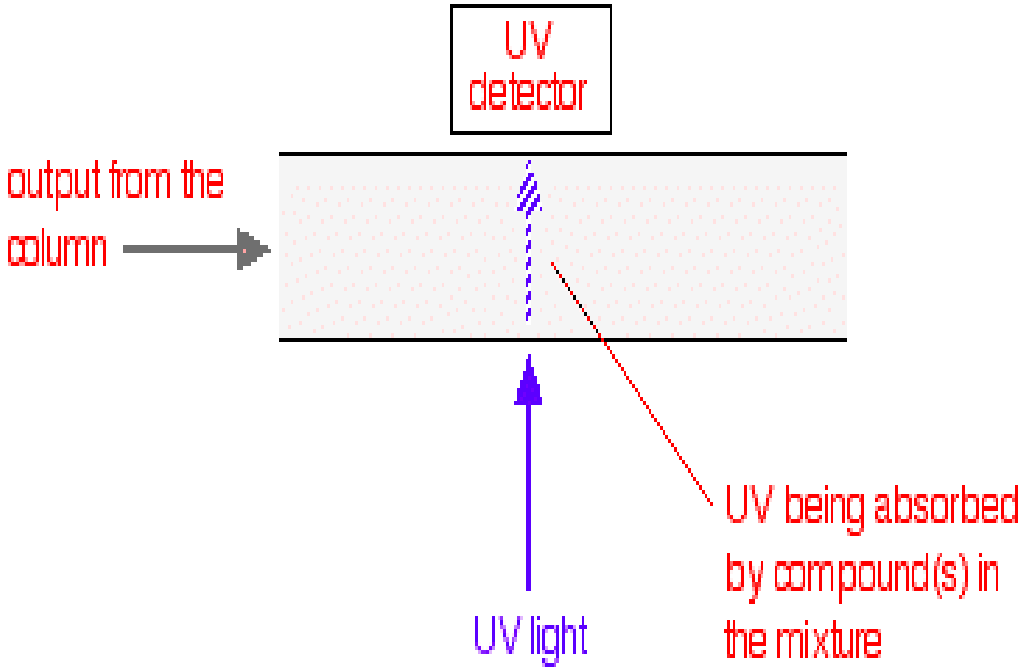


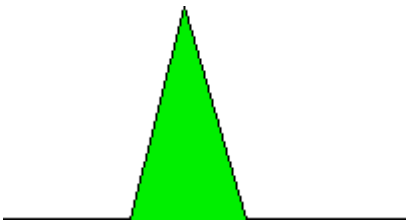
Figure 2.1: schematic diagram of UV detector

Methanol, for example, absorbs at wavelengths below 205 nm, and water below 190 nm. If you were using a methanol-water mixture as the solvent, you would therefore have to use a wavelength greater than 205 nm to avoid false readings from the solvent.[31]

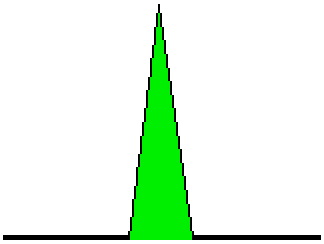
5.2.0 Interpreting the output from the detector

The output will be recorded as a series of peaks - each one representing a compound in the mixture passing through the detector and absorbing UV light. As long as you were careful to control the conditions on the column, you could use the retention times to help to identify the compounds present - provided, of course, that you (or somebody else) had already measured

them for pure samples of the various compounds under those identical conditions. But you can also use the peaks as a way of measuring the quantities of the compounds present. Let's suppose that you are interested in a particular compound, X.If you injected a solution containing a known amount of pure X into the machine, not only could you record its retention time, but you could also relate the amount of X to the peak that was formed.The area under the peak is proportional to the amount of X which has passed the detector, and this area can be calculated automatically by the computer linked tothe display. The area it would measure is shown in green in the (very simplified) diagram.



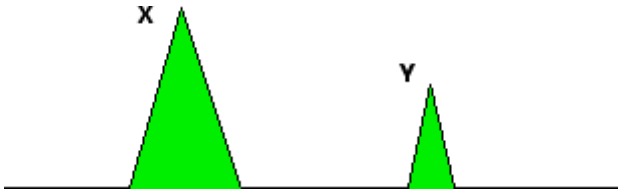
If the solution of X was less concentrated, the area under the peak would be less although the retention time will still be the same. For example:



This means that it is possible to calibrate the machine so that it can be used to find how much of a substance is present - even in very small quantities.Be careful, though! If you had two different

substances in the mixture (X and Y) could you say anything about their relative amounts? *Not if you were using UV*

absorption as your detection method.



In the diagram, the area under the peak for Y is less than that for X. That may be because there is less Y than X, but it could equally well be because Y absorbs UV light at the wavelength you are using less than X does. There might be large quantities of Y present, but if it only absorbed weakly, it would only give a small peak.

5.2.1 Advantages of HPLC:

• It includes both aspects of analysis i.e qualitative and quantitative analysis.

• HPLC method evaluates almost all the molecules of same family.

• For example in one single run all the mono-amines like dopamine, epinephrine, serotonin can be estimated. Single run for steroids in one sample gives the data of all the steroids in that sample.

• Molecules with small differences in absorption wavelengths can be detected well due to their differences in separation time. i.e one which travels faster is measured prior to the other which is

measured later. This is the prime advantages if HPLC which makes it non-replaceable. Substances in very low concentration like nano and picograms can be detected due to the sensitivity of HPLC detectors used like electrochemical detector, fluorescence detector etc.

• Due to its high separation efficiency, the quality of substance obtained by preparative mode or technique (prep hplc) is of high purity.[31]

5.2.2 Disadvantages of HPLC

• It's an expensive technique as it requires costly HPLC instrumentation, columns and also use of highest grade of purity solvents, buffers, chemicals etc termed as HPLC grade.

• Working on HPLC requires heavy processing before estimation like mixing, homogenization, filtration, degassing, derivatization etc. These techniques are also to be performed with proper care to avoid problems in estimation.

• The systems operation requires prior HPLC training and effective HPLC troubleshooting skills. So prior practice is essential to run this chromatography systems.

• HPLC Data obtained is non-homogenous and is never without any noise (fluctuation) and errors during estimation.

• Its time consuming and you must have good amount of patience[31].

• Alteration in temperature and presence of dust in chromatography lab can greatly vary the result out put. So strict maintenance of experimental conditions is required through out the process.

5.3 Method development of Vildagliptin by HPLC

The present work aims to a simple, rapid and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of vildagliptin in pure form and in tablet dosage form using Agilent XDB C18, 150 × 4.6 mm, 5 µm, column.

5.3.1 Procedure

●Wave Length: 210 nm

●Buffer: 0.5 ml Tri-fluroacetic Acid for 500 ml water.

●Mobile Phase (MP): Buffer : Methanol

=20 : 80

●Flow rate: 1.00 ml/min

50.00 mg (Vildagliptin) ----------50 ml (MP) ------1ml--------10 ml (MP)

100µl (methanolic sample of seed) ----------10 ml (MP)

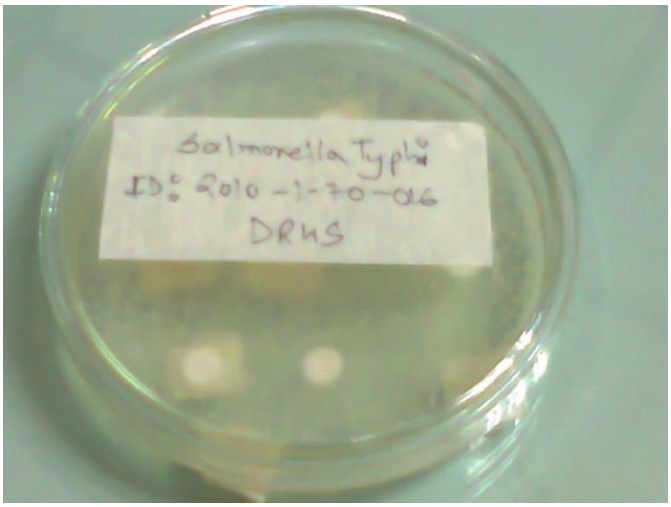
CHAPTER FOUR RESULT & DISCUSSION

5.4 RESULTS

5.4.1 RESULTS OF ANTIMICROBIAL SCREENING

Table 2.2 : This table shows the antibacterial activity of different fractions of *Atrocarpus heterophyllus*

Test organism	n-hexane	DCM (Dichloromethane)	Diethyl ether	Butanol	Chloroform	Ethyl acetate	Methanol
1. *Escherichia coli*	----	----	----	----	-----	----	-----
2. *Staphylococcus aureus*	-----	----	---	----	-----	----	----
3. Salmonella typhi	----	----	---	----	-----	----	----
4. Beta-hemolytic streptococcus	----	-----	---	9mm	9mm	----	----
5. Candida albicans	----	-----	---	----	-----	----	----



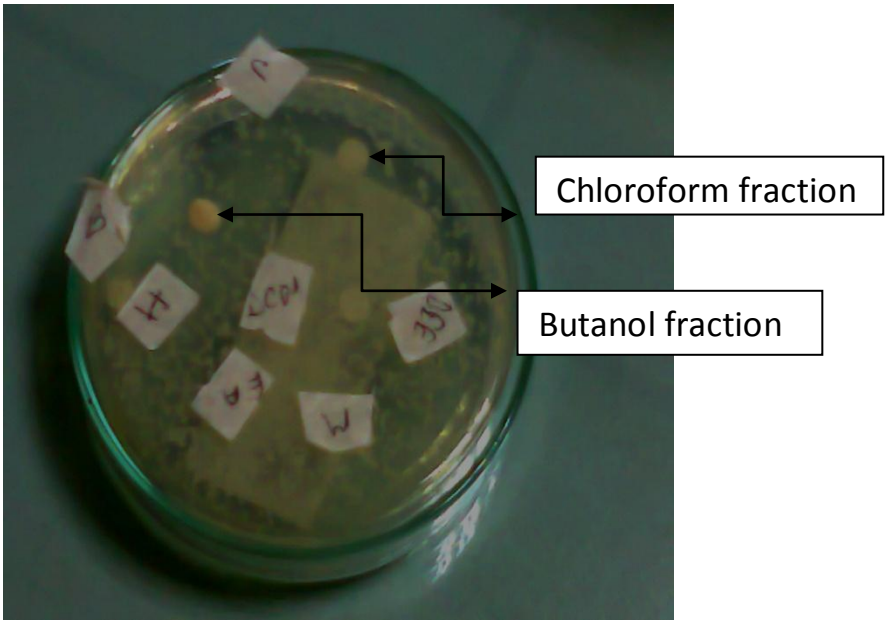


Figure 2.2 : Zone of inhibition for *Salmonella typhi* by anti-microbial screening

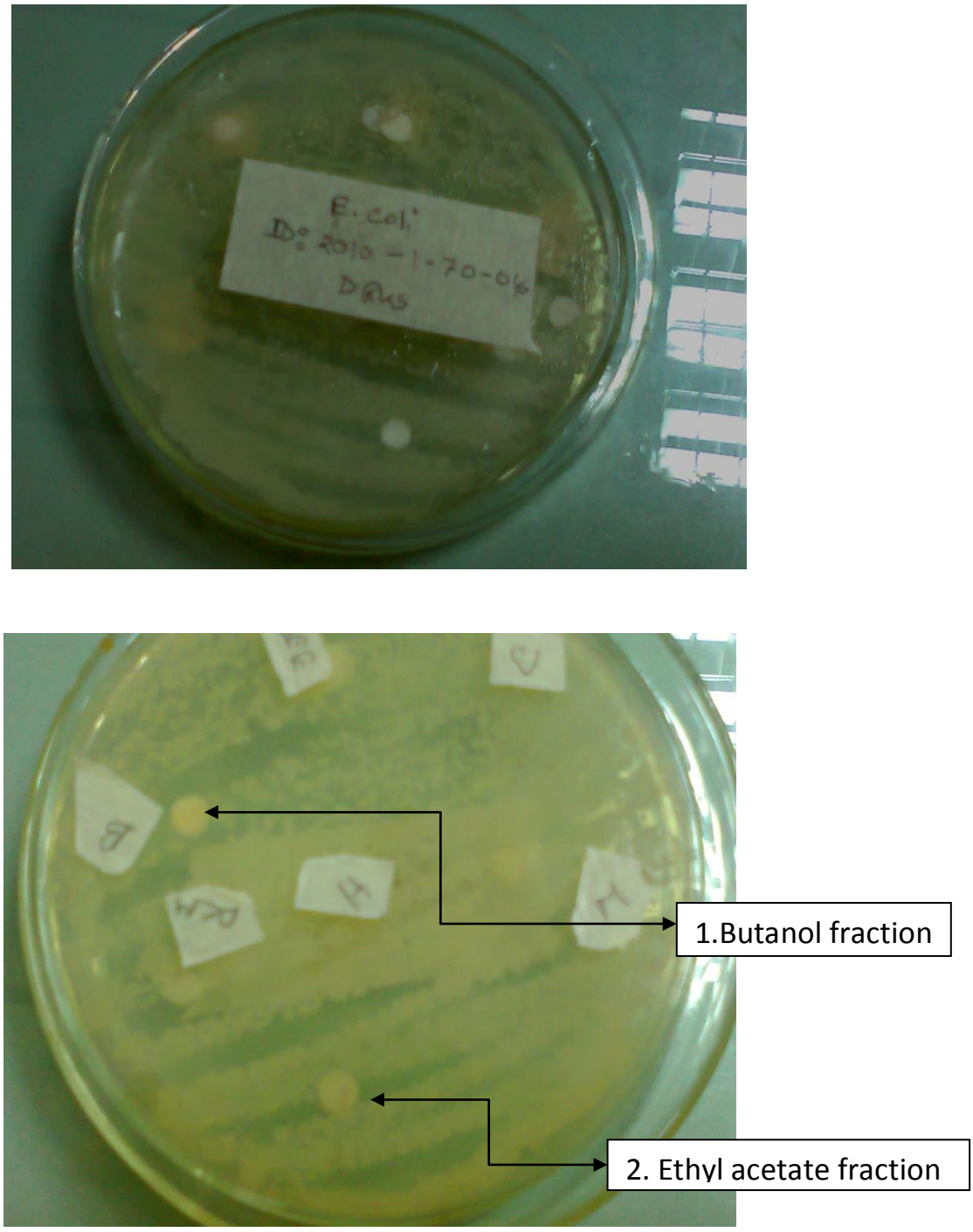
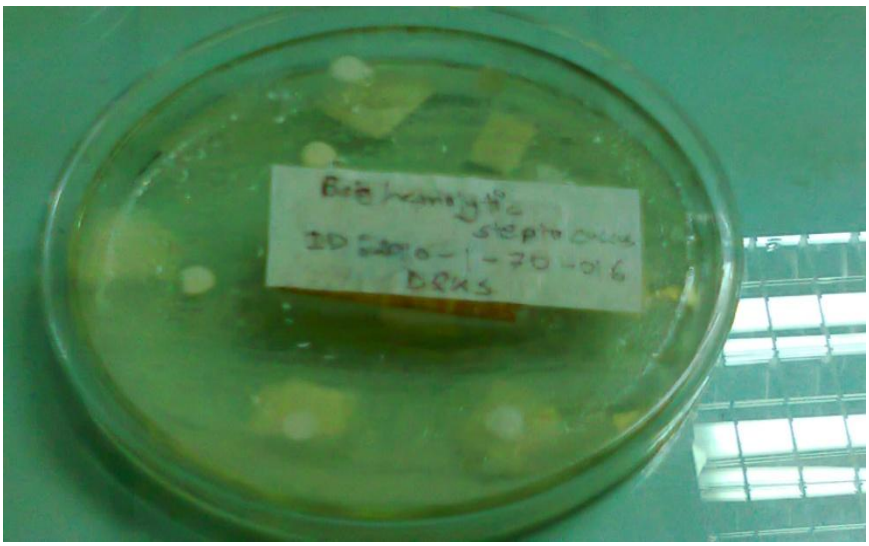


Figure 2.3 : Zone of inhibition for *Escherichia coli* by anti-microbial screening



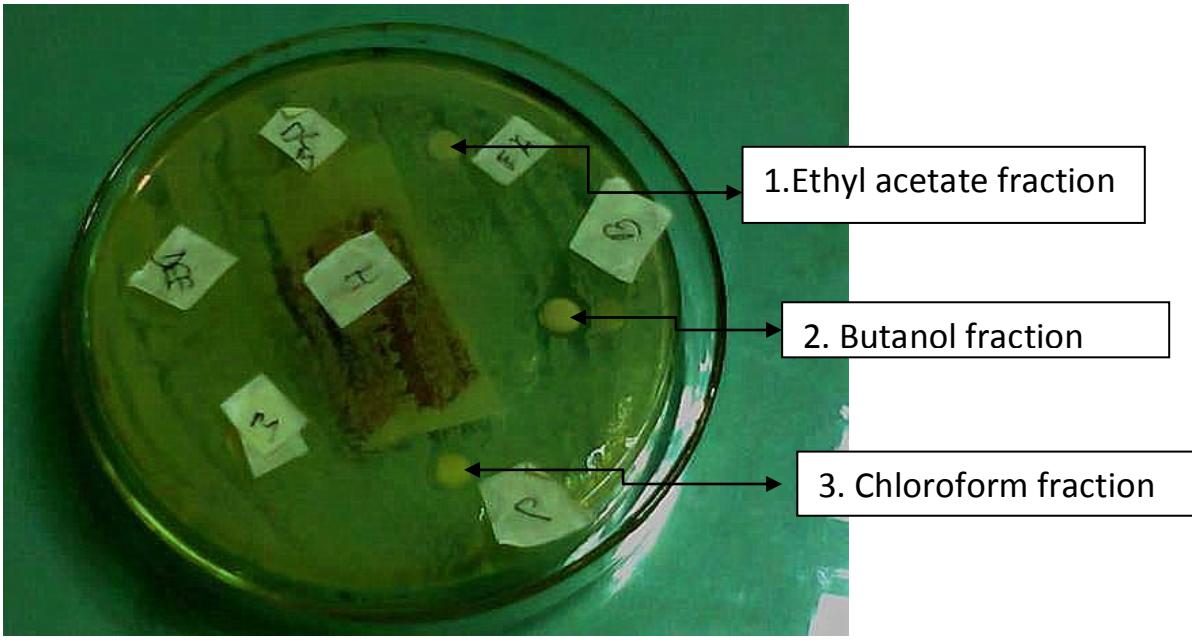


Figure 2.4 : Zone of inhibition for *Beta heamolytic streptococcus* by anti-microbial screening

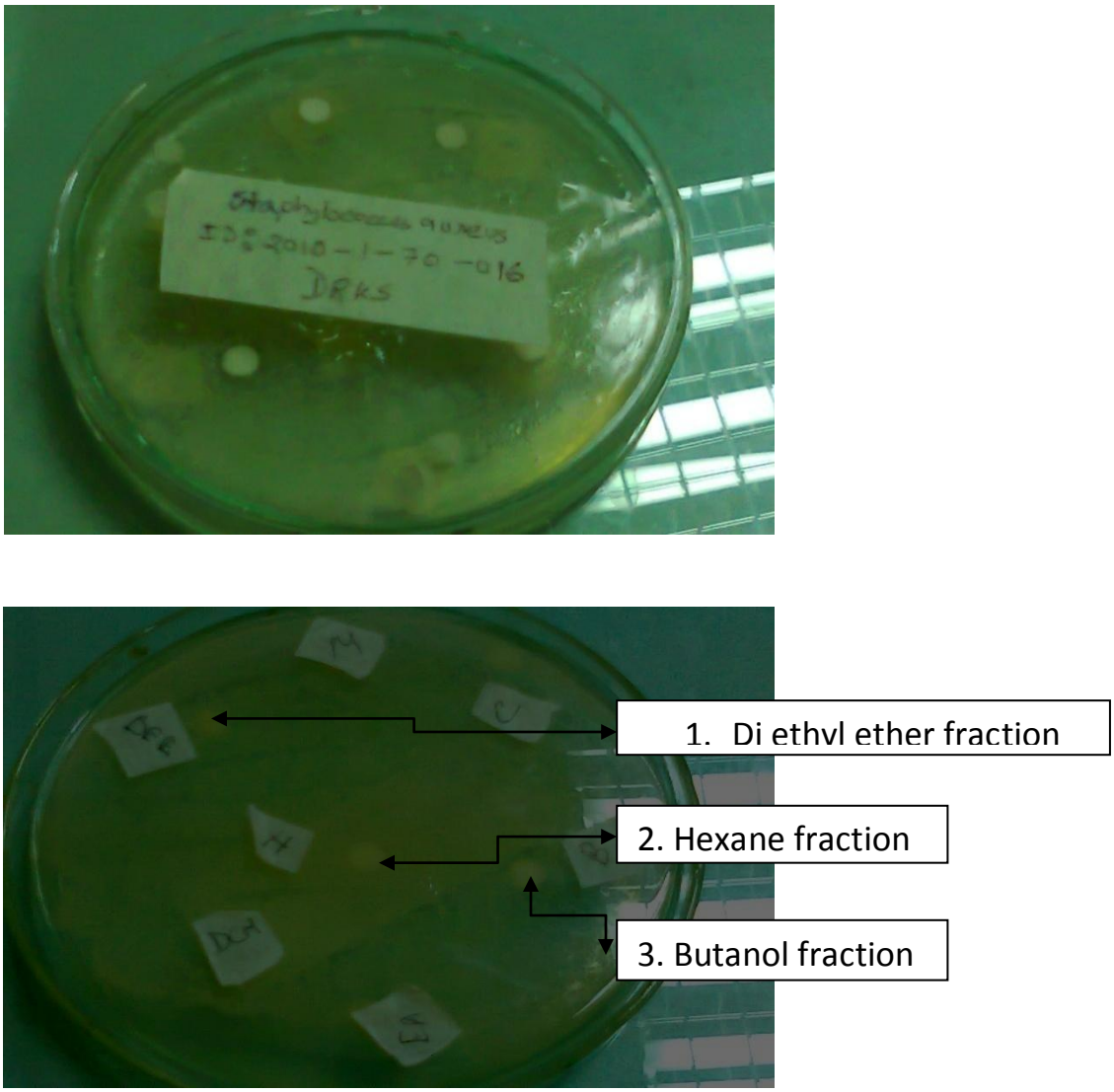


Figure 2.5: Zone of inhibition for *Staphylococcus aureus* by anti-microbial screening

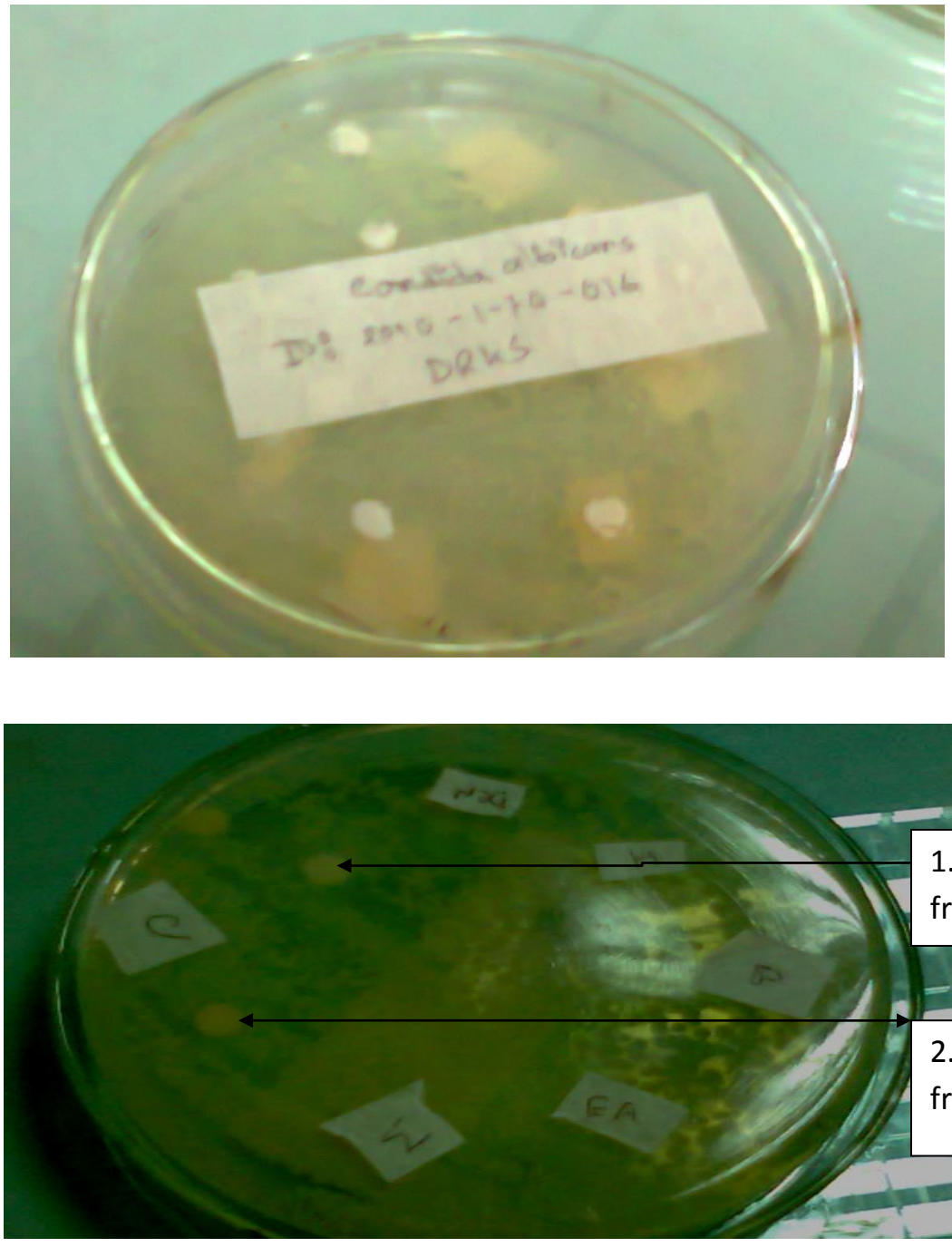


Figure 2.6 : Zone of inhibition for *Candida albicans* by anti-microbial screening

The disc-diffusion method is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture.Hence, from these results it is observed that the seed of jackfruit possess slight antimicrobial activity.Specially butanol and chloroform fraction showed antimicrobial activity against one species.May be it was due to the presence of some valuable compounds(for example; tannins,colchicines;catechols;eugenol and essential oil etc.) in butanol and chloroform fraction.Other fractions did not show any activity. Recent study reported that many varities of jackfruit possess moderate antimicrobial property. In this study,only two fraction gave zone of inhibition (9mm) against one gram(+ve) species. May be it was due to the change in varities of jackfruit.[32]

5.4.2 THIN LAYER CHROMATOGRAPHIC ANALYSIS USING DIFFERENT FRACTIONS OF METHANOLIC EXTRACT of *Artocarpus heterophyllus* SEEDS

TLCs were conducted on methanolic extract of the Artocarpus heterophyllus seed by using all the three types of solvent system & the best results were obtained by using the solvent system 1,which is the non-polar solvent system.The pictures of the plates tht were developed are displayed below:

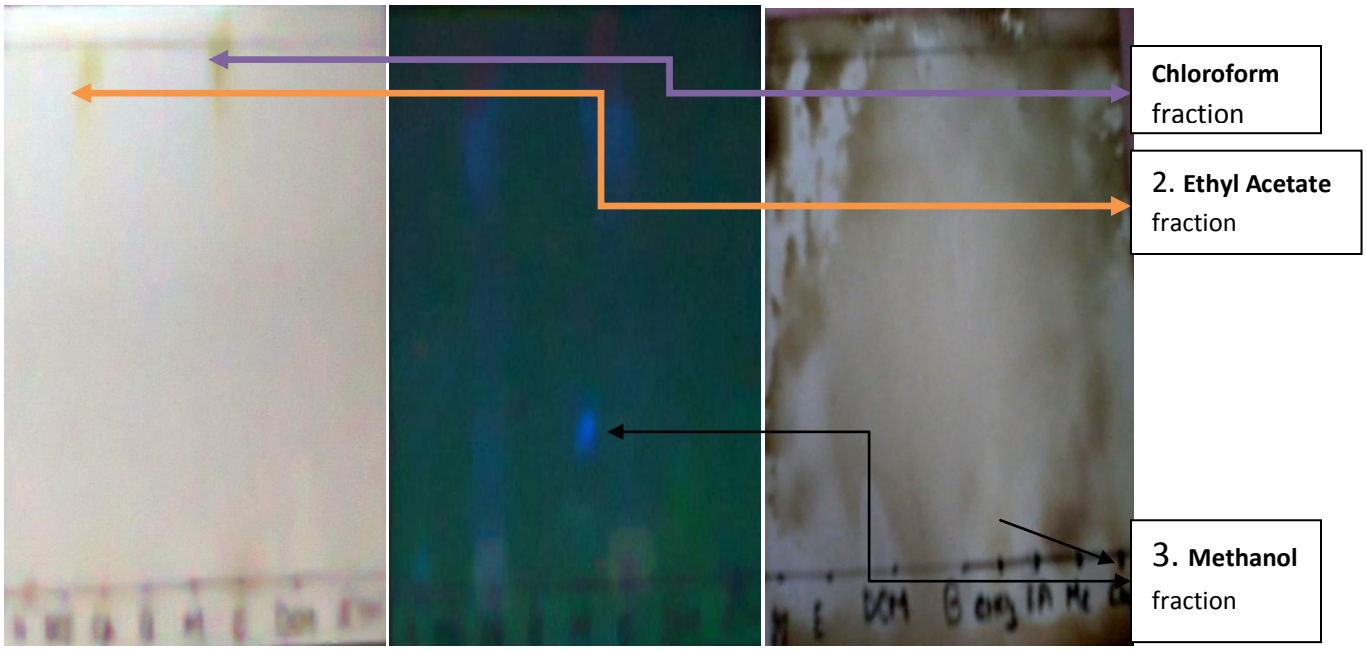
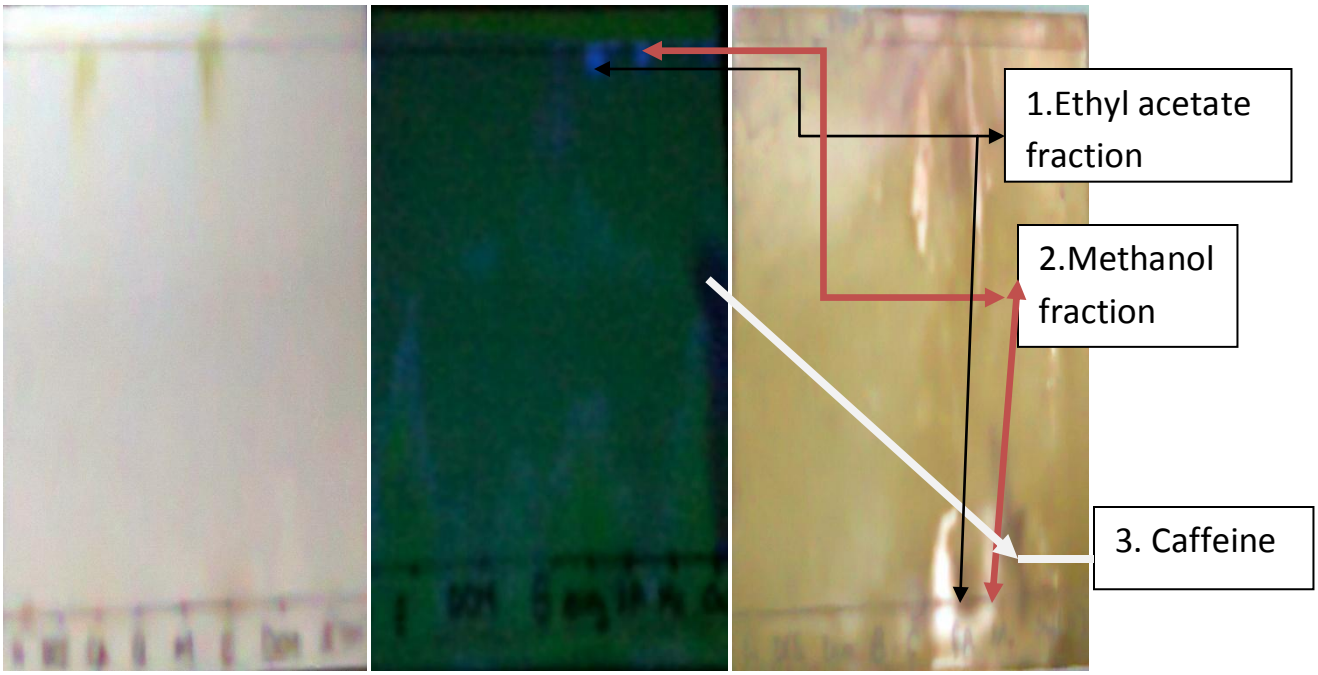


Figure 2.7 : Results for TLC in nonpolar basic solvent (A = naked eye view; B = UV light view; C = after Charring)

Here chromatograms developed after separating the methanolic extract of *Artocarpus heterophyllus* using the nonpolar basic solvent system containing benzene, ,ethanol & ammonium hydroxide in the ratios of 9:1:0.1.The naked eye view of the TLC was mentioned in the plate A which showed clear spot. Then the plate was observed under UV which is shown in the plate B. It showed some spots which indicate the presence of different compounds in that sample(Chloroform fraction and ethyl acetate fraction). Charring process done in 10% conc. Sulfuric acid on plate C showed brown black spot which indicates the presence of different

compounds in that sample.This brown-black spot indicates the presence of valuable compounds that may be present in the methanolic extract of *Atocarpus heterophyllus* seeds.



(D) (E) (F)

1.Hexane fraction ; 2.Di ethyl ether fraction; 3. Di chloro methane fraction; 4.Butanol fraction; 5.Chloroform fraction; 6.Ethyl acetate fraction; 7.Methanol fraction; 8. Standard (Caffeine)

Figure 2.8 : Results for TLC in polar basic solvent (D = naked eye view; E = UV light view; F = after Charring)

The naked eye view of the TLC was mentioned in the plate D which showed clear spot. Then the plate was observed under UV which is shown in the plate E. It showed some spots which indicate the presence of different compounds in that sample (Chloroform fraction and methanol fraction). Charring process done on plate F showed brown black spot(Chloroform fraction and methanol fraction) which indicates the presence of different compounds in that sample.[32]

5.4.3 THIN LAYER CHROMATOGRAPHIC ANALYSIS USING DIFFERENT FRACTIONS OF METHANOLIC EXTRACT AGAINST A STANDARD (Vildagliptin)

The chloroform fraction gave clear spot In TLC using media 1 wherein Vildagliptin is used as a standard.

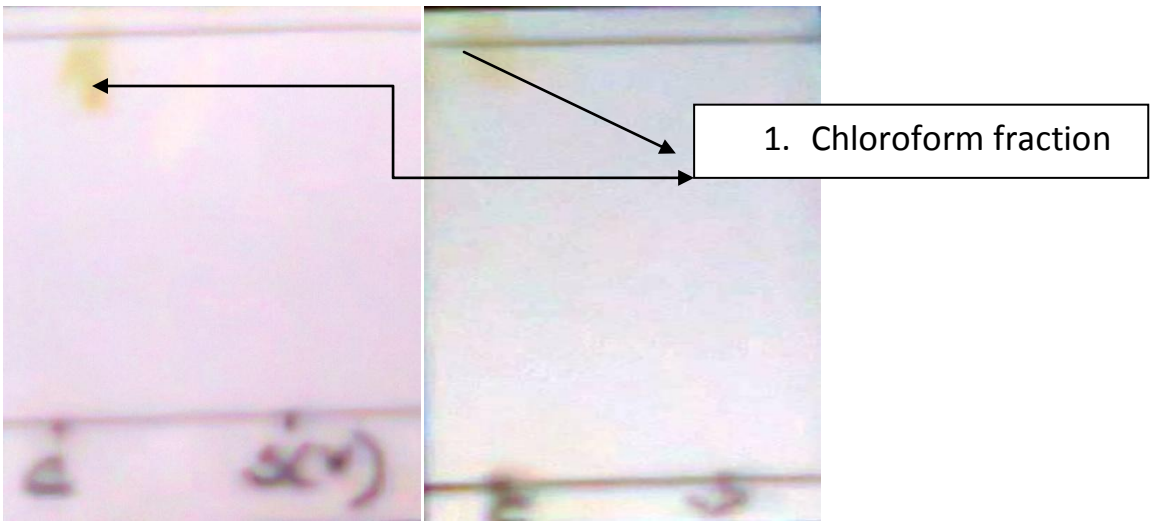


Figure 2.9 : TLC Using solvent media 1(Naked eye view)

The yellow spot present in the plate indicates the presence of valuable compound in the chloroform fraction which is spotted against an anti-diabetic drug(Vildagliptin).

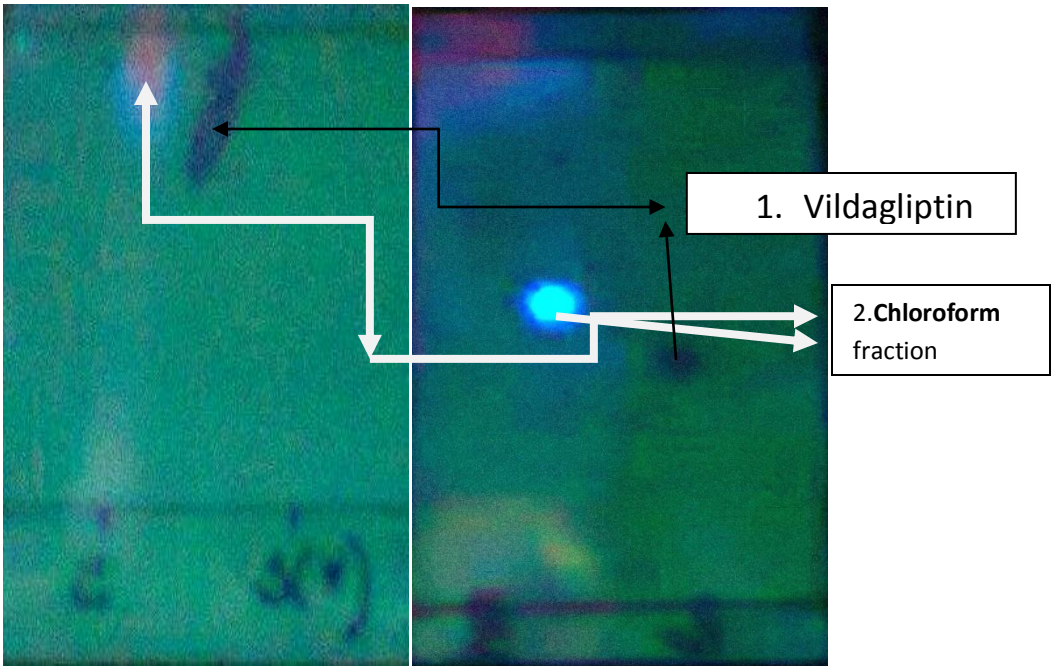
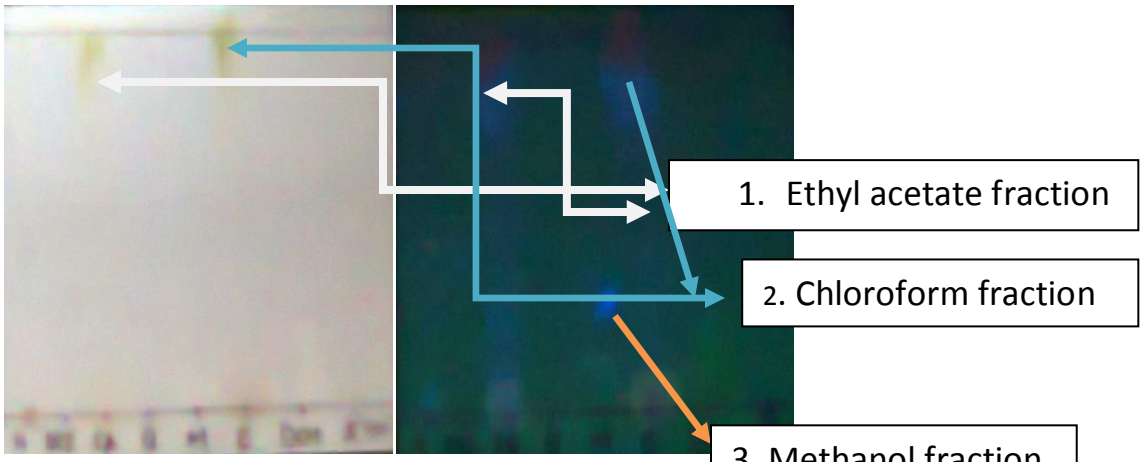


Figure 3.0 : Under UV light

Rf value of Chloform = 0.452

Rf value of Vildagliptin = 0.401

The formation of white spot on the plate(chloroform fraction) and dark blue spot(Vildagliptin) indicates the extensive presence of valuable compounds which may can maintain the glucose level in blood.The Rf value of both chloroform & vildagliptin is nearly same,but they flurosence at two different color (white and dark blue).From which we can have a preliminary idea of the valuable compounds that may be present in the methanolic extract of *Atocarpus heterophyllus* seeds.



(G) (H)

1.Hexane fraction ; 2.Di ethyl ether fraction; 3. Di chloro methane fraction; 4.Butanol fraction; 5.Chloroform fraction; 6.Ethyl acetate fraction; 7.Methanol fraction; 8. Standard (Vildagliptin & Meropenem)

Figure 3.1 : Naked eye view (G); Under UV light (H)

Rf value of methanol = 1.4 cm

Rf value of Ethyl Acetate = 3.7 cm

Rf value of Chloroform = 3.6 cm

Rf value of standard = 3.1 cm

Standard : Vildagliptin & Meropenem

5.4.4 Brine Shrimp lethality test

Table 2.3: This table shows the cytotoxic activity of different fractions of *Atrocarpus heterophyllus*

Sample	Concentration	No. of nauplii alive	% of nauplii alive
DCM	5	0	0
	2.5	0	0
	1.25	0	0
	0.625	1	14.28
	0.3125	3	42.85
	control	7	100
Di ethyl ether	5	0	0
	2.5	0	0

	1.25 0.625 0.3125 control	1 2 2 7	14.28 28.57 28.57 100
ethyl acetate	5 2.5 1.25 0.625 o.3125 control	0 0 0 0 0 7	0 0 0 0 0 100
butanol	5 2.5 1.25 0.625 0.3125 control	1 1 2 1 4 7	14.28 14.28 28.57 45.09 57.14 100

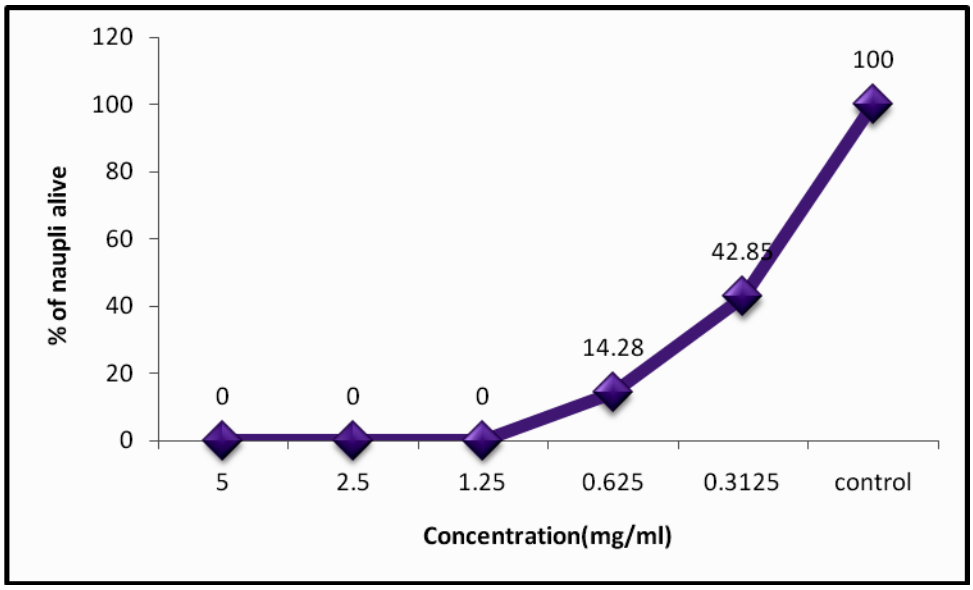


Figure 3.3 : Curve of Cytotoxic activity of DCM showing relationship between % of naupli alive and concetration

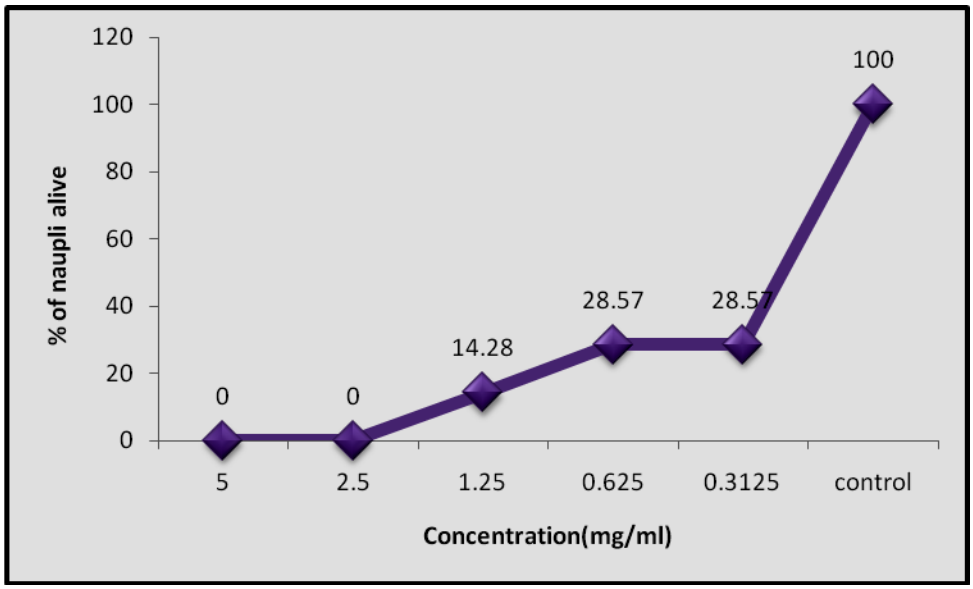


Figure 3.4 : Curve of Cytotoxic activity of Di ethyl ether showing relationship between % of naupli alive and concetration

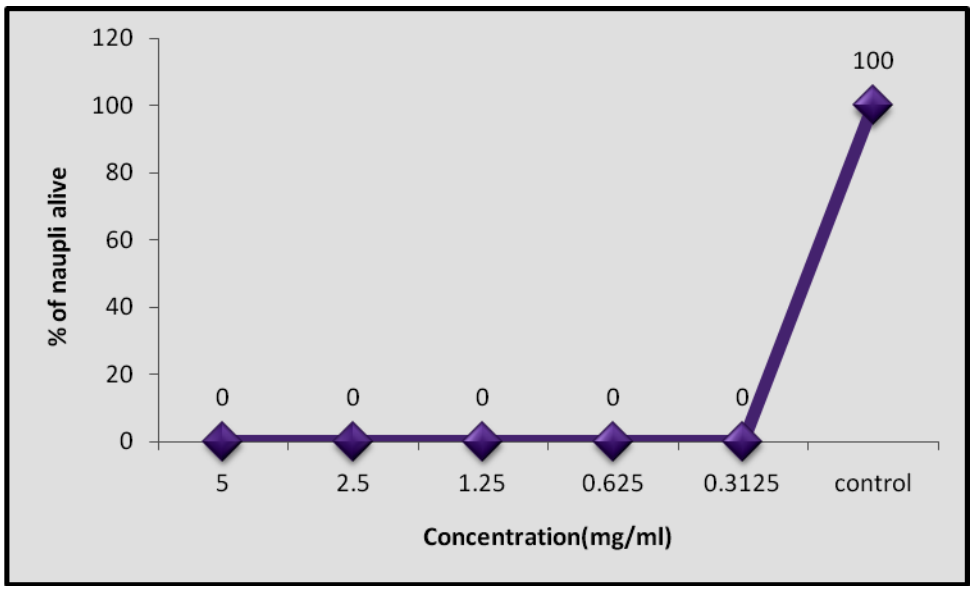


Figure 3.5 : Curve of Cytotoxic activity of Ethyl acetate showing relationship between % of naupli alive and concetration

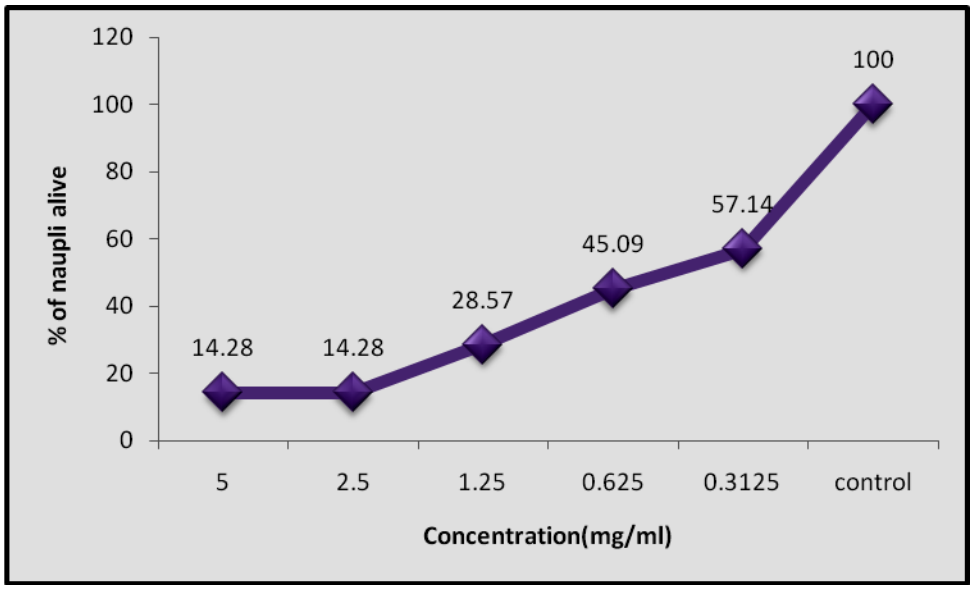


Figure 3.6 : Curve of Cytotoxic activity of Butanol showing relationship between % of naupli alive and concetration

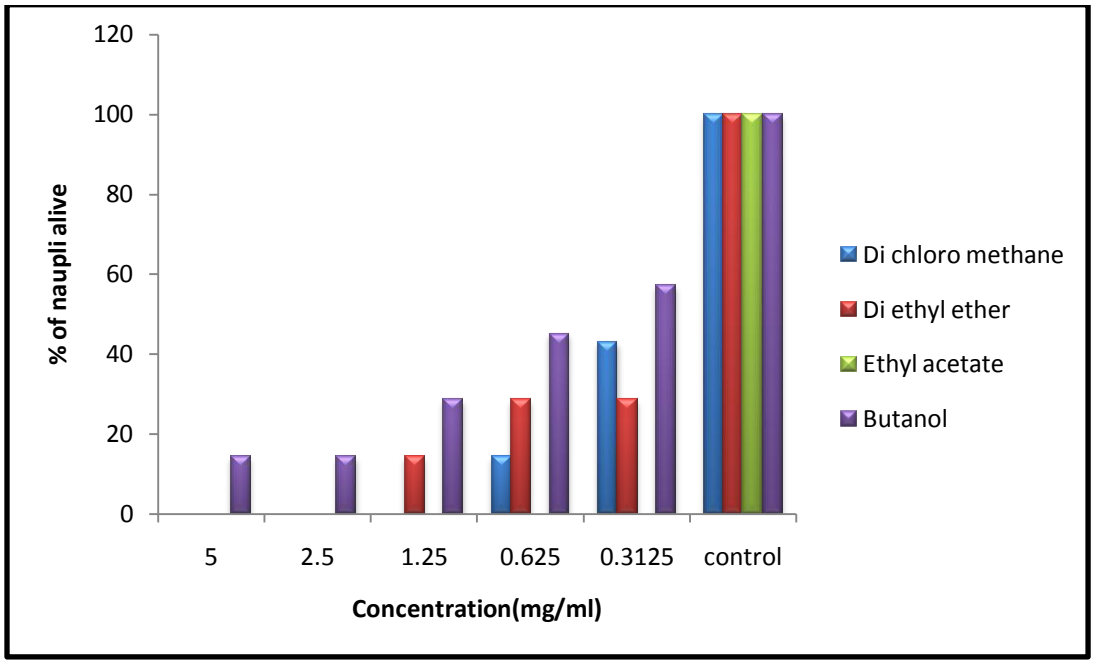


Figure 3.7 : Column diagram of comparison of cytotoxic activity of Di chloro methane; Di ethyl ether; Ethyl acetate and butanol extract.

From this graph, we can see that, ethyl acetate fraction was found to give most cytotxic activity(no naupli left alive in different concentration) and butanol fraction was found to give least cytotoxic activity(maximum number of nauple left alive in different concentration) among other fractions of jackfruit seeds. The cytotoxic activity showed by the methanolic extracts of Artocarpus heterophyllus seeds may be attributed due to the presence of their high antioxidant activity since antioxidants have a dual role in fighting cancer [],From this, it can be said that there is an association between the observed cytotoxic properties and degree of antioxidant activity for each fraction.

5.4.5 DPPH Test

The crude methanolic extract of *Artocarpus heterophyllus* (seeds) was subjected to free radical scavenging activity and it showed significant free radical scavenging activity.

Table 2.4: Determination of free radical scavenging capacity for the standard (ascorbic acid) 2

Name	Concentration (µg/µl)	Absorbance	% of inhibition	IC50 (mg/ml)
Ascorbic Acid (S1)	40	0.114	**45.71**	
Ascorbic Acid (S2)	80	0.108	**48.57**	**0.082**

Ascorbic Acid (S3)	120	0.103	**50.95**	
Ascorbic Acid (S4)	160	0.100	**52.39**	
Ascorbic Acid (S5)	**200**	**0.098**	**53.33**	

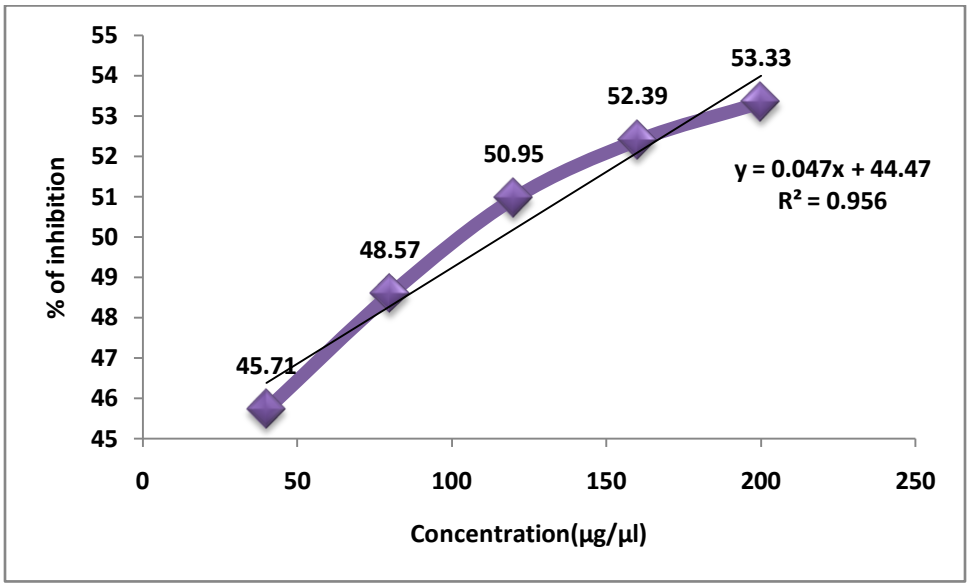


Figure 3.6: DPPH scavenging potential & IC50 value of ascorbic acid

Table 2.5: Determination of free radical scavenging capacity for *Artocarpus heterophyllus* crude methanolic extract

Name	Concentration (µg/µl)	Absorbance	% of inhibition
Butanol 10	5	0.115	**57.72**
Butanol 20	10	0.105	**62.5**
Butanol 30	15	0.103	**62.13**
Butanol 40	20	0.076	**72.06**
Butanol 50	25	0.008	**97.06**
Ethyl acetate 10	5	0.113	**58.45**
Ethyl acetate 20	10	0.112	**58.82**
Ethyl acetate 30	15	0.109	**59.65**
Ethyl acetate 40	20	0.106	**61.03**
Ethyl acetate 50	25	0.100	**63.23**
Dichloromethane 10	5	0.115	**57.73**
Dichloromethane 20	10	0.114	**58.08**
Dichloromethane 30	15	0.113	**58.83**
Dichloromethane	20	0.109	**59.93**

40			
Dichloromethane	**25**	0.102	**62.5**
50			

Name	IC 50 value(mg/ml)
1. Butanol	**0.00431**
2. Ethyl acetate	**0.00427**
3. Di chloro methane	**0.00433**

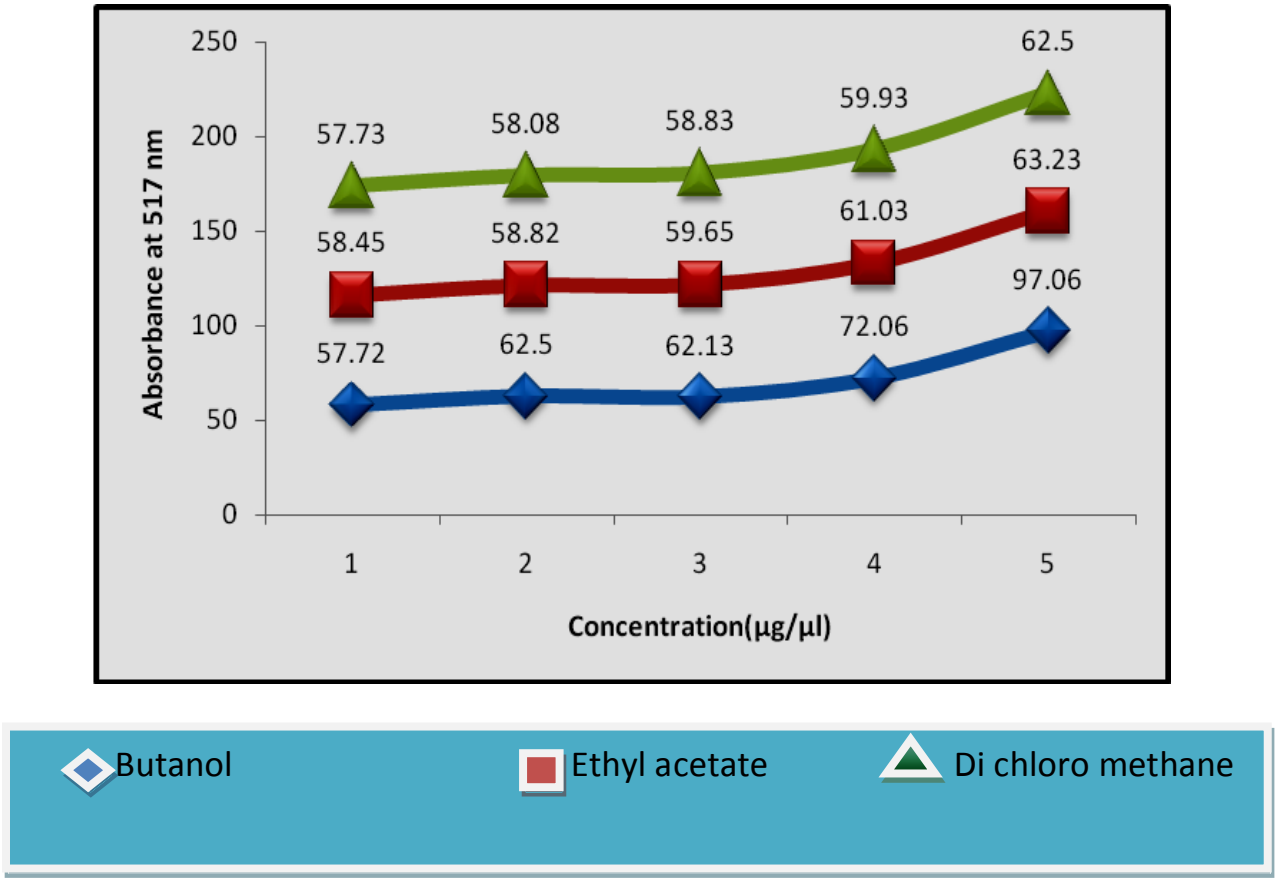


Figure 3.7 : DPPH scavenging potential of Butanol extract; Ethyl acetate extract & Di chloro methane extract

From the analysis, we found the scavenging effect in a concentration dependent manner. A higher DPPH radical-scavenging activity is associated with a lower IC50 value.The results show that the IC 50 of ascorbic acid is 0.082 mg/ml and seed methanolic extract is 0.00431(Butanol); 0.00427(Ethyl acetate) and 0.00433(Di chloro methane) respectively. These results show that the seeds of *Artocarpus heterophyllus* possess antioxidant free radical scavenging activity. This indicates that there are many compounds present in them that have antioxidant potential.DPPH is a very good free radical scavenger for other radicals; therefore,reduction of DPPH upon addition gives a very good measure of antioxidant activity.

5.4.6 Method development of Vildagliptin by HPLC

The method was statistically validated for its linearity, precision, accuracy, stability, specificity, LOD and LOQ. Due to its simplicity, rapidness, high precision and accuracy,the proposed RP-HPLC method may be used for determining vildagliptin in pure form and in tablet formulation.In this method we can separate our compound present in the crude methanolic extract of Seed of *Artocarpus heterophyllus*.

5.4.7 Calculation of active materials

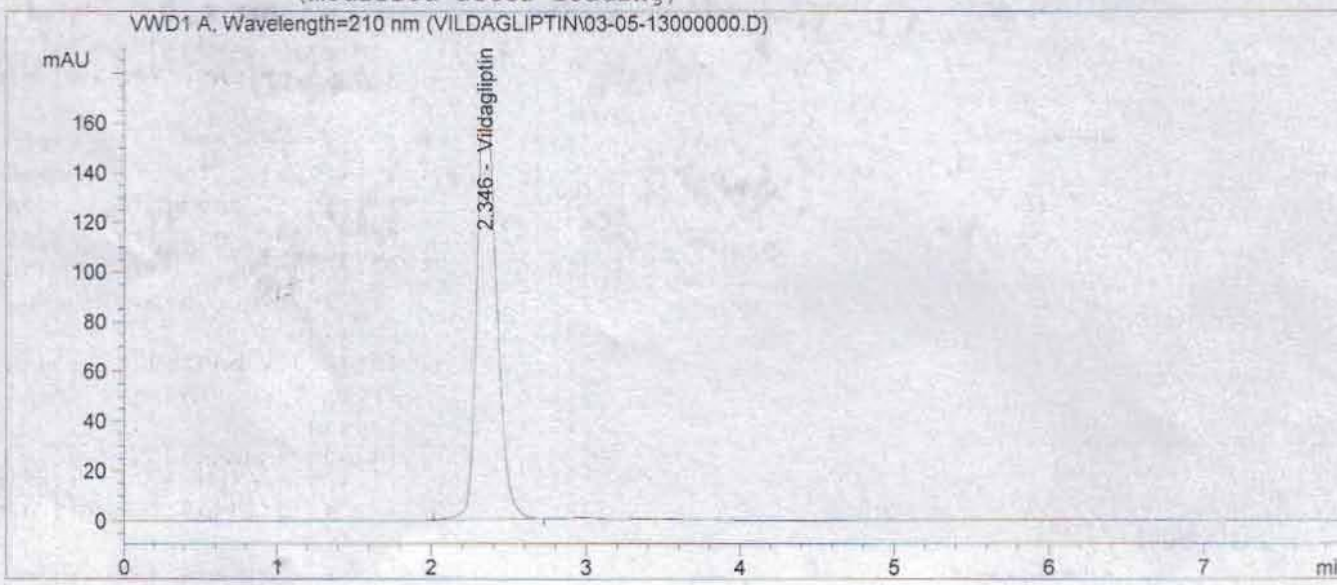
$$\frac{\text{Peak area of sample}}{\text{Peak area of standard}} \text{ X standard concentration X dilution factor}$$

$$= \frac{1268.45239}{1519.58557} \text{ X } 0.1 \text{ X } 10.21 = 0.852\ \%$$

The Chromatogram of Vildagliptin and the sample of methanolic extract of seed of *Artocarpus heterophyllus* are given in below:

Data File C:\CHEM32\1\DATA\VILDAGLIPTIN\03-05-13000000.D
Sample Name: Vildagliptin Std

```
==========================================================================
Acq. Operator    : Iffat Ara Zabeen
Acq. Instrument  : Instrument 1                    Location : Vial 1
Injection Date   : 5/3/2013 10:46:42 AM
Acq. Method      : C:\CHEM32\1\METHODS\VILDAGLIPTIN.M
Last changed     : 5/3/2013 9:59:35 AM by Iffat Ara Zabeen
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CALCIROL.M
Last changed     : 5/6/2013 1:06:55 PM by Nurun Naher
                   (modified after loading)
```




```
==========================================================================
                      External Standard Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      5/6/2013 1:06:53 PM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     20.00000  [ng/µl]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=210 nm

RetTime  Type     Area      Amt/Area    Amount   Grp   Name
 [min]          mAU   *s                [ng/µl]
-------|------|----------|----------|----------|--|------------------
  2.346 BB     1519.58557 6.58074e-3   10.00000    Vildagliptin

Totals :                                10.00000


1 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
```

```
:\CHEM32\1\DATA\VILDAGLIPTIN\03-05-13000006.D
me: Vildagliptin MP

================================================================================
cq. Operator    : Iffat Ara Zabeen
Acq. Instrument : Instrument 1                    Location : Vial 1
Injection Date  : 5/3/2013 11:30:26 AM
Acq. Method     : C:\CHEM32\1\METHODS\VILDAGLIPTIN.M
Last changed    : 5/3/2013 11:29:52 AM by Iffat Ara Zabeen
                  (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\CALCIROL.M
Last changed    : 5/6/2013 1:05:55 PM by Nurun Naher
                  (modified after loading)
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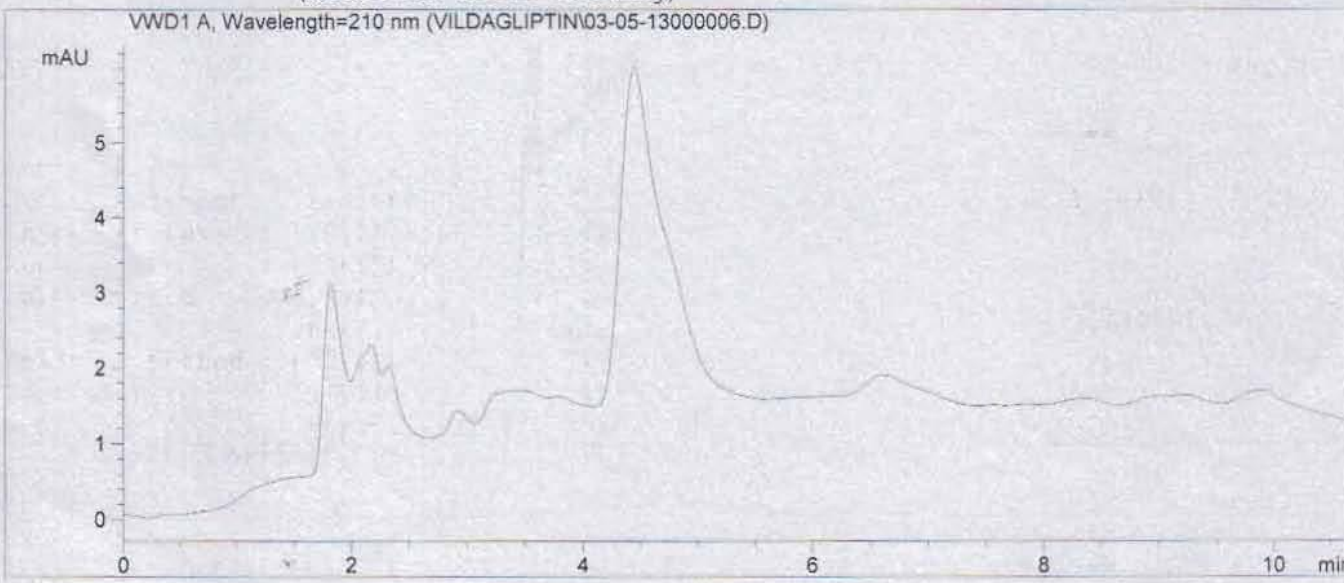



```
================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     20.00000  [ng/µl]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
================================================================================
================================================================================
                          Area Percent Report
================================================================================

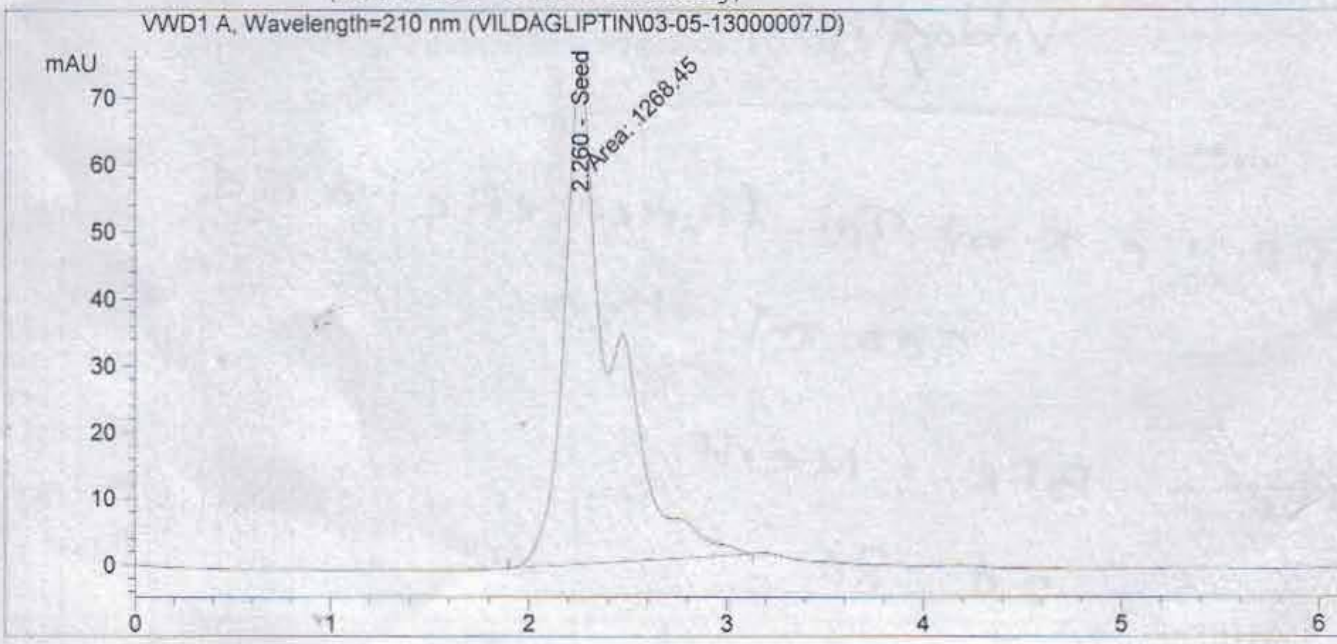
Sorted By             :      Signal
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     20.00000  [ng/µl]    (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

No peaks found

================================================================================
                          *** End of Report ***
```

```
C:\CHEM32\1\DATA\VILDAGLIPTIN\03-05-13000007.D
Name: Vildagliptin Seed

Acq. Operator    : Iffat Ara Zabeen
Acq. Instrument  : Instrument 1                    Location : Vial 1
Injection Date   : 5/3/2013 11:41:49 AM
Acq. Method      : C:\CHEM32\1\METHODS\VILDAGLIPTIN.M
Last changed     : 5/3/2013 11:41:11 AM by Iffat Ara Zabeen
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\CALCIROL.M
Last changed     : 5/6/2013 11:28:28 AM by Nurun Naher
                   (modified after loading)
```




```
                    External Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      5/6/2013 11:28:26 AM
Multiplier:                   :      1.0000
Dilution:                     :      1.0000
Sample Amount:                :     20.00000  [ng/µl]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=210 nm

RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          mAU   *s                [ng/µl]
-------|------|----------|----------|----------|--|-----------------
  2.260 MM     1268.45239 9.35380e-3   11.86485     Seed

Totals :                                11.86485
```

Figure 3.8 : HPLC Chromatogram of Vildagliptin and the sample of methanolic extract of seed of *Artocarpus heterophyllus* at 210 nm

Both Vildagliptin & Artocarpus heterophyllus showed different constituents at two retention time(2.346 & 2.260 min) respectively. These two peaks represent the main constituents present in them. Their retention time is almost similar and it proves that there is a characteristic similarity present between them.So seeds of Artocarpus heterophyllus can be used as an effective tool to treat diabetic and further investigation is needed to ensure it.

HPLC is a sensitive and accurate tool that widely used for the quality assessment of plant extract and its derived product.This method can be coupled to NMR analyses in order to completely identify the compounds detected and get some insight into their structure.

CHAPTER FIVE CONCLUSION

5.4.8 CONCLUSION

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines.

Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectancy.

Crude methanolic extract of *Artocarpus heterophyllus* seeds of the family *Moraceae* is traditionally used in various disease conditions. Such as osteoporosis; diabetic mellitus. Also used for thyroid disorder, anemia, gonorrhea, dysuria, worms in children, hepatic and intestinal colic, and for gastric catarrh. In my experiment it shows very positive result for Anti-oxidant activity, Anti-diabetic activity, cytotxic activity. Moderate antimicrobial activity.There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.[32]

CHAPTER SIX REFERENCE

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