"Cytotoxic and Antimicrobial Activity of *Argemone mexicana*"

Submitted By

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In Partial Fulfillment of the Requirements for the Award of the Degree

Bachelor of Pharmacy

Under the Guidance of

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Cytotoxic and Antimicrobial Activity of *Argemone mexicana*"** is an authentic and genuine research work carried out by me under the guidance of **Mr. Apurba Sarker Apu,** Senior lecturer, Department of Pharmacy, East West University, Dhaka, Bangladesh.

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ENDORSEMENT BY HEAD OF THE DEPARTMENT

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THIS RESEARCH PAPER IS

DEDICATED

TO

MY MOTHER, SISTERS,

BROTHER IN LAWS, NIECES

&

MY BELOVED

S. SHEFAUL ISLAM

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ABSTRACT

Argemone mexicana belonging to the family of Papaveraceae has been studied in this research work. The work was carried out for evaluation of biological activities of methanol, ethyl acetate and n-hexane crude extracts of this plant with special emphasis on cytotoxicity, and antimicrobial activity. In the brine shrimp lethality bioassay, the cytotoxicity exhibited by methanolic extract was promising. On the other hand, ethyl acetate and n-hexane demonstrated moderate cytotoxic activity, in comparison to positive control ($KMnO_4$). Against the test micro-organisms the zone of inhibition produced by methanolic, ethyl acetate and n-hexane crude extract of *Argemone mexicana* was partially active and inactive with different concentrations.

Keywords: *Argemone mexicana*, crude extracts, brine shrimp, LC_{50}, zone of inhibition.

CHAPTER 1:

INTRODUCTION

1. Introduction

1.1 *Argemone mexicana* :

Argemone mexicana (family - Papaveraceae) known as Ghamoya is an indigenous herb found in India. It is a commonly occurring weed present in many regions of the country, being presence of many noxious constituents it is composition of many traditional remedies Ghamoya (Bangla-shialkanta) has occupied a pivotal position in Indian culture and folk medicine. It has been used in all most all the traditional system of medicine, such in ayurveda, unani and sidha. The wide therapeutic application of Weed can made researcher to study this plant in details [1,2].

In India it is introducd and naturalised and occur as wasteland weed in almost every part of India. The genus Argemone includes 12 species. Some major species are: *A. alba* (used medicinally in North America), *A. platyceras*, *A. grandiflora*[2].

1.1.1 Common names [1, 2]:

Bangla name: Shialkanta

English name: Mexican prickly poppy

Hindi: Shialkanta, Satyanashi

Gujrati: Darudi

Danarese: Balurakkisa, Datturi, Pirangi, datturi

Marathi: Daruri, Firangi-kote-pavola, dhotara.

Sanskrit: Brahmadandi, Pitopushpa, Srigalkanta, Svarnakshiri.

Malyalam: Ponnummattu, Kantankattiri

Tamil: Kutiyotti, Ponnummuttai

Telugu: Brahmadandicettu

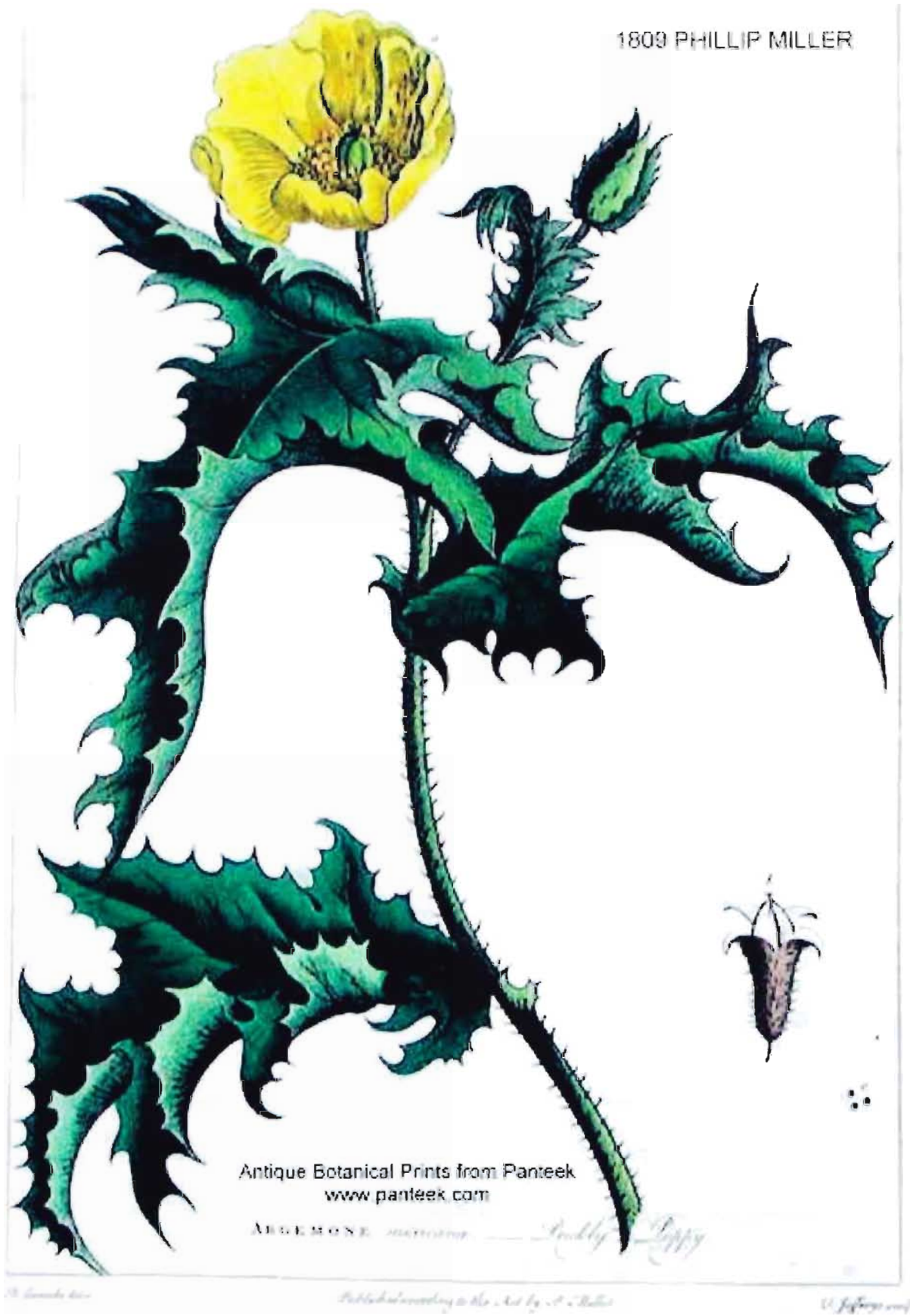

Figure1.1: An antique botanical prints of *Argemone mexicana*

1.1.2 Scientific classification[3]:

Argemone mexicana L.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Magnoliidae*
Order	*Papaverales*
Family	*Papaveraceae* – Poppy family
Genus	*Argemone* L. – pricklypoppy
Species	*Argemone mexicana* L. – Mexican pricklypoppy

1.1.3 Plant description:

It is a prickly, glabrous, branching herb with yellow juice and showy yellow flowers, The Sanskrit name svarnakshiri is given because of the yellow juice (Svarna - Gold; Kshiri - Juice). The height of this plant varies between 0.3 to 0.12 meters, Leaves are thistle like. Stem clasping, Oblong, sinuately pinnatifid, spinous and viens are white. Flowers are terminal, yellow and of 2.5–5.0 cm diameter. Fruits are capsule. Seeds numerous, globosely, netted and brownish black. Flowering time is all round the year in

Indian conditions. Stems 2.5-10 dm long, branched, sparsely to moderately cover with prickles. Leaves glaucous, oblong-oblanceolate, pinnately lobed, 1/2-3/4 to midrib, both surfaces sparsely covered with prickles .Buds subglobose, 1.2-1.6 cm long, sparsely prickly; petals bright yellow, 1.7-3 cm long; Capsules oblong to broadly ellipsoid, 3-4.2 cm long, each valve with 9-15 prickles, the longest one 7-10 mm long. Seeds numerous, 1.2-1.5 mm in diameter [1,2].

Figure1.2: Different parts of *Argemone mexicana*

1.1.4 Growth and development:

The plant is self-fertile. The plant prefers light (sandy) soils, requires well-drained soil and can grow in nutritionally poor soil. The plant also prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires dry or moist soil and can tolerate drought[1].

In the tropics, *Argemone mexicana* flowers and fruits throughout the year. The flowers open early in the morning, and last for 2–3 days. Small stingless bees are the main pollinators, but *Argemone mexicana* is predominantly self-pollinated. Most seeds fall around the base of the parent plant where they may form a carpet of seedlings. The seed is light, has a waxy coat and is pitted, and may be dispersed by wind and water and is known to spread quickly in irrigation schemes. Dispersal also occurs by soil adhering to farm machinery and by man and livestock. Seeds can remain dormant for many years[4].

1.1.5 Properties of *Argemone mexicana*[2,4]:

Argemone mexicana contains numerous isoquinoline alkaloids of the protoberberine type and related types, including sanguinarine. The total alkaloid fraction in the dried roots and stems is 0.25%, mainly consisting of protopine and berberine. The alkaloid 6-acetonyldihydrochelerythrine has recently been isolated from whole plant extracts and was found to have significant anti-HIV activity.

The alkaloids berberine, protopine, protopine hydrochloride, sanguinarine and dihydrosanguinarine have been isolated from the seeds. Protopine is considered a narcotic and it reduces morphine-withdrawal effects significantly. Protopine and sanguinarine showed molluscicidal properties against *Lymnaea acuminata* and *Biomphalaria glabrata*. Berberine has improving effects on the circulation in small doses and also has hallucinogenic properties. An overdose, however, produces death by

paralysis of the central nervous system. Other pharmacological effects of berberine include spasmolytic, antibacterial and to some degree antifungal and antiprotozoal activities. Most berberine is formed in the flowers. The alkaloid fraction from the roots showed anti-inflammatory activity in rabbits and rats. Leaf extracts showed in-vitro anti-plasmodial activity.

The seeds of *Argemone mexicana* contain 35–40% of an orange-yellow oil which consists mainly of linoleic acid (54–61%) and oleic acid (21–33%). It also contains poisonous sanguinarine in concentrations as high as 10 g/l. Accidental mixing of *Argemone mexicana* seed with grain and oil seeds has caused deaths in several countries, including South Africa. The seed oil has a significant nematicidal effect on larvae of the genus *Meloidogyne*. An aqueous mixture of the oil (0.2%) applied to the soil of okra (*Abelmoschus esculentus* (L.) Moench) significantly reduced nematode infection and nematode concentrations in roots and soil, thereby increasing okra growth. When sprayed on the leaves the effect was even more striking, showing the systemic effect of the spray.

Leaf extracts show antifeedant activity against insects, including the large cabbage-heart caterpillar (*Crocidolomia binotalis*), the cluster caterpillar (*Spodoptera litura*), the cotton aphid (*Aphis gossypii*) and also larvae of the southern house mosquito (*Culex quinquefasciatus*). Dried plant extracts significantly reduced nematode damage on seedlings of tomato and eggplant. Tomatoes treated with a leaf extract showed significantly less fruit rot caused by *Aspergillus niger*. A flower extract induced a high level of resistance to tomato virus × in *Chenopodium album* L. Extracts also showed antibacterial activity in vitro against *Bacillus subtilis*, *Escherichia coli* and *Streptococcus faecalis*.

Aqueous leaf and flower extracts inhibit the germination and growth of many cultivated crops, such as tomato, cucumber, mustard, radish and pearl millet. Allelopathic effects of the residues on Bambara groundnut and sorghum have been observed in the field.

1.1.6 Medicinal uses:

According to Ayurveda the plant is diuretic. purgative and destroys worms. It cures lepsory, skin-diseases, inflammations and bilious fevers. Roots are anthelmintic. Juice is used to cure ophthalmia and opacity of cornea. Seeds are purgative and sedative. Seeds resemble mustard seeds and in India it is used to adulterate mustard seed. Seed yield non edible toxic oil and causes lethal dropsy when used with mustard oil for cooking.

In Homoeopathic system of medicine, the drug prepared from this herb is used to treat the problem caused by tape-worm [1].

The whole plant is analgesic, antispasmodic, possibly hallucinogenic and sedative. The fresh yellow, milky, acrid sap contains protein-dissolving substances and has been used in the treatment of warts, cold sores, cutaneous affections, skin diseases, itches etc. The root is alterative and has been used in the treatment of chronic skin diseases. The flowers are expectorant and have been used in the treatment of coughs. The seed is demulcent, emetic, expectorant and laxative. An infusion, in small quantities, is used as a sedative for children, but caution is advised since the oil in the seed is strongly purgative. The seed has also been used as an antidote to snake poisoning. The oil from the seed is purgative. It has been used in the treatment of skin problems [1,2].

Table1.1: Different parts of *Argemone mexicana* in medicinal uses.

Plant parts	Medicinal uses
All parts	Dropsy, swellings, oedema, gout; generall healing; kidneys, diuretics; liver, etc.; pain killers; sedatives, etc.
Leaf	Pulmonary troubles
Root	Abortifacients, ecbolics; eye treatments; tumours, cancers
Sap	Ear treatments
Sap, root	Cutaneous, subcutaneous parasitic infection
Seed	Diarrhoea, dysentery; emetics; laxatives, etc.
Latex	Applied externally to treat Eczema

1.2 Brine Shrimp Lethality Test:

Over the last decade, interest in drugs of plant origin has been growing steadily. The study of bioactive compounds from plant sources and extracts in the chemical laboratory is often hampered by the lack of a suitable, simple, and rapid screening procedure. There are, of course, many procedures for bioassay, but unless collaborative programs with biologists or pharmacologists are in place, the typical chemical laboratory is not suitably equipped to perform the usual bioassays with whole animals or isolated tissues and organs, as well aseptic techniques [5].

When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the

investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds [6].

The *in vivo* lethality in a simple zoological organism, such as the brine shrimp lethality test (BST), developed for Meyer *et al.* [7], might be used as a simple tool to guide screening and fractionation of physiologically active plant extracts, where one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. This general bioassay detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply pharmacology at a higher dose, thus if we find toxic compounds, a lower, non-toxic, dose might elicit a useful, pharmacological, perturbation on a physiologic system [19]. However, it has been demonstrated that BST correlates reasonably well with cytotoxic and other biological properties [8]. Brine shrimp have been previously utilized in various bioassay systems. There have been many reports on the use of this animal for environmental studies [9-11], screening for natural toxins [12, 13] and as a general screening for bioactive substances in plant extracts [6].

1.3 Antimicrobial susceptibility test[14]:

The disc diffusion method of is the most practical method and is still the method of choice for the average laboratory. Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	**Dilution**
Stokes method	Minimum Inhibitory Concentration
Kirby-Bauer method	i) Broth & ii) Agar Dilution

1.3.1 The Kirby-Bauer test:

The Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what treatment of antibiotics should be used when treating an infection. This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic, is placed on the medium. The organism will grow on the agar plate while the antibiotic "works" to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the National Committee for Clinical Laboratory Studies (NCCLS). Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S) [14-16].

1.3.2 Useful considerations for Kirby-Bauer testing [14-16]:

The culture used in this test has to be the Mueller-Hinton agar because it is an agar that is thoroughly tested for its composition and its pH level. Also, using this agar ensures that zones of inhibitions can be reproduced from the same organism, and this agar does not inhibit sulphonamides. The agar itself must also only be 4mm deep. This further ensures standardization and reproducibility.

Factors that influence zones of inhibition:

- Concentration of bacteria spread onto agar plate

- Pathogen susceptibility
- Agar depth
- Growth temperature
- Nutrient availability

Factors that influence the diffusion of the antibiotic:

- Concentration of crude extract/antibiotic
- Molecular weight of extract/antibiotic
- Water solubility of extract/antibiotic
- pH and ionization
- Binding to agar

The size of the inoculated organism must also be standardized (using barium sulfate standards, McFarland standards). The reasons are because if the size of the inoculum is too small, the zone of inhibition will be larger than what it is supposed to be ("the antibiotics will have a distinct advantage") and if the inoculums is too large, the zone of inhibition will be smaller [14-16].

CHAPTER 2:
LITERATURE REVIEW

2.1 Phytochemistry of *Argemone mexicana* Linn.

The research works or phytochemical studies on *Argemone mexicana* are very rich. There are a lot of studies that are related to *Argemone mexicana*. Some of them are listed below-

In 1981 some researchers of the Department of Chemistry, University of Delhi (India) were isolate Phenolics from the seeds of *Argemone mexicana*. Two new phenolic compounds, 5, 7, 2', 6'-tetrahydroxyflavone and 5, 7-dihydroxychromone 7- neohesperidoside have been characterized from the seeds of *Argemone mexicana* [17].

In 1983 Jeffrey and Christine, the researchers of Phytochemical Unit, Plant Science Laboratories (U.K.) were isolated Flavonoids from the seeds of *Argemone mexicana*. According to that study, Re-examination of the seed extract of *Argemone mexicana* for the newly reported 5,7,2',6'-tetrahydroxy-flavone failed to indicate the presence of any novel flavones. The major seed flavone is, in fact, luteolin and this is accompanied by the related flavanone, eriodictyol. The danger of relying entirely on spectral measurements for identifying new flavonoids is highlighted by these findings [18].

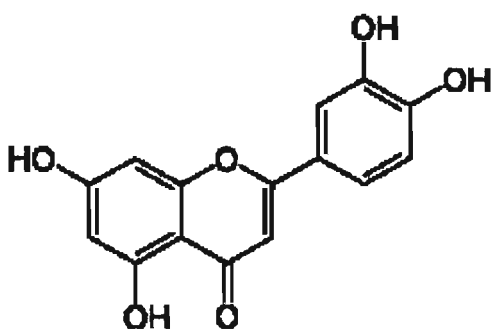

Figure2.1: Structure of luteolin

In 1998 Mr. Naresh K. Sangwan and Mr. Mangel S. Malik were isolated a long chain alcohol from *Argemone mexicana.* They Were worked under the Department of Chemistry and Biochemistry, CCS Haryana Agricultural University (Hisar, India). An extract of aerial parts of *Argemone mexicana* afforded a new monohydric alcohol, triacontan-11-ol (**1**), in addition to a known dihydric alcohol, triacontane-6,11-diol. The structures were elucidated on the basis of spectral, analytical and degradative experiments [19].

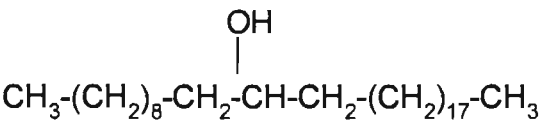

Figure2.2: Structure of triacontan-11-ol

In 2003 some researchers of Department of Chemical Engineering, Kao Yuan Institute of Technology (Kaohsiung, Taiwan) were isolated two new protopine-type alkaloids, argemexicaine A (1) and argemexicaine B (2), along with thirteen known alkaloids from MeOH extracts of Formosan *Argemone mexicana* L. (Papaveraceae). Physical and spectral analyses, particularly IR and thermo-modulated 1D and 2D NMR, were used to determine the transannular conformations of the isolated protopine-type alkaloids. The known benzo[c]phenanthridine (+/-)-6-acetonyldihydrochelerythrine (5) exhibited significant anti-HIV activity in H9 lymphocytes with EC50 and TI (Therapeutic Index) values of 1.77 microg/mL and 14.6, respectively[20].

In 2010 some researchers of Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India were isolated four quaternary isoquinoline alkaloids, dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine, from the whole plant of *Argemone mexicana* Linn. (Papaveraceae) and their structures established by spectral evidence. This is the first report of these alkaloids (dehydrocorydalmine, jatrorrhizine, columbamine, and oxyberberine) from *Argemone mexicana* and the Argemone genus[21]

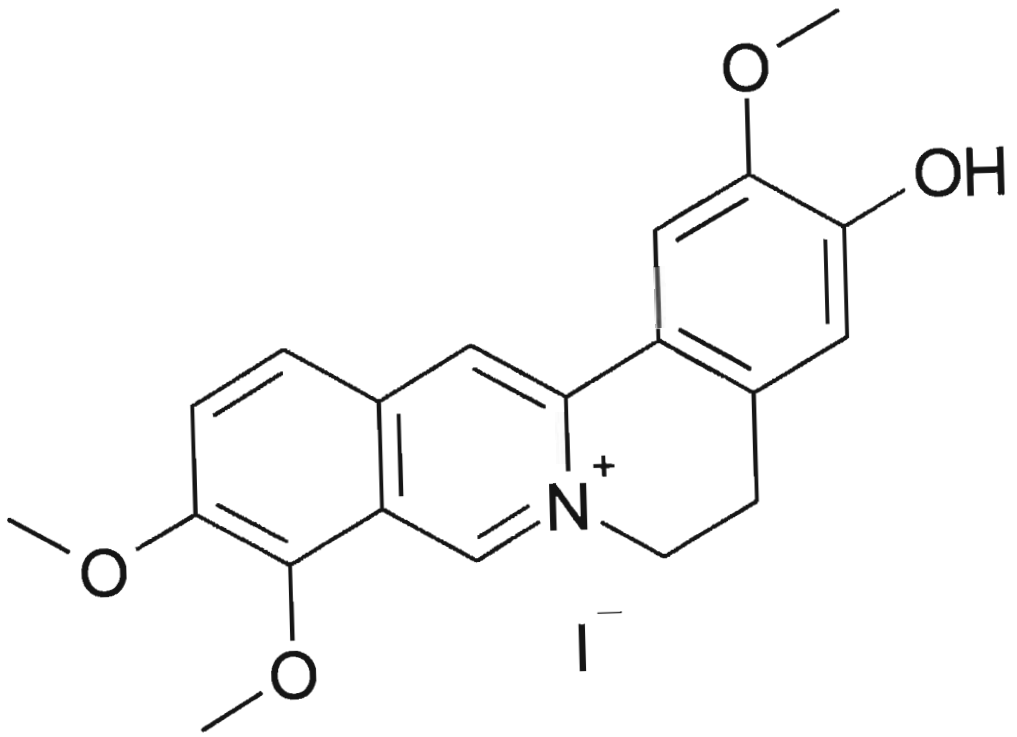

Figure2.3: Structure of jatrorrhizine

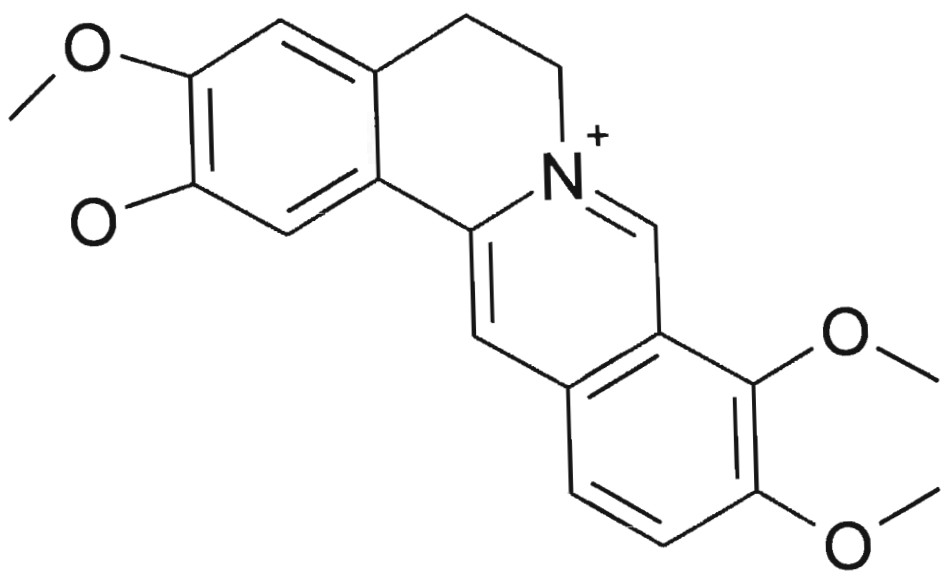

Figure2.4: Structure of columbamine

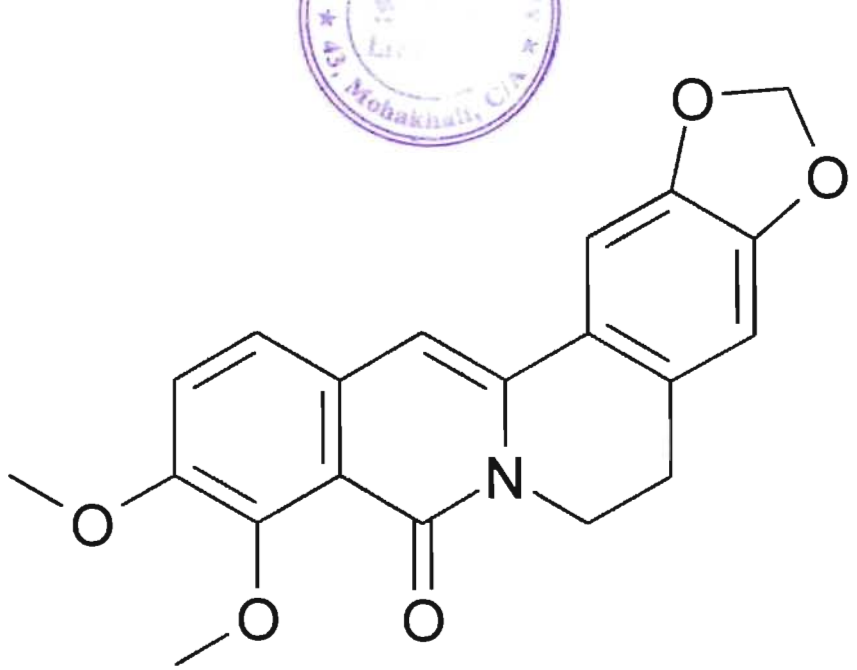

Figure2.5: Structure of oxyberberine

In the same year researchers of two different countries (USA & Germany) were characterized two methylenedioxy bridge-forming cytochrome P450-dependent enzymes of alkaloid formation in the Mexican prickly poppy *Argemone mexicana.* Formation of the methylenedioxy bridge is an integral step in the biosynthesis of benzo[*c*]phenanthridine and protoberberine alkaloids in the Papaveraceae family of plants. This reaction in plants is catalyzed by cytochrome P450-dependent enzymes. Two cDNAs that encode cytochrome P450 enzymes belonging to the CYP719 family were identified upon interrogation of an EST dataset prepared from 2-month-old plantlets of the Mexican prickly poppy *Argemone mexicana* that accumulated the benzo[*c*]phenanthridine alkaloid sanguinarine and the protoberberine alkaloid berberine. CYP719A13 and CYP719A14 are 58% identical to each other and 77% and 60% identical, respectively, to stylopine synthase CYP719A2 of benzo[*c*]phenanthridine biosynthesis in *Eschscholzia californica*. Functional heterologous expression of *CYP719A14* and *CYP719A13* in *Spodoptera frugiperda* Sf9 cells produced recombinant enzymes that catalyzed the formation of the methylenedioxy bridge of (*S*)-

cheilanthifoline from (*S*)-scoulerine and of (*S*)-stylopine from (*S*)-cheilanthifoline, respectively. Twenty-seven potential substrates were tested with each enzyme. Whereas CYP719A14 transformed only (*S*)-scoulerine to (*S*)-cheilanthifolin, CYP719A13 converted (*S*)-tetrahydrocolumbamine to (*S*)-canadine, (*S*)-cheilanthifoline to (*S*)-stylopine and (*S*)-scoulerine to (*S*)-nandinine. These results indicate that although CYP719A14 participates in only sanguinarine biosynthesis, CYP719A13 can be involved in both sanguinarine *and* berberine formation in *A. mexicana*[22].

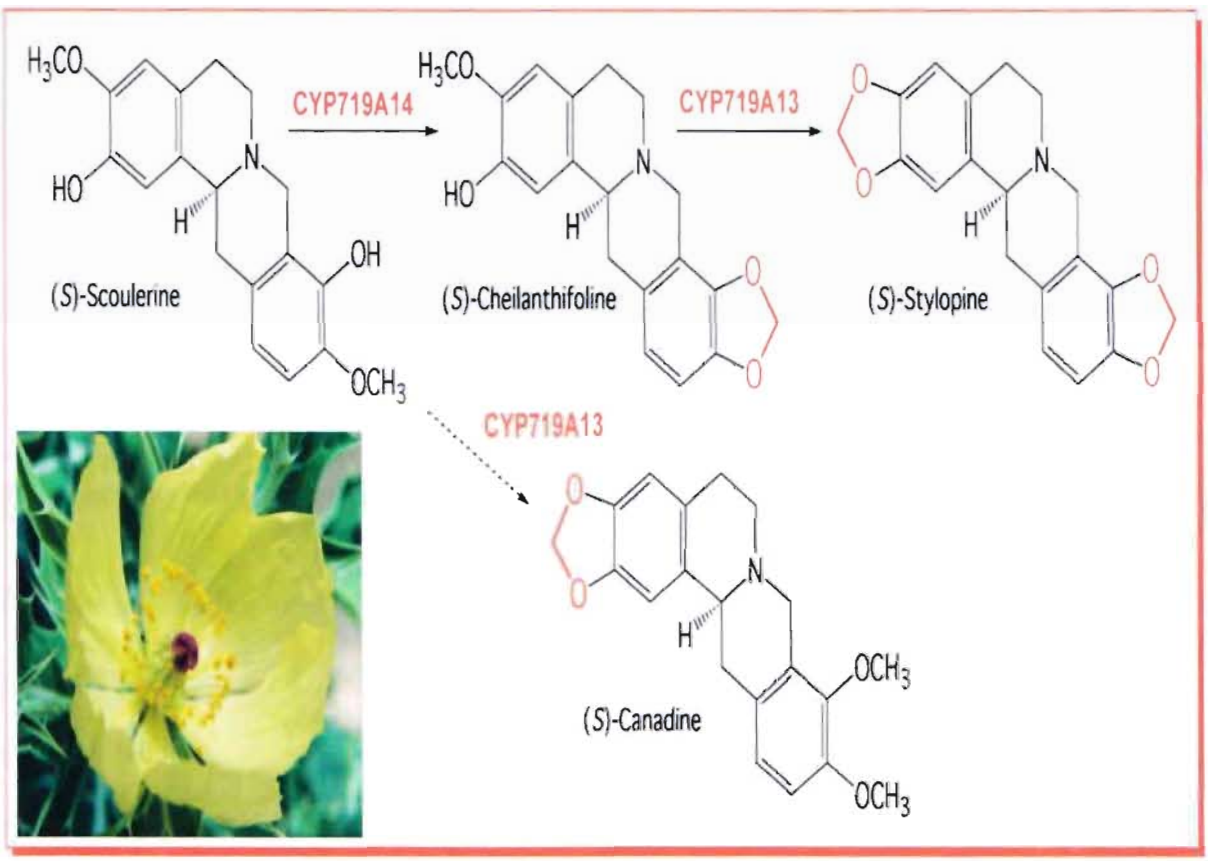

Figure2.6: Graphical view of CYP719A13 participates in both sanguinarine and berberine biosynthesis. Prepared from 2-month-old plantlets of the Mexican prickly poppy *Argemone Mexicana*.

Table2.1: Summary of the Phytochemical Studies on *Argemone mexicana*

Year	Author(S) /Researcher(S)	Parts of The Plants	Compound(S)
1981	D.K. Bhardwaj, M.S. Bisht, R.K. Jain and Anita Munjal	Seeds	Two new phenolic compounds
1983	Harborne B. H. & Williams A. C	Seeds	Flavonoids
1998	Sangwan K. N. & Malik S. M.	Aerial Part	Long Chain Alcohol
2003	Chang YC, Hsieh PW, Chang FR, Wu RR, Liaw CC, Lee KH & Wu YC		Protopines
2010	Singh S, Singh TD, Singh VP, Pandey VB	Whole Plant	Isoquinoline Alkaloids
2010	Maria Luisa Díaz Chávez, Megan R., Andreas G. and Toni M. K.	2-month-old plantlet	benzo[*c*]phenanthridine alkaloid

2.2 Pharmacological studies on *Argemone mexican*:

The research works or Pharmacological findings on *Argemone mexicana* are extremely well-off. There are a lot of studies that are associated to *Argemone mexicana*. Some of them are listed below-

In 1985 Upreti KK, Das M and Khanna SK.(Dyes and Food Adulterant Toxicology Laboratory, Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow, India) reported four cases manifesting features characteristic of epidemic dropsy following body massage with contaminated mustard oil. A transcutaneous route of absorption for the toxin (sanguinarine) resulting in epidemic dropsy has not been documented previously in man. Oil used for body massage was found to be adulterated with Argemone mexicana oil,

while hydrogenated vegetable fat used for cooking did not reveal any contamination. Diagnosis of the disease was confirmed by establishing the presence of sanguinarine in the urine and serum of all four cases [23].

In 1988, some researchers from the same laboratory reported a Consumption of edible oils contaminated with Argemone mexicana seed oil causes various toxic manifestations. In this investigation the in vivo effect of argemone oil on NADPH-dependent enzymatic and Fe^{2+}-, Fe^{2+}/ADP- or ascorbic acid-dependent non-enzymatic hepato-subcellular lipid peroxidation was studied. Parenteral administration of argemone oil (5 ml/kg body weight) daily for 3 days produced a significant increase in both non-enzymatic and NADPH-supported enzymatic lipid peroxidation in whole homogenate, mitochondria, and microsomes. Lipid peroxidation aided by various pro-oxidants, namely Fe^{2+}, Fe^{2+}/ADP and ascorbic acid also revealed a significant enhancement in the whole homogenate, mitochondria and microsomes of argemone oil-treated rats. Further, when compared with whole homogenate, the hepatic mitochondria and microsomes of either control or argemone oil-treated rats showed a 4- and 6-fold increase in non-enzymatic, and a 5- and 18-fold increase in NADPH-dependent enzymatic lipid peroxidation, respectively. Similarly, both mitochondrial and microsomal fractions showed a 5- and 7-fold increase in Fe^{2+}-, and a 12- and 15-fold increase in either Fe^{2+}/ADP- or ascorbic acid-aided lipid peroxidation, respectively. These results suggest that the hepatic microsomal as well as the mitochondrial membrane is vulnerable to the peroxidative attack of argemone oil and may be instrumental in leading to the hepatotoxicity symptoms noted in argemone poisoning victims[24].

In 2001 researchers group of the Department of Forensic Medicine and Toxicology, University College of Medical Sciences & G.T.B. Hospital (Delhi, India) were found the *Argemone mexicana* poisoning during doing autopsy of two patients. Epidemic dropsy, a

disease due to *Argemone mexicana* poisoning, is characterized by pathological accumulation of diluted lymph in body tissues and cavities. Recently, the largest epidemic of the disease in India affected Delhi and its neighboring states during the months of August–September 1998. Over 3000 persons fell ill, and more than 65 died in the state of Delhi alone. Two cases belonging to the same family died, out of the large number of cases admitted in this tertiary care teaching hospital situated in eastern part of Delhi. Autopsy findings of these two cases are presented and discussed here along with the review of toxicity due to this poisoning[25].

In 2003 M. Sakthivadivel and D. Thilagavathy, the researchers of M.S. Swaminathan, Research Foundation & Gandhigram Rural Institute (Tamil Nadu, India) find out the Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L. seed. This activity occurred at higher concentrations (200, 100, 50 and 25 ppm). Chemosterilant activity, including reduction in blood meal utilization (27.70%), reduction in fecundity (19.00%), formation of larval–pupal intermediates, formation of pupal–adult intermediates, adult mortality and sterility of first generation eggs (100%), occurred at low concentration (10 ppm)[26].

In 2004 some research group from Department of Biochemistry and Molecular Biology, University of Ferrara, Ferrara, Italy, work on the Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene in which *A. mexicana* was also included. In this study the determination the activity of extracts from Bangladeshi medicinal plants (Emblica officinalis, Aegle marmelos, Vernonia anthelmintica, Oroxylum indicum, Argemone mexicana) on human breast tumor cell lines. Extracts from E. officinalis and O. indicum displayed anti-proliferative activity on MCF7 and MDA-MB-231 breast cancer cell lines, while extracts from A. mexicana were active on MCF7 cells, exhibiting on the contrary low

antiproliferative effects on MDA-MB-231 cells. Extracts from A. marmelos and V. anthelmintica were antiproliferative on both cell lines, but at higher concentrations. The accumulation of estrogen receptor alpha (ERalpha) mRNA, a marker of neoplastic status, was analysed by quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). The data obtained demonstrated that only extracts from E. officinalis induce an increase of ERalpha mRNA in MCF7 cells. When MDA-MB-231 cell line was employed, extracts from E. officinalis, V. anthelmintica and A. mexicana were found to be inducers of the increase of ERalpha mRNA accumulation. Since activation of ERalpha gene expression could have clinical impact, our results suggest a possible use of extracts from medicinal plants to identify compounds of possible interest in the treatment of breast cancer [27].

In 2007 some researchers of Antenna Technologies, Geneva, Switzerland done A prospective, dose-escalating, quasi-experimental clinical trial which was conducted with a traditional healer using a decoction of *Argemone mexicana* for the treatment of malaria in Mali. The remedy was prescribed in three regimens: once daily for 3 days (Group A; $n = 23$); twice daily for 7 days (Group B; $n = 40$); and four times daily for the first 4 days followed by twice daily for 3 days (Group C; $n = 17$). Thus, 80 patients were included, of whom 80% were aged <5 years and 25% were aged <1 year. All presented to the traditional healer with symptoms of malaria and had a *Plasmodium falciparum* parasitaemia >2000/μl but no signs of severe malaria. The proportions of adequate clinical response (ACR) at Day 14 were 35%, 73% and 65% in Groups A, B and C, respectively ($P = 0.011$). At Day 14, overall proportions of ACR were lower in children aged <1 year (45%) and higher in patients aged >5 years (81%) ($P = 0.027$). Very few patients had complete parasite clearance, but at Day 14, 67% of patients with ACR had a parasitaemia <2000/μl. No patient needed referral for severe disease. Only minor side effects were observed. Further research

should determine whether this local resource could represent a first-aid home treatment in remote areas [28].

In 2008 Reddy NP, Das M. (Formerly Food Toxicology Division Indian Institute of Toxicology Research, Industrial Toxicology Research Centre), Lucknow, India, showed Interaction of sanguinarine alkaloid, isolated from argemone oil, with hepatic cytochrome p450 in rats. In this study interaction of sanguinarine (SAN) alkaloid, isolated from AO, with rat hepatic P450 was investigated. Hepatic microsomes prepared from 3-methylcholantherene (3MC) treated rats when incubated with SAN (1-3 muM) resulted in a spectral peak at 385 nm and a trough at 415 nm, indicative of Type I binding. Incubation of SAN (50-200 muM) with hepatic microsomes prepared from phenobarbitone (PB) treated rats also showed a Type I spectra with a peak at 395 nm and a trough at 420 nm. Relative binding efficiency (DeltaA(max)/K(s)(app) factor) of SAN with P450 was found to be 1540 and 1030 absorbance units/nmol CYP/M for 3MC and PB induced microsomes, respectively. In a P450 spectral inhibition study SAN showed higher affinity towards 3MC eliciting inhibition at much lesser concentrations (0.25-5 muM) as compared to PB (100-300 muM). The IC50s of SAN with different catalytic markers of P450 isoforms, i.e. ethoxyresorufin-O-deethylase (EROD) for CYP1A1, was 2.8 muM and for methoxyresorufin-O-deethylase (MROD) for CYP1A2 was 2.2 muM in 3MC induced microsomes, while benzoyloxyresorufin-O-deethylase (BROD) for CYP 2B1/1A1 showed an IC50 of 50 muM but pentoxyresorufin-O-deethylase (PROD) for CYP2B1 showed no inhibition even at higher concentrations of SAN (> 60 muM) in PB-induced microsomes. These results indicate that higher affinity of SAN binding towards the CYP1A family may have a role in SAN toxicity [29].

In 2010 researchers of Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Lucknow, India studied Potentiation of tumour promotion by topical application of argemone oil/isolated sanguinarine alkaloid in a model of mouse skin carcinogenesis. In this study, the effect of AO/SANG was investigated on the development of tumour formation in mice using 7,12-dimethylbenz (a) anthracene (DMBA) initiated followed by tetradecanoyl phorbol acetate (TPA)-promoted skin tumour protocol. Single application of AO (300mul) or SANG (4.5mumol) when used during initiation phase in DMBA/TPA group did not reveal substantial difference in tumourigenic response. However, twice weekly application of AO (100mul) or SANG (1.5mumol) during promotion phase (25 weeks) resulted in enhanced tumourigenic response by >/=30% in DMBA/TPA treated group along with significant decrease in dermal tyrosinase (45-49%), histidase (30-32%), superoxide dismutase (53-56%), catalase (41%), GSH reductase (37-40%) and GSH-peroxidase activity (29-33%) compared to control. Furthermore, significant decrease of epidermal GSH (64-66%) content and enhanced formation of lipid peroxides (96-121%) was noticed following AO or SANG treatment during promotion phase to DMBA/TPA induced animals indicating the modified pro-oxidant status in skin. Although dermal biochemical parameters were also altered by AO or SANG when used during initiation phase in DMBA/TPA treated animals, nonetheless, the response in these parameters were relatively more when AO or SANG were used during promotion phase in DMBA/TPA treated animals. These results clearly suggest that AO and SANG have the ability to enhance the tumourigenic response, which may have relevance to its carcinogenic potential[30].

Table2.2: Summary of the pharmacological Studies on *Argemone mexicana*

Years	**Author/Rsearchers Name**	**Work Title**
1985	Sood NN,	Epidemic dropsy following transcutaneous

	Sachdev MS, Mohan M, Gupta SK, Sachdev HP.	absorption of *Argemone mexicana* oil.
1988	Upreti KK, Das M, Khanna SK.	Biochemical toxicology of argemone alkaloids. III. Effect on lipid peroxidation in different subcellular fractions of the liver.
2001	Verma S.K., Dev G., Tyagi A.K., Goomber S. & Jain G.V.	*Argemone mexicana* poisoning: autopsy findings of two cases
2003	Sakthivadivel M, Thilagavathy D.	Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana L* seed Bioresour Technol.
2004	Lambertini E, Piva R, Khan MT, Lampronti I, Bianchi N, Borgatti M, Gambari R.	Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene.
2007	Willcox L. M. , Graz B. , Falquet J. , Sidibé O. , Forster M & Diallo D.	*Argemone mexicana,* decoction for the treatment of uncomplicated falciparum malaria.
2008	Reddy NP, Das M.	Interaction of sanguinarine alkaloid,

		isolated from argemone oil, with hepatic cytochrome p450 in rats Toxicol Mech Methods.
2010	Ansari KM, Das M	Potentiation of tumour promotion by topical application of argemone oil/isolated sanguinarine alkaloid in a model of mouse skin carcinogenesis.

CHAPTER 3:
METHODS & MATERIALS

3. Method and materials

3.1 Plant extraction:

3.1.1 Plant collection and identification:

Argemone mexicana was collected in the month of February, 2011 from Manikganj, a District of Bangladesh. Collected plant was identified by a taxonomist from Bangladesh National Herbarium, Mirpur (Dhaka). The accession number was DACB 35574. A duplicate specimen has been deposited in the Bangladesh National Herbarium.

Figure3.1: The identification plate of *Argemone mexicana*.

3.1.2 Chopping, drying and grinding of the plants:

About 1.5 kilograms plants were collected. At first these plant was chopping to small pieces with a scissor and were dried under sun-light for about 2 weeks. After drying, the dried plants were grinded into grinding machine to get fine powder. After grinding the weight of the plants were measured and the weight was about 256 grams. All grinded powder was preserved in a glass container covered with aluminum foil paper.

Figure3.2: Cutting and drying of *Argemone mexicana.*

3.1.3 Selection of Solvent:

Methanol, ethyl-acetate and n-hexane were selected as the solvent for extraction of *Argemone mexicana* according to their polarity index.

Properties of the solvents

Solvent	Appearance	Chemical formula	Boiling point	Solvent type
Methanol	Colorless liquid	CH_3OH	65 °C	Strongly polar
Ethyl acetate	Colorless liquid	$CH_3COOCH_2CH_3$	77 °C	Polar
N-hexane	Colorless liquid	C_6H_{14}	69 °C	Non-polar

3.1.4 Methanolic extraction process of *Argemone mexicana:*

86.6 gm powder of *Argemone mexicana* was taken into a 500 ml conical flask and it was soaked with methanol. Then the top of the conical flask was covered with aluminum foil paper for further prevention of evaporation of solvent and volatile constituents from the mixture. It was kept for three days and everyday it was shacked for several times and stirred with a clean glass rod to ensure the maximum amounts of constituents present in the grinded plants become soluble into methanol. After three days, the mixture was filtered. For filtration, filter paper (Double Rings 102- 11cm, Germany) was used and after filtration two parts were obtained- The residue portion over the filter and the filtrate. The filtrate, which contains the substance soluble in methanol, was putted into a 1000ml round bottom flask (BOROSIL, Japan), then the flask was placed it in a rotary evaporator (brand name, country of origin). The evaporation was done at 65 °C temperature. The number of rotation per minute was selected as 110 RPM. The pressure of the vacuum pumper machine

(Biometra) was 6 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate. When the evaporation seemed to be satisfactory, then the methanolic extract was collected by using di-ethyl ether. The whole process was done again with the residue portion by adding 100ml more methanols to get pure extract than first time. 1.81 mg (2.09%) crude extract of *Argemone mexicana* was found from this methanolic extraction. The crude extracts were then preserved in a 25ml beaker covered with aluminum foil paper for the cytotoxicity study/brine shrimp lethality bioassay and antimicrobial investigations.

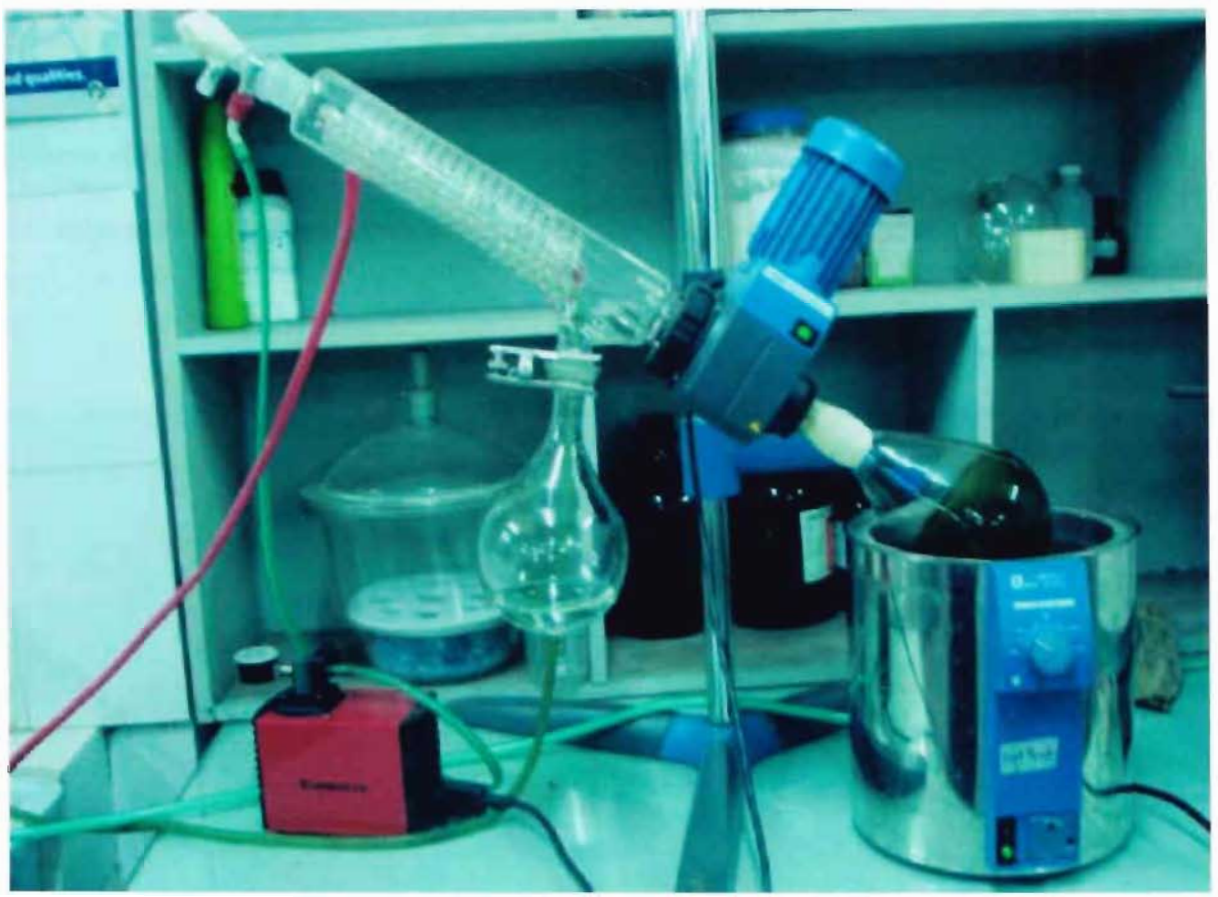

Figure3.3: Rotary Evaporator (IKA ®RV05 Basic, Biometra, Germany)

3.1.5 Ethyl acetate extraction process of *Argemone mexicana*:

72.1 gm powder of *Argemone mexicana* was taken into a 500 ml conical flask and it was soaked with ethyl acetate. Then the extraction process was done as methanolic extraction process. But here 75 °C temperature was selected for the rotary evaporator. 1.51mg crude extract was found and preserved according to previous manner.

3.1.6 N-hexane extraction process of *Argemone mexicana*:

99.8 gm powder of *Argemone mexicana* was taken into a 500 ml conical flask and it was soaked with n-hexane. Then the extraction process was done as methanolic extraction process. But here 68 °C temperature was selected for the rotary evaporator. 0.56 mg crude extract was found and preserved according to previous manner.

Figure3.4: Crude extracts of *Argemone mexicana*

3.1.7 Apparatus & reagent used for plant extraction:

- Plant sample
- Scissor (for cutting the plants into small pieces for drying)
- Blender machine (for obtain the powder of plant)
- Acetone (Merck, Germany)
- Glass container (storing of plant powder)
- Glass rod
- Aluminum foil (covered the beaker and conical flask)
- Electric Balance; SHIMADZU AY220 & SCALTEC SPB31
- Conical flask; 1000ml
- Methanol, ethyl acetate and n-hexane (Merck, Germany)
- Filter paper (Double Rings 102 – 11cm, HANGZHOU XINHUA PAPER Industry Co. Ltd., China)
- Volumetric Flask; 250ml, 500ml and 1000ml
- Round Bottle Flask; BOROSIL 1000ml
- Rotary evaporator (IKA RRV05 Basic, Biometra, Germany)
- Di-ethyl-ether (Merck, Germany)
- Beaker; 25ml

3.2 Brine shrimp lethality test of *Argemone mexicana*:

3.2.1 Hatching of *Artemia salina* leach (brine shrimp eggs):

38 gm of pure NaCl was dissolved in distilled water and then the volume made up to 1000ml by distilled water in a 1000ml beaker to prepared sea water for *Artemia salina* hatching. 1-2 drops of NaOH solution of 1N was added with a dropper to obtain the pH 8.4 as sea water. Then 0.25gm of *Artemia salina* leach was added in the artificial sea water.

Oxygen was supply through an air pump and a table lamp was placed near the beaker. After 24 hours incubation at room temperature, newly hatched free-swimming red-colored nauplii were harvested from the bottom outlet and place into another beaker with a dropper where previously made sea water was present. As the cyst capsules floated on the surface, this collection method ensured pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay.

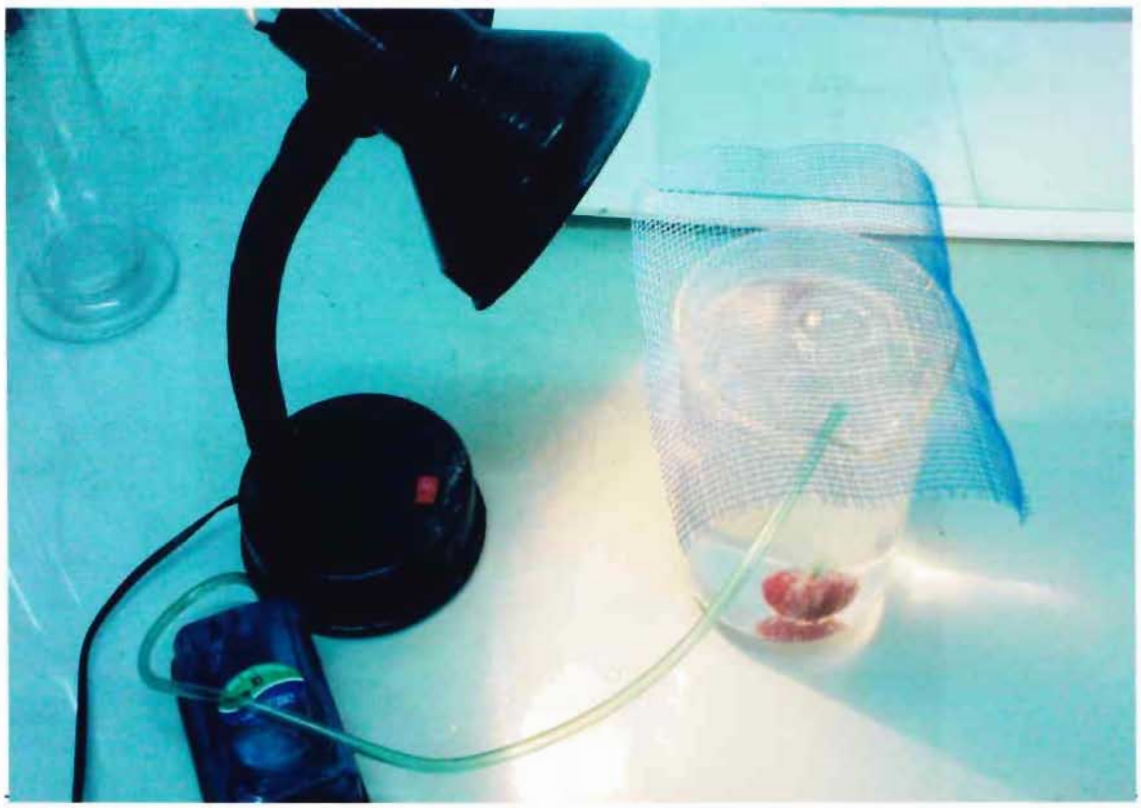

Figure3.5: Hatching of *Artemia salina*

3.2.2 Collection of *Artemia salina*:

50 test tubes were cleaned with acetone and then every test tube was filled with 10ml of water and then the water level was tagged with masking tape. Then the water removed from

all the test tube and kept for the assay. A serial number was also written on the masking tape for maintaining the drugs concentration. 20 test tubes were for positive and negative control test and 30 test tubes for the cytotoxic test with the three solvents extracts of *Argemone mexicana*. After prepared the test tube 10 nauplii was taken into each test tube with a dropper from the freshly hatched free-swimming red nauplii. And the sea water level in the test tube was below 10 ml for the further adjustment with crude drugs.

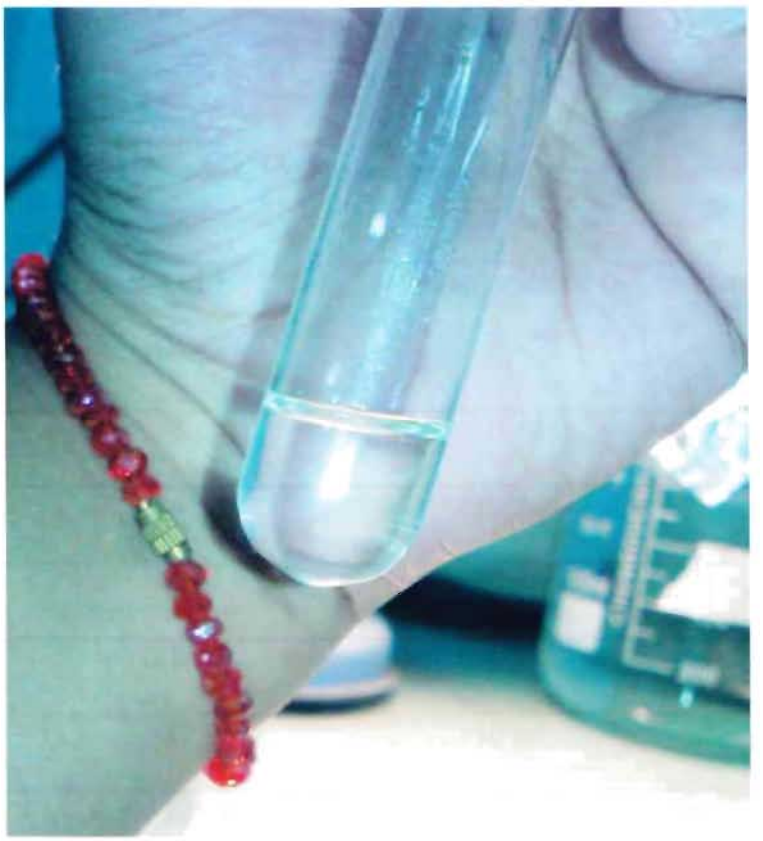

Figure3.6: Collection of nauplii in a test tube

3.2.3 Positive control:

10 test tubes were taken in which 10 nauplii were taken previously. For positive control $KMnO_4$ were used as the standard drug. 50 mg KMnO4 were dissolve 10 ml of sea water.

So that 5000 µg of $KMnO_4$ were dissolved in 1000 µg of sea water. The concentration of $KMnO_4$ in this sea-water was 5µg/ml. Then 2 µl of $KMnO_4$ containing this sea-water were taken into the first test tube with a micro pipette for positive control and then the test tube were adjusted with normal sea-water up to 10ml, so the final concentration of the first test tube was 1 µg/µl. This solution were given to the other test tubes for positive control test in the same manner but the final concentration varied as different amount of solution to each test tubes. Then these test tubes were kept for 24 hours to get the result of brine shrimp lethality test.

Table3.2: Preparation of test solution for positive control

Serial number	**Amount of KMnO4 solution**	**Final volume**	**Final concentration**
1	2 µl	10 ml	4 µg/ml
2	4 µl	10 ml	8 µg/ml
3	6 µl	10 ml	12 µg/ml
4	8 µl	10 ml	16 µg/ml
5	10 µl	10 ml	20 µg/ml
6	12 µl	10 ml	24 µg/ml
7	14 µl	10 ml	28 µg/ml
8	16 µl	10 ml	32 µg/ml
9	18 µl	10 ml	36 µg/ml
10	20 µl	10 ml	40 µg/ml

3.2.4 Adding of *Agemone mexicana* extracts:

200 mg of each solvent extracts were taken into a 25ml different beaker and each solvent extracts was dissolved in 10 ml of dimethyl sulfoxide (DMSO) and two drops of TWEEN 80. 30 test tubes were taken in which 10 nauplii were taken previously. 8 test tubes for each solvent extracts. For the first test tube was adjusted 10 µl of solution were taken and then the test tube was adjusted with fresh seawater up to 10 ml to get the final concentration. Micro tips and micro pipette were used to transfer the crude extracts into each test tube for accurate measurement. After that the test tubes were filling up to 10ml with the previously made fresh sea water. Then these test tubes were kept for 24 hours to get the result of brine shrimp lethality test.

Table3.3: Preparation for *Argemone mexicana* extracts for brine shrimp test

Amount of methanol extracts	**Amount of ethyl acetate extracts**	**Amount of n-hexane extracts**	**Final volume**	**Final concentration µg/ml**
10 µl	10 µl	10 µl	10 ml	20
25 µl	25 µl	25 µl	10 ml	50
37.5 µl	37.5 µl	37.5 µl	10 ml	75
75 µl	75 µl	75 µl	10 ml	150
100 µl	100 µl	100 µl	10 ml	200
150 µl	150 µl	150 µl	10 ml	300
175 µl	175 µl	175 µl	10 ml	350
200 µl	200 µl	200 µl	10 ml	400

3.2.5 Negative control:

10 test tubes were taken in which 10 nauplii were taken previously. For negative control 2 drops of TWEEN80 were added with 10 ml of DMSO which was the mother solution for negative control test. Then in each test tube this solution was added according to the volume calculated as the *Agemone mexicana* extracts. Then these test tubes were kept for 24 hours to get the result of brine shrimp lethality test.

Figure3.7: Prepared test tubes for negative control

3.2.6 Apparatus & Reagent used for brine shrimp lethality test:

- Pure NaCl (Merck, Germany)
- Distilled water
- Measuring cylinder
- Brine shrimp eggs
- Crude extracts
- Test tubes and racks
- Aluminum foil
- Electric Balance; SHIMADZU AY220 & SCALTEC SPB31

- 1000 ml beaker
- Glass rod
- Table lamp
- Aquarium air pump (SB2488, Sovo)
- Filter paper (Double Rings 102 – 11cm, HANGZHOU XINHUA PAPER Industry Co. Ltd., China)
- DMSO (Merck, Germany)
- TWEEN80 (Merck, Germany)
- $KMnO_4$ (Merck, Germany)

3.3 Antimicrobial susceptibility test of *Argemone mexicana:*

3.3.1 Sterilization of Petri dishes:

9 Petri dishes were wrapped with paper and placed inside an autoclave machine (HIRAYAMA, Japan) for sterilization at 121°C for 15 minutes. After that, Petri dishes were washed with detergent soap properly. After washing, allowed them to dry and then placed these Petri dishes into hot air oven (FN-500, Niive) for 20 minutes. Then these Petri dishes were place into the laminar flow cabinet for prevention of further contamination.

3.3.2 Preparation of agar solution:

A standard rule is, 28 gram Nutrient Agar should dissolve in 1000 ml of water. 8.4 gram Nutrient Agar was weighed and then 300 ml of distilled water was added to prepare 300 ml Agar solution. This preparation was kept in a 400 ml glass container. And then the glass

container was kept in the autoclave machine for 15 minutes at 121°C temperatures. After that the agar solution were replaced in the laminar air flow cabinet.

Figure3.8: Autoclave (HIRAYAMA, Japan) and hot air oven (FN-500, Niive)

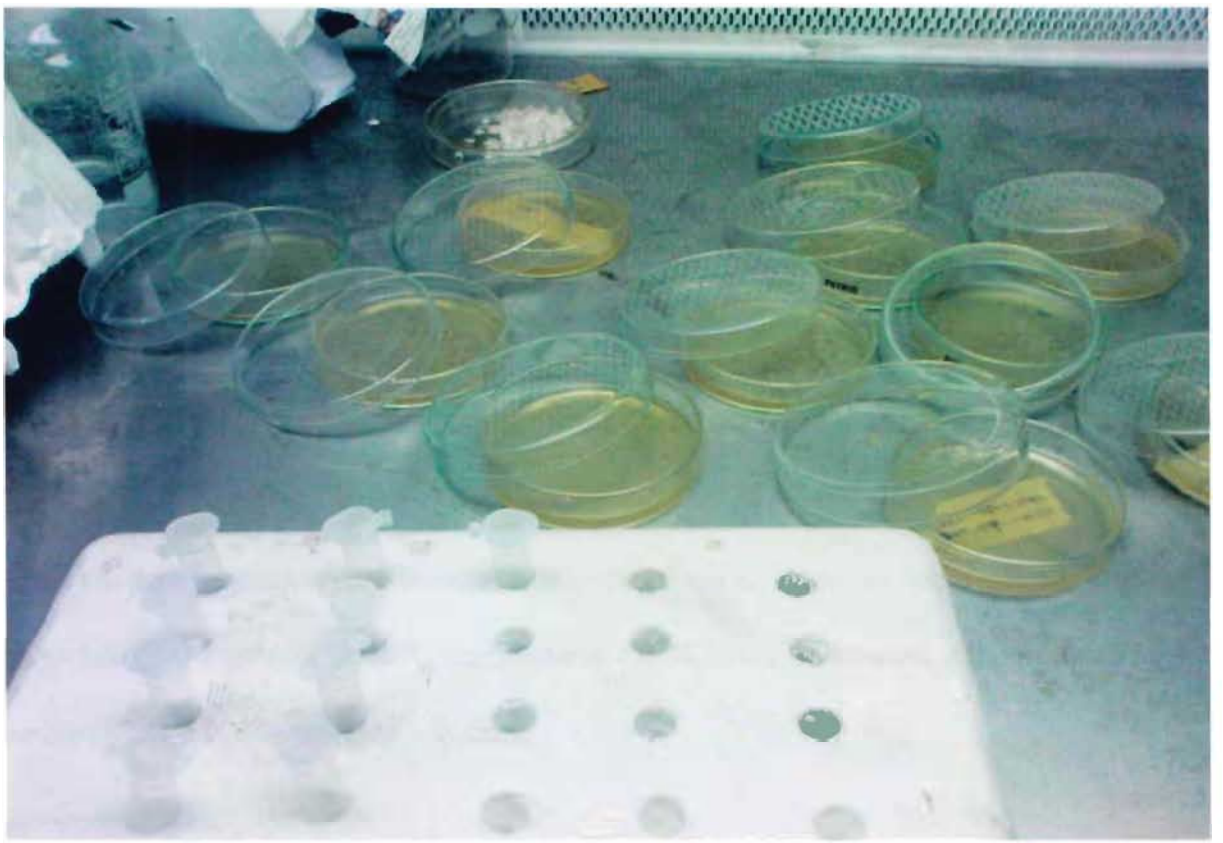

Figure3.9: Prepared agar media for bacterial cultures

3.3.3 Inoculations:

The prepared Agar solution was poured into each of the nine Petri dishes in a way so that each Petri dish gets 12-15 ml agar medium. Agar medium was dispensed into each Petri dish to get 3-4 mm depth of agar media in each Petri dish. After pouring the agar medium, all Petri dishes were kept in room temperature so that agar medium can become properly solidified. *Candida albicans, Salmonella dysenteriae, Salmonella dysenteriae* were used as test micro-organisms and the concentration of the suspension of test micro-organism was 1480 CFU/ml for *Candida albicans,* 320CFU/ml for *Salmonella dysenteriae* and 1000 for *Salmonella dysenteriae.* Petri dishes were labelled with the name of the microorganisms.

0.5 ml of bacterial suspension was taken from the each vial with micropipette and place on the surface of the agar media on each Petri dish according to their labelled name. After that the suspension was spread with glass rod spreader gently on the agar media. Then the ciprofloxacin disc and other two discs (one discs having 500 µg and another having 1000 µg of crude drugs) containing crude extracts were placed into the agar media. All these work were done in the laminar air flow cabinet.

3.3.4 Incubation:

Then all the prepared agar plates with respective microorganisms were placed inside a bacteriological incubator at 36°C temperatures for 24 hours for obtaining the result of anti-susceptibility test of *Argemone mexicana.*

Figure3.10: Laminar air flow cabinet (ESCO, Singapore)

Figure3.11: Incubator

3.3.4 Apparatus & reagent used for antimicrobial susceptibility test:

- Microorganisms suspensions
- Laminar air flow cabinet (ESCO, Singapore)
- Petri dishes
- Sterile forceps
- vials
- Autoclave (HIRAYAMA, Japan)
- Hot air oven (FN-500, Niive)
- Nutrient Agar (TECHNO PHARMCHEM, India)
- Filter paper (Double Rings 102 – 11cm, HANGZHOU XINHUA PAPER Industry Co. Ltd., China)
- Measuring Cylinder (10ml & 100ml)
- Hole puncher
- Filter paper discs
- Inoculating loop
- Crude extracts of experimental plant
- Micropipette (Eppendrof, Germany)
- Micropipette tips (Eppendrof, Germany)
- Spirit burner
- Hand gloves
- Incubator
- Distilled water

CHAPTER 4:

RESULTS & DISCUSSION

4. Result and discussion

4.1 Brine shrimp lethality test:

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors were counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product was expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the crude extracts that produces death in half of the test subjects after a certain exposure period.

Table 4.1, 4.2, 4..3 and 4.4 gives the results of the brine shrimp lethality after 24 hours exposure to the positive control $KMnO_4$, methanol, ethyl acetate and n-hexane extracts of *Argemone mexicana* respectively. The positive control, compared with the negative control was lethal, giving significant mortality to the shrimp as there was no mortality found in the negative control.

The lethal concentration LC_{50} of the test samples after 24 hrs was obtained by a plot of percentage of the shrimps killed against the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration to highly significant with the highest concentration (400µg/ml). Maximum mortalities took place at a concentration of 400µg/ml, whereas least mortalities were at lowest concentration. In other words, mortality increased gradually with the increase in concentration of the test samples.

Table4.1: Effect of $KMnO_4$ on brine shrimp nauplii.

Concentration (µg/ml)	Total population	Survivors	Death	% Mortality	LC_{50} (µg/ml)
4	10	10	0	0	
8	10	9	1	10	
12	10	8	2	20	
16	10	8	2	20	23
20	10	7	3	30	
24	10	5	5	50	
28	10	4	6	60	
32	10	3	7	70	
36	10	0	10	100	

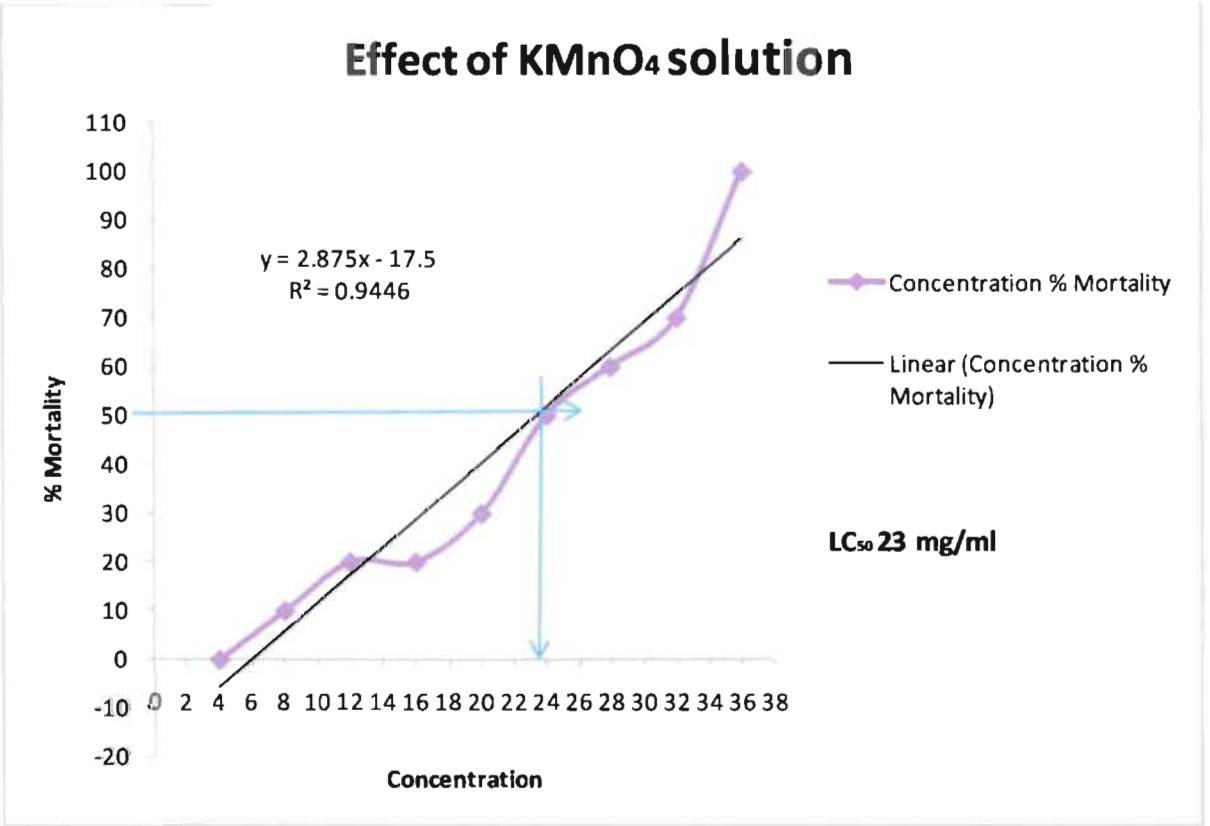

Figure4.1: Effect of KMnO4 on brine shrimp nauplii.

Table4.2: Effect of methanol extracts of *Argemone mexicana* on brine shrimp nauplii.

Concentration (µg/ml)	Total population	Survivors	Death	% Mortality	LC_{50} (µg/ml)
20	10	10	0	0	254
50	10	10	0	0	
75	10	8	2	20	
150	10	7	3	30	
200	10	5	5	50	
300	10	6	4	40	
350	10	4	6	60	
400	10	0	0	100	

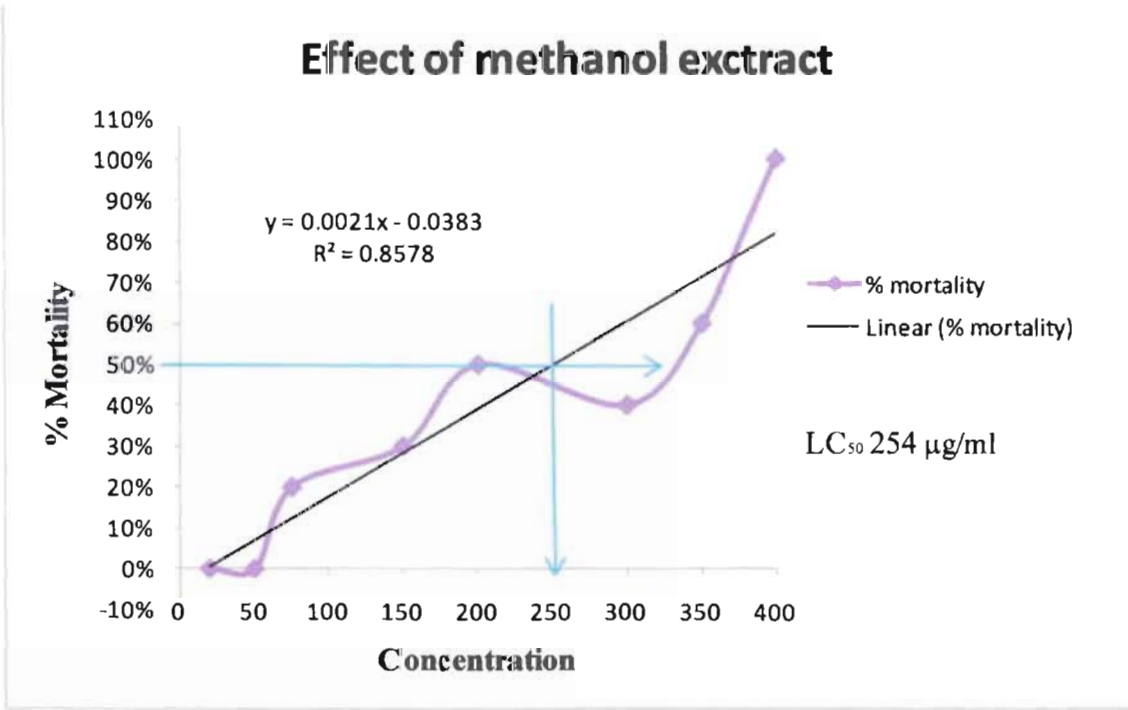

Figure4.2: Effect of methanol extracts of *Argemone mexicana* on brine shrimp nauplii.

Table4.3: Effect of ethyl acetate extracts of *Argemone mexicana* on brine shrimp nauplii.

Concentration (µg/ml)	Total population	Survivors	Death	% Mortality	LC_{50} (µg/ml)
20	10	10	0	0	
50	10	10	0	0	
75	10	8	2	20	
150	10	7	3	30	196
200	10	5	5	50	
300	10	6	4	40	
350	10	4	6	60	
400	10	0	0	100	

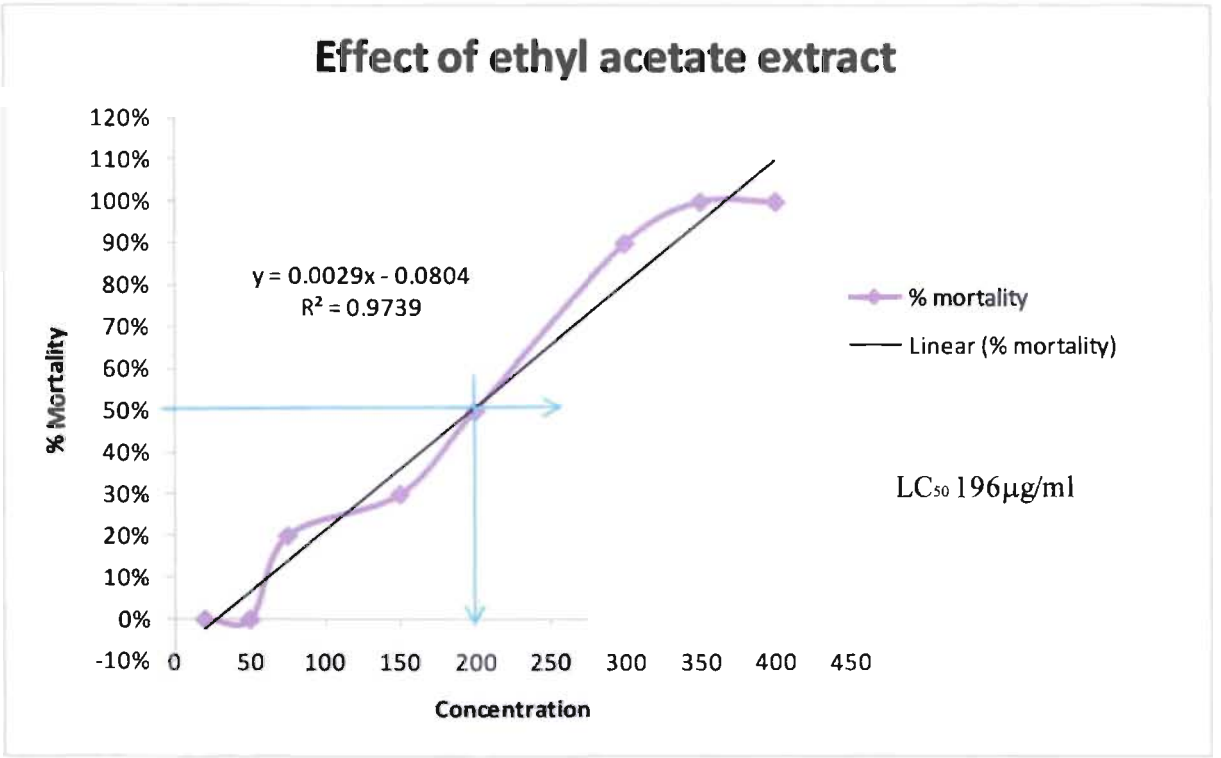

Figure4.3: Effect of ethyl acetate extracts of *Argemone mexicana* on brine shrimp nauplii.

Table4.4: Effect of n-hexane extracts of *Argemone mexicana* on brine shrimp nauplii.

Concentration (µg/ml)	Total population	Survivors	Death	% Mortality	LC_{50} (µg/ml)
100	10	10	0	0	273
125	10	10	0	0	
200	10	8	2	20%	
250	10	7	3	30%	
300	10	5	5	50%	
350	10	3	7	70%	
400	10	0	10	100%	

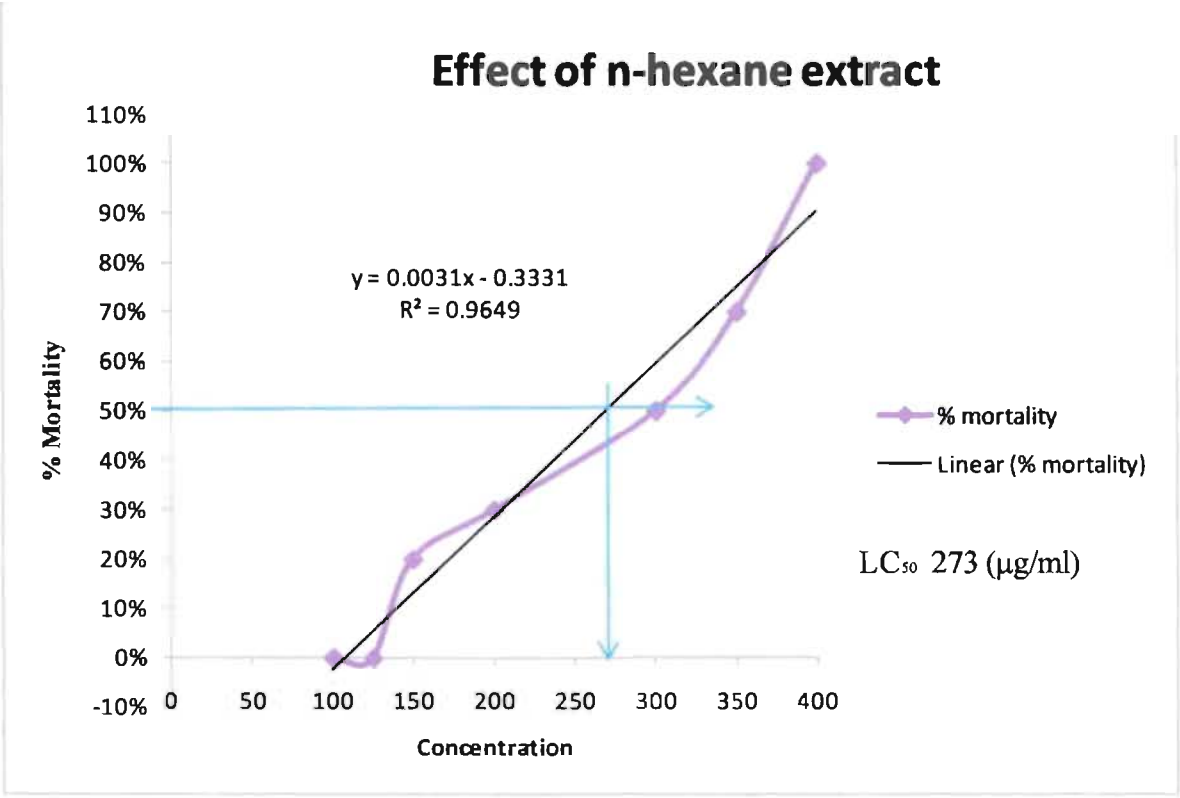

Figure4.4: Effect of n-hexane extracts of *Argemone mexicana* on brine shrimp nauplii.

Table4.5: Results of the brine shrimp test of extract of *Argemone mexicana.*

Sample	LC_{50} (µg/ml)	Regression equation	R^2
$KMnO_4$ (Std.)	23	y = 2.875x– 17.5	0.9446
Methanol extract	196	y = 0.0021x – 0.0383	0.8578
Ethyl acetate extract	254	y = 0.0029x – 0.0804	0.9739
N-hexane extract	273	y = 0.0031x – 0.3331	0.9649

4.2 Antimicrobial susceptibility test:

Values (mean of three replicates) are diameter of zone of inhibition (mm): <9, inactive; 9–12, partially active; 13–18, active; >18, very active [31].

Against the test micro-organisms the zone of inhibition produced by methanolic extract was ranges from 6-7 mm and 8-9 mm at a concentration of 500 µg/disc and 1000 µg/disc respectively, which means it was partially active at 1000 µg/disc and in active at 500 µg/disc.

The ethyl acetate extracts of the plant exhibited very low activity against the tested micro organisms. The average zone of inhibition produced by ethyl acetate extract was 8-7 mm and 10-12 mm at a concentration of 500 µg/disc and 1000 µg/disc respectively, which means it was partially active at 1000 µg/disc and in active at 500 µg/disc.

The n-hexane extracts of the plant exhibited no activity against the tested *Staphylococcus aureus* and *Shigella dysenteriae* with the concentration of 500 µg, however it was partially

active with 1000 µg. The range of zone of inhibition for these two microorganisms was 9-13mm with 1000 µg/disc.

N-hexane extracts also exhibited partial activity against *Candida albicans* with both concentrations. The produced zone of inhibition was 9 mm and 13 mm at a concentration of 500 µg/disc and 1000 µg/disc respectively, which means it has partially activity and active respectively.

Table4.6: Antimicrobial activity of methanolic crude extract.

Microorganisms	Zone of inhibition (mm)		
	500µg/disc	1000µg/disc	Ciprofoxacin (Std.)
Staphylococcus aureus (gram +ve bacteria)	6	8	42
Shigella dysenteriae (gram -ve bacteria)	7	9	38
Candida albicans (fungi)	6	8	47

Table4.7: Antimicrobial activity of ethyl acetate crude extract.

Microorganisms	Zone of inhibition (mm)		
	500µg/disc	1000µg/disc	Ciprofoxacin (Std.)
Staphylococcus aureus (gram +ve bacteria)	8	12	42
Shigella dysenteriae (gram -ve bacteria)	7	10	38
Candida albicans (fungi)	7	11	47

Table4.8: Antimicrobial activity of n-hexane crude extract.

Microorganisms	Zone of inhibition (mm)		
	500µg/disc	1000µg/disc	Ciprofoxacin (Std.)
Staphylococcus aureus (gram +ve bacteria)	0	9	42
Shigella dysenteriae (gram -ve bacteria)	0	10	38
Candida albicans (fungi)	9	13	47

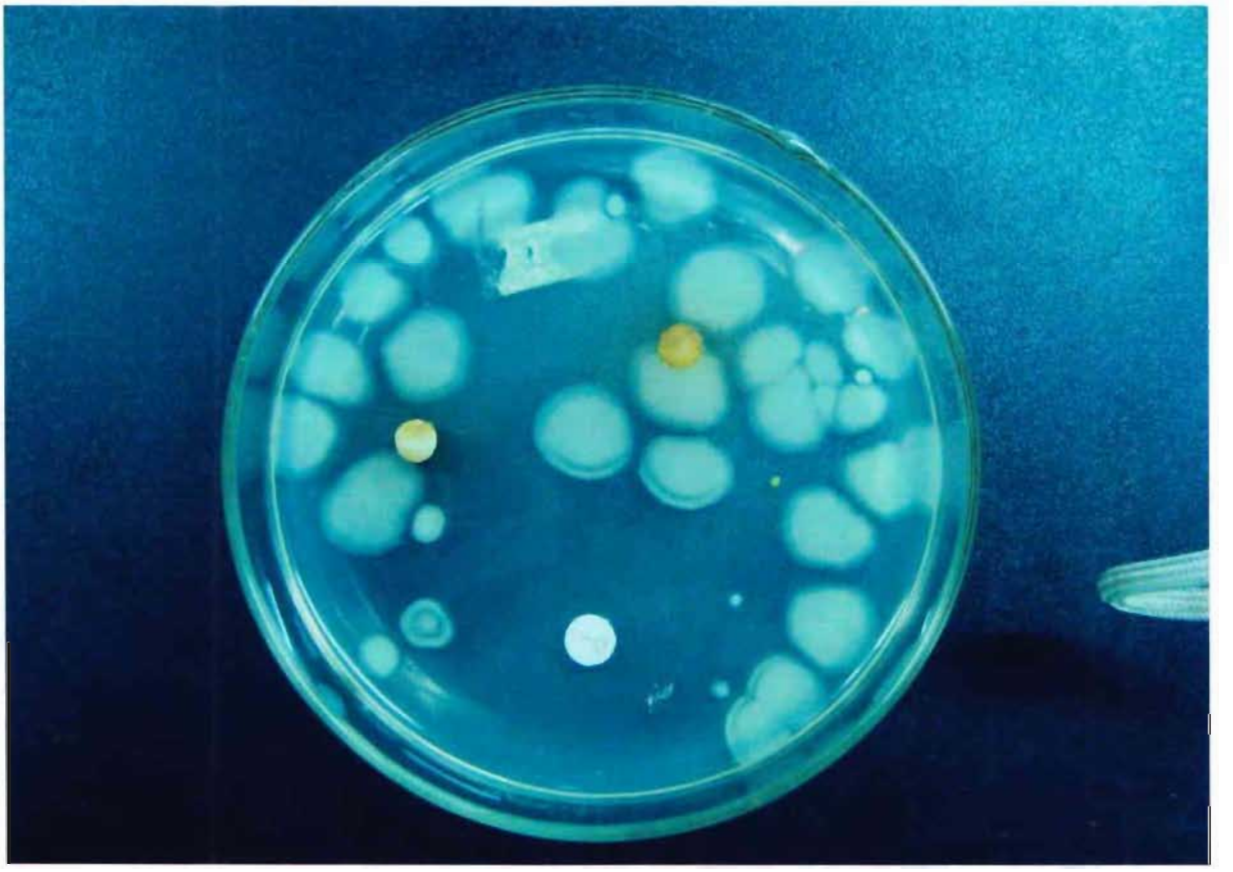

Figure4.5: Methanolic crude extracts against *Staphylococcus aureus*

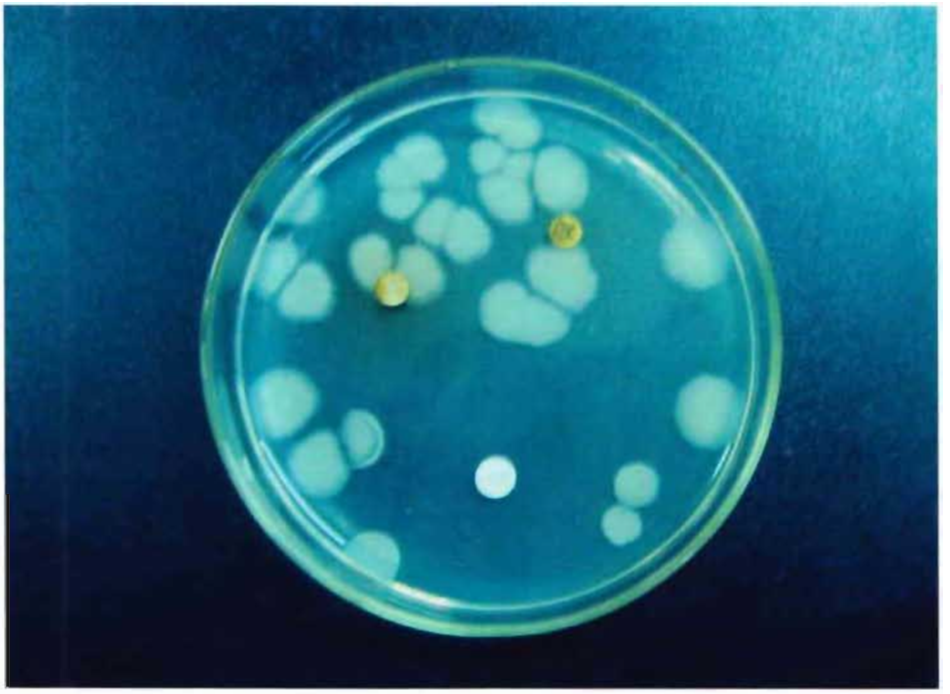

Figure4.6: Methanolic crude extracts against *Shigella dysenteriae*

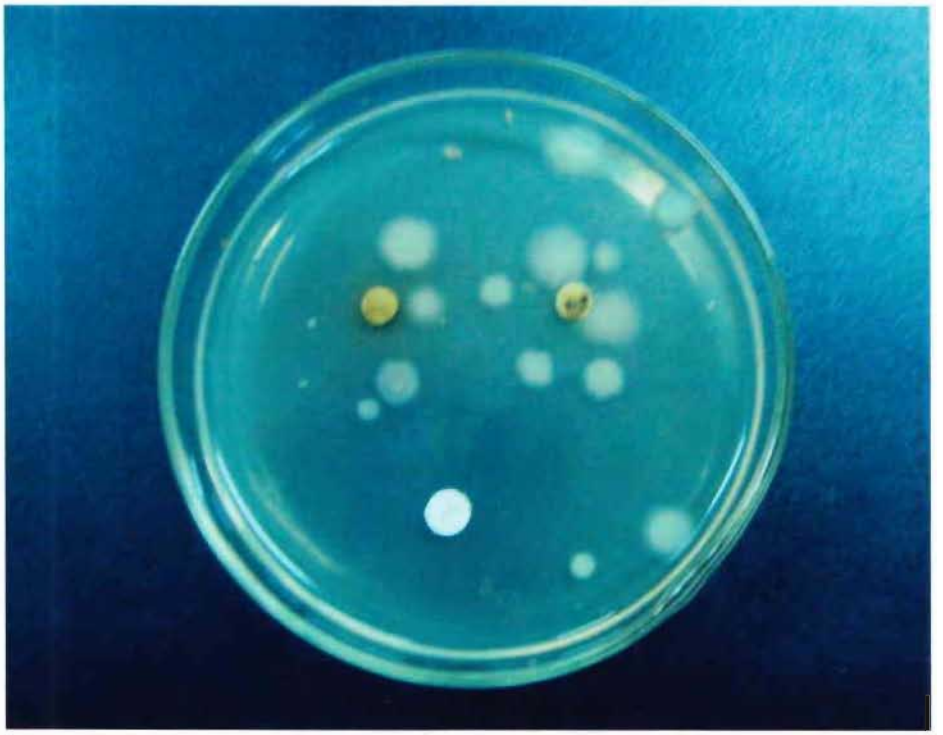

Figure4.7: Methanolic crude extracts against Candida *albicans*

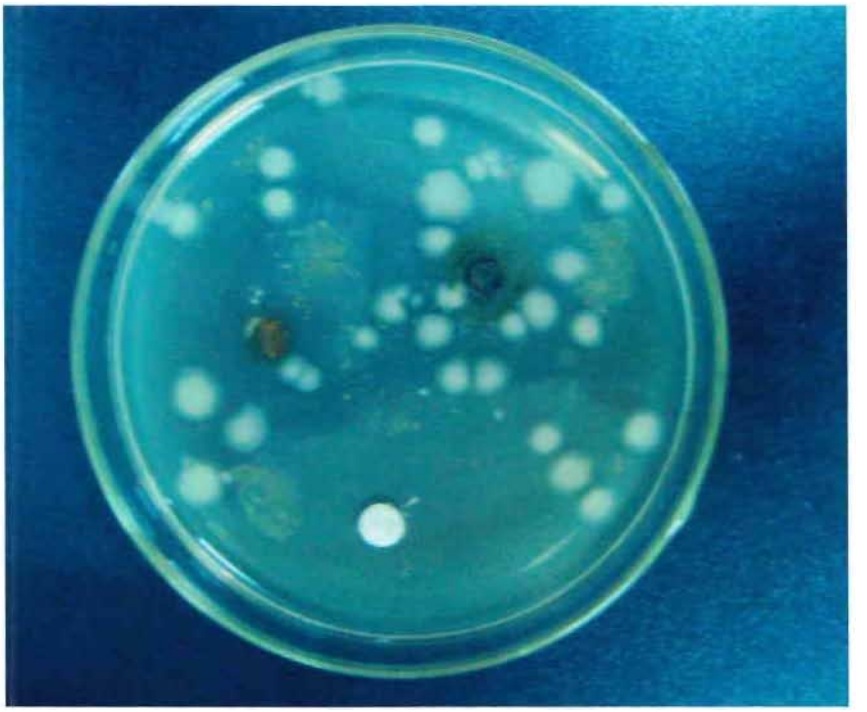

Figure4.8: Ethyl acetate crude extracts against *Staphylococcus aureus*

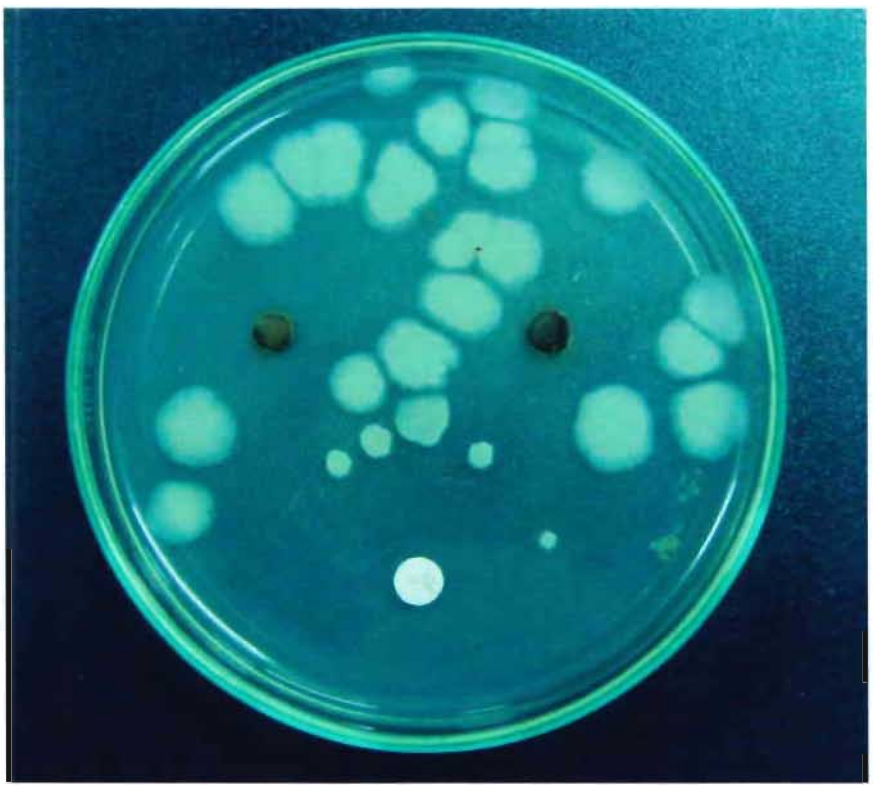

Figure4.9: Ethyl acetate crude extracts against *Shigella dysenteriae*

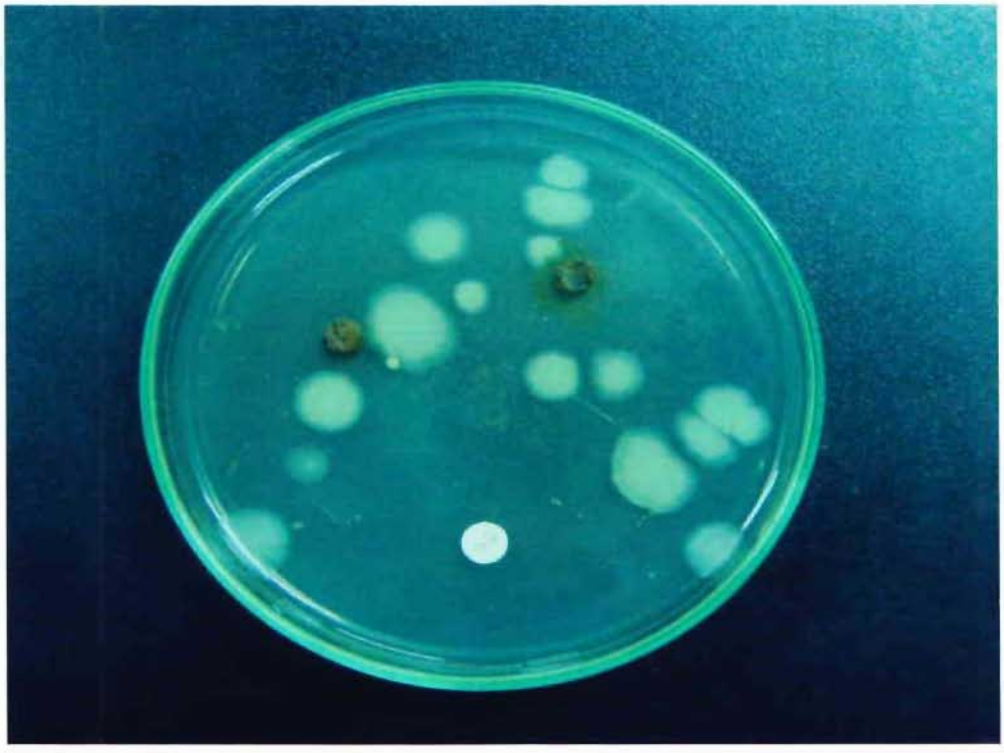

Figure4.10: Ethyl acetate crude extracts against *Candida albicans*

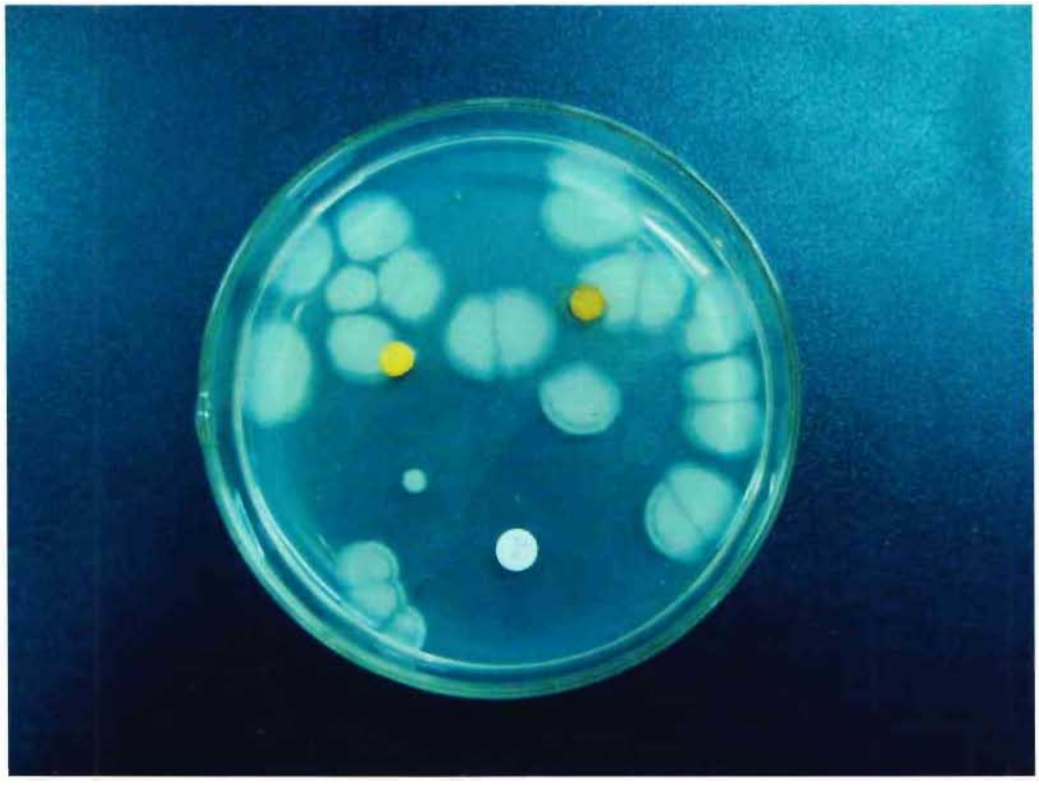

Figure4.11: N-hexane crude extracts against *Staphylococcus aureus*

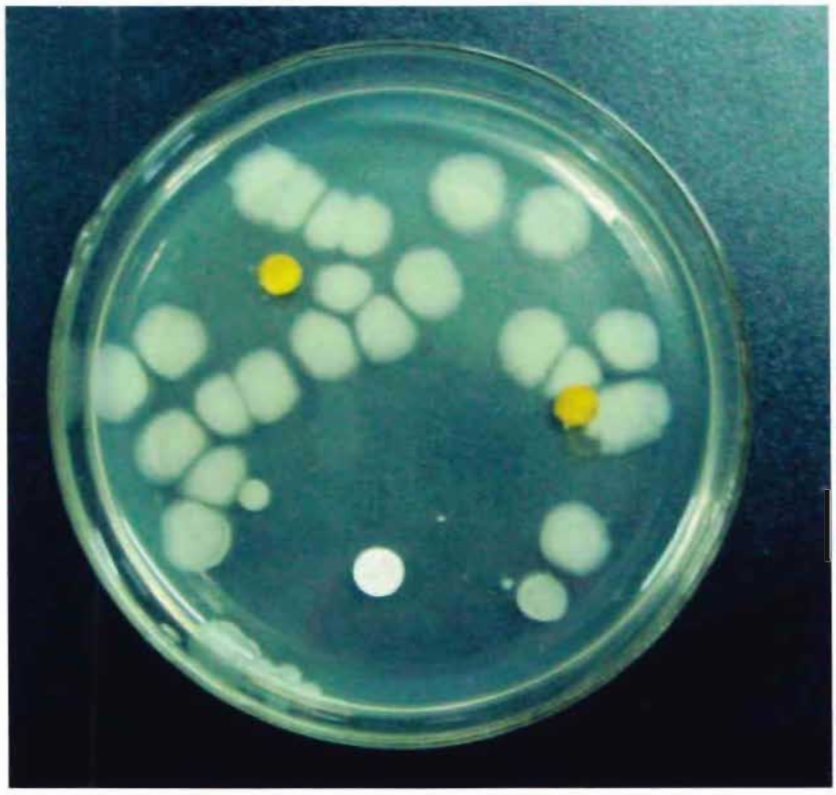

Figure4.12: N-hexane crude extracts against *Shigella dysenteriae*

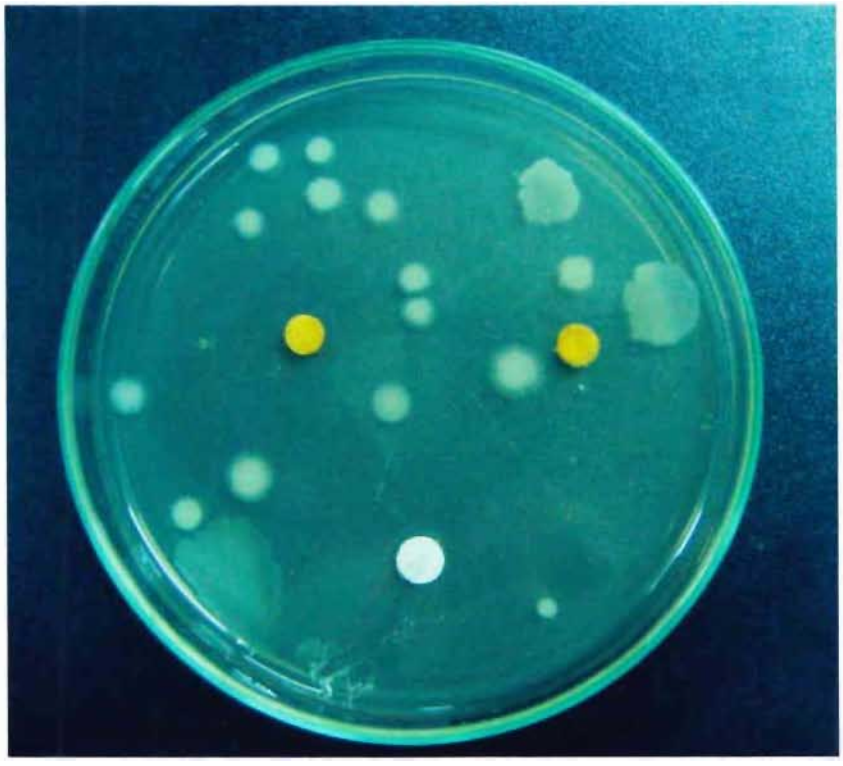

Figure4.13: N-hexane crude extracts against *Candida albicans*

CHAPTER 5:

CONCLUSION

5. Conclusion

The brine shrimp bioassay and microbial screening of *Argemone mexicana* gives some significant data on *Argemone mexicana* is used as a medicinal plant in several countries for several purposes. The use of plant extracts and phytochemicals, with known antibacterial properties, may be of immense importance in therapeutic treatments. In the past few years, a number of studies including phytochemicaly and pharmacologicaly have been conducted in different countries to prove such efficiency, but further investigation is needed to identify other activities of this plant.

REFERENCES

1. Dwivedi S., Abhishek D. & Kapadia R. (2008). Vol. 6 Issue 6 ; 2008
2. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses; 2nd edition.
3. Plants profile from United States Department of Agriculture, Natural Resources Conservation Service, retrieved from- http://plants.usda.gov/java/profile?symbol=ARME4
4. Bosch, C.H., 2007. *Argemone mexicana L.* In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands.
5. Sam, T.W. (1993) Bioactive Natural Products18: 441-56.
6. Hamburger, M. & K. Hostettmann (1991) *Phytochemistry* 30: 3864-74
7. Meyer, B.N., N.R. Ferrigni, J.E. Putman, L.B. Jacobson, D.E. Nichols & J.L. McLaughlin (1982) *Planta Med.* 45: 31-4
8. McLaughlin, J.L. (1991) *Methods Plant Biochem.* 6: 1-32
9. Price, K.S., G.T. Waggy & R.A. Conway (1974) *J. Water Pollut. Control Fed.* 46: 63-5.
10. Sorgeloos, P., C. Remiche-van der Wielen & G. Persoone (1978) Ecotoxicol. Environ. Saf. 2: 249-51
11. Harwig, J. & P.M. Scott (1971) *Appl. Microbiol.* 21: 1011-12.
12. Zillioux, E.J., H.R. Foulk, J.C. Prager & J.A. Cardin (1973) J. Water Pollut. Control Fed. 45: 2389-91
13. Singh, C.D. (2010). Preliminary Phytochemical and Antimicrobial Investigations of the Aqueous Extract of *Ixoracoccinea* Linn. And *Commelinabenghalensis* L. on Gram-

Positive and Gram-Negative Microorganism. Middle-East Journal of Scientific Research, 6(5), 436-439.

14. Antibiotic Disk Susceptibilities (Kirby-Bauer Disk-Diffusion Method); BSCI 424 — Pathogenic Microbiology; 2000

15. Cannon, M.; Reviewed by the American College of Rheumatology Patient Education Task Force; *Cyclosporine (*Gengraf, Neoral, Sandimmune*)*, Updated November 2009

16. Howland, R. D., & Mycek, M. J. (2007). Lippincott's Illustrated Reviews: Pharmacology (3rd ed.). New York, NY: Lippincott Williams & Wilkins.

17. D.K. Bhardwaj, M.S. Bisht, R.K. Jain and Anita Munjal. Phenolics from the seeds of *Argemone mexicana* ;Phytochemistry;Volume 21, Issue 8, 1982, Pages 2154-2156.

18. Harborne B. J. & Williams A. C.(1983). Flavonoids in the seeds of *Argemone mexicana* a reappraisal. Phytochemistry, 22(6), 1520-1521.

19. Sangwan K. N. & Malik S. M. (1998). A long chain alcohol from *Argemone Mexicana,* Phytochemistry, 49(3), 755-756.

20. Chang YC, Hsieh PW, Chang FR, Wu RR, Liaw CC, Lee KH, Wu YC. Two new protopines argemexicaines A and B and the anti-HIV alkaloid 6-acetonyldihydrochelerythrine from formosan Argemone mexicana. Planta Med. 2003 Feb; 69(2):148-152.

21. Singh S, Singh TD, Singh VP, Pandey VB . Quaternary alkaloids of Argemone mexicana. Pharm Biol. 2010 Feb; 48(2):158-60.

22. Maria Luisa Díaz Chávez, Megan Rolf, Andreas Gesell and Toni M. Kutchan. Archives of Biochemistry and Biophysics; Characterization of two methylenedioxy bridge-forming cytochrome P450-dependent enzymes of alkaloid formation in the Mexican prickly poppy *Argemone Mexican;* Volume 507, Issue 1, March 2011, Pages 186-193.

23. Sood NN, Sachdev MS, Mohan M, Gupta SK, Sachdev HP. Epidemic dropsy following transcutaneous absorption of Argemone mexicana oil. Trans R Soc Trop Med Hyg. 1985;79(4):510-2

24. Upreti KK, Das M, Khanna SK. Biochemical toxicology of argemone alkaloids. III. Effect on lipid peroxidation in different subcellular fractions of the liver. Toxicol Lett. 1988 Sep;42(3):301-8

25. Verma S.K., Dev G., Tyagi A.K., Goomber S. & Jain G.V. Argemone mexicana poisoning: autopsy findings of two cases; Forensic Science International, Volume 115, Number 1, 1 January 2001, pp. 135-141(7)

26. Sakthivadivel M, Thilagavathy D. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from Argemone mexicana L. seed Bioresour Technol. 2003 Sep; 89(2):213-6.

27. Lambertini E, Piva R, Khan MT, Lampronti I, Bianchi N, Borgatti M, Gambari R. Effects of extracts from Bangladeshi medicinal plants on in vitro proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene. Int J Oncol. 2004 Feb; 24(2):419-23.

28. Willcox L. M. , Graz B. , Falquet J. , Sidibé O. , Forster M. & Diallo D. (2007) *Argemone mexicana,* decoction for the treatment of uncomplicated falciparum malaria, Transactions of the Royal Society of Tropical Medicine and Hygiene Volume 101, Issue 12, December 2007, Pages 1190-1198

29. Reddy NP, Das M. Interaction of sanguinarine alkaloid, isolated from argemone oil. with hepatic cytochrome p450 in rats Toxicol Mech Methods. 2008 Jan;18(8):635-43

30. Ansari KM, Das M. Potentiation of tumour promotion by topical application of argemone oil/isolated sanguinarine alkaloid in a model of mouse skin carcinogenesis. Chem Biol Interact. 2010 Aug 5.

31. Smaˆnia Juˊnior A, Delle Monache F, Smaˆnia EFA, Gil ML, Benchetrit LC, Cruz FS. J Ethnopharmacol 1995; 45:177.