"Phytochemical Screening & *in vitro* Antioxidant and Thrombolytic Activities of *Argemone mexicana* Extracts"

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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July, 2012

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"**Phytochemical Screening & *in vitro* Antioxidant and Thrombolytic Activities of *Argemone mexicana* Extracts**"** 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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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled **"**Phytochemical Screening & *in vitro* Antioxidant and Thrombolytic Activities of *Argemone mexicana* Extracts**"** is a bonafide research work done by **Ahamed Hasan Al-Baizyd,** in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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ENDORSEMENT BY CHAIRPERSON

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ABSTRACT

Purpose: In present study the leaves extracts of *Argemone mexicana* (Papaveraceae) have been screened for their photochemical constituents, antioxidant, total phenolic content and *in vitro* thrombolytic activities.

Methods: The *n*-hexane, ethyl acetate and methanol extracts were screened for the presence of phytochemicals, using *in vitro* assay methods and their inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was used to evaluate their free radical scavenging activity at 517 nm. Nitric oxide scavenging capacity was measured by sodium nitroprusside and griss reagent. The total phenolic content (TPC) measured by the Folin-Ciocalteu method. An *in vitro* thrombolytic model was used to check out the clot lysis effects of three extracts using streptokinase as a positive control.

Results: Phytochemical screening of the plant showed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins. All the extracts inhibited DPPH, indicating their antioxidant activity. In DPPH and nitric oxide scavenging assay both *n*-hexane extract showed highest IC_{50} values 73.73 µg/ml, 152.73µg/ml and the lowest IC_{50} value was 12.54 µg/ml of ethyl acetate extract and 65.56µg/ml of methanol extract. It appears that, it has greater free radical scavenging capacity. Ethyl acetate extract had the highest total phenolic content of 106.65 mg GAE/100 g extract. All the extracts showed significant % of clot lysis effect (p<0.001) with reference to negative control.

Conclusion: The outcomes of the study are an indication of present of phytochemicals and may be responsible for some of the therapeutic uses of these plants.

Keywords: *Argemone mexicana*, Phytochemical screening, DPPH, Nitric oxide, Phenolic content, *In vitro* Thrombolytic.

CHAPTER 1:

INTRODUCTION

1. INTRODUCTION

Moreover, it is estimated that only 500 medicinal plant species had been recorded in Bangladesh out of approximately 1,900 species regarded as having medicinal value. There are several studies on the botanical aspects of the plants of Bangladesh. However, although plants are used by a great segment of the population; scarce investigation has been done on their biological activities. In more recent years, with considerable research, it has been found that many plants do indeed have medicinal values[1].

The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Approximately, half of the world's 25 best-selling pharmaceutical agents are derived from natural products. Thus, emphasis is now given on the standardization of herbal medication by screening of biological activities of medicinal plants and isolating active principles from them[2].

New drugs of plant origin and new methods of producing them will continue to be an important parts of the service and thus Plants are considered as are of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates[3].

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 have 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].

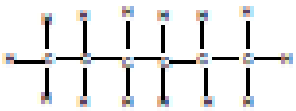
Table1.1: Different parts of *Argemone mexicana* in medicinal uses.

Plant parts	**Medicinal uses**
All parts	Dropsy, swellings, oedema, gout; general 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

Solvent System

1. *n*-hexane



Chemical structure of *n*-hexane

Applications

• *n*-hexane is widely used as cheap, relatively safe, largely unreactive, and easily evaporated non-polar solvent.

• It is used in the extraction of mainly lipophilic compounds from many plant extracts.

• It is also used as an alcohol denaturant and as a paint diluent.

• *n*-hexane is also used to extract oil from grains as well as protein from soy and hexane can persist in the final food product created (McCaine, 1990).

Physical and chemical properties of *n*-hexane

Appearance: Colorless liquid

Molecular formula: C6H14

Molecular weight: 86.10 g mol-1

Density: 0.660 g/cm3

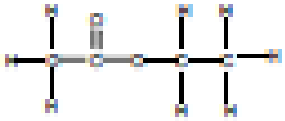
Boiling point: 68.95°C

Melting point: -95.3°C

Vapor pressure: 25°C

Solubility in water: 13 mg/L at 20°C

2. Ethyl acetate



Chemical structure of Ethyl acetate

Applications

• Ethyl acetate is used as the solvent for various purposes in different sectors, e.g. as electroplating-vapor degreasing solvent, solvent for dilution and extraction in laboratories, solvents for Flexography and Gravure Printing, varnish solvent etc.

• Ethyl acetate is reasonably polar and it is chosen to extract a reasonably polar compound from a mixture, is like a good rule of thumb for extractions.

Physical and chemical properties of ethyl acetate

Appearance: Colorless liquid

Molecular formula: CH3COOC2H5

Molecular weight: 88

Boiling point: 77ºC (171ºF)

Melting point: -83ºC (-117ºF)

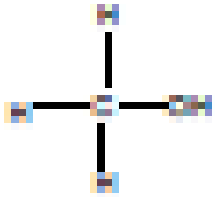
Vapor pressure (mm Hg): 76 at 20ºC (68ºF)

Solubility: 1 ml/10ml water at 25ºC

Odor: Fruity odor

pH **:**Acidic and about 5-7

3. Methanol



Chemical structure of Methanol

Applications

• Methanol, a common laboratory solvent, is especially useful for HPLC, UV/VIS spectroscopy.

• The largest use of methanol by far is in making other chemicals. About 40% of methanol is converted to formaldehyde, and from there into products as diverse as plastics, plywood, paints, explosives, and permanent press textiles.

• Other chemical derivatives of methanol include dimethyl ether, which has replaced chlorofluorocarbons as an aerosol spray and propellant, acetic acid. Dimethyl ether (DME) also can be blended with liquefied petroleum gas (LPG) for home heating and cooking, and can be used as a diesel replacement for transportation fuel (Blum, 2010).

Table1.2: Properties of the solvents

Solvent	**Physical Appearance**	**Chemical formula**	**Polarity Index**	**Boiling point**
Methanol	Colorless liquid	CH_3OH	5.1	65 °C
Ethyl acetate	Colorless liquid	$CH_3COOCH_2CH_3$	4.4	77 °C
N-hexane	Colorless liquid	C_6H_{14}	0.1	69 °C

Phytochemical Screening

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al*., 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few. The phytochemicalconstituent of a plant will often determine the physiological action on the human body (Pamplona-Roger, 1998). The most important of these bioactive constituents of plants are alkaloids, flavonoids, tannins, phenolic compounds etc. (Hill, 1952).

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity, and their absence in this plant tend to lower the risk of poisoning by the plant. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2. Flavonoids serve as health promoting compound. Steroids which are very important compounds especially due to their relationship with compounds such as sex hormone 19. These phenolic compounds contribute to their anti-oxidative properties and thus the usefulness of these in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol (Shrivastava & Leelavathi, 2010). Saponins, which are present in plants, have been suggested as possible anticarcinogens. They possess surface-active characteristics that are due to the amphiphilic

nature of their chemical structure. The mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune-modulatory effects, bile acid binding and normalization of carcinogen-induced cell proliferation. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Shrivastava & Leelavathi, 2010).

Some methods for identification the chemical constituents are given in the following table: **(**Vinod *et a*l., 2010; Reuben *et a*l., 2008; Shrivastava & Leelavathi, 2010)

Some popular methods used for the detection of various phytochemicals

Chemical Constituents	Test
Alkaloid	Hager's test
Flavonoid	Ammonia test (modified)
Steroid	Salkowski test
Terpenoid	Salkowski test (modified)
Carbohydrates	Fehling's (Reducing sugar) test (modified)
Saponins	Frothing test
Tannins	FeCl3 test
Cardiacglycoside	Killer-Killani's test
Anthraquinones	Chloroform layer test

Antioxidants

Anti-oxidants are substances that delay or inhibit oxidative damage to a target molecule, also capable to mop up free radicals and prevent them from causing cell damage. Antioxidants cause protective effect by neutralizing free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age.

Increasing the antioxidant intake can prevent diseases and lower the health problems. Phytoconstituents are important source of antioxidant and capable to terminate the free radical chain reactions. Antioxidants prevent cell and tissue damage as they act as scavenger (Sen *et al*., 2010) and thus help body fight against the pathophysiology of aging and a multitude of diseases, such as cancer, Alzheimer's disease and Parkinson's disease.

DPPH free radical scavenging assay

Free radical scavenging activity is evaluated with the spectrometric method where the DPPH reagent (2, 2-diphenyl-1-picrylhydrazyl), is mixed with the serial diluations of theextracts, is utilized to determine the antioxidant potential (Guerrero et al., 2004). DPPH free radical scavenging was first reported by Alexander Prokhorov in 1963.

Nitric oxide scavenging

The procedure is based on the method, where the compound sodium nitropruside is known to decompose in aqueous solution at physiological pH (7.2) and spontaneously generates nitric oxide (NO•). Under aerobic condition, NO• interacts with oxygen to produce stable products nitrate and nitrite ions, which can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthyl ethylenediamine dihydrochloride can be immediately read at 546 nm (Balakrishnan *et al*., 2009).

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical, nitric oxide scavenging test (O'Brian *et al*., 1998). Nitric oxide is implicated for inflammation, cancer and other pathological conditions. Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. This nitric acid gets

converted to nitrous acid and further forms nitrite ions which diazotize with sulphanilic acid and couple with naphthylethylenediamine (Griess reagent), producing pink color which can be measured at 546 nm (Marococci *et al.,* 1994). Hence nitric oxide scavenging capacity may help to arrest the chain of reactions initiated by excess generation of nitric oxide that are detrimental to the human health (Moncada *et al*., 1991).

Total phenolic content

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties (Javanmardi *et al*., 2003).

Polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid per oxidation, are the most crucial. The antioxidant activity of apples is highly correlated to the total phenolic content (TPC) measured by the Folin-Ciocalteu method (Tsao *et al*., 2005).

Total phenolic content is determined by the Folin–Ciocalteu method, which was adapted from Swain and Hillis (1959) (Thaipong *et al*., 2006). The extracts oxidized with Folin-Ciocalteu reagent, and the reaction neutralized with sodium carbonate. The absorbance of the resulting blue color is measured at 765 nm (Hodzic *et al*., 2009). The Folin-Ciocalteu method is an electron transfer (ET) based assay and measures reducing capacity, which has normally been used to expressed as phenolic contents of biological materials (Huang *et al*.,2005).

Thrombolytic Activity

Since ancient times, herbal preparations have been used for the treatment of several diseases. The leaves and or twigs, stem, bark and underground parts of plants are most often used for

traditional medicines (Gesler, 1992). Considerable efforts have been directed towards the discovery and development of natural products from various plant and animals which have anti platelet, anticoagulant, antithrombotic, and thrombolytic activity (Demro & Briggs 1995, 2001). This method was done to investigate whether extract from plants possess thrombolytic activity or not.

One of the major causes of blood circulation problem is the formation of blood clots. Thrombo embolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries. Thrombi can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction or even death of the tissues (necrosis) in that area. Atherothrombotic diseases such as myocardial or cerebral infarction are also serious consequences of the thrombus formed in blood vessels (Thrombus, 2011).

Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels. One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction or even death of the tissues (necrosis) in that area. Thrombolytic therapy reduces mortality.

Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots (Anwar *et al*., 2011). Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic altreplase in most infarct patients while having the advantages of being much less expensive. Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators (Khan *et al*., 2011). Streptokinase forms a complex with plasminogen (Figure 14) which then

converts plasminogen to plasmin. Plasmin breaks down clots as well as fibrinogen and other plasma proteins (Banerjee *et al*., 2004).

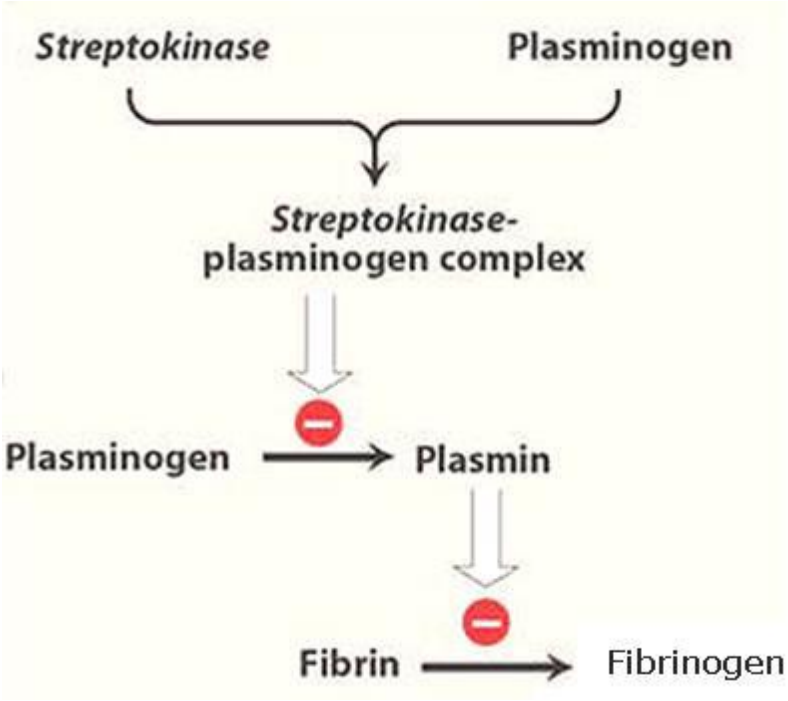


Figure 1.3: Mechanism of anticoagulation of streptokinase

Another synthetic molecule, clopidogrel thieno [3,2-c] pyridine-5(4H)-acetic acid, a(2 chlorophenyl)-6,7-dihydro-,methyl ester,(*S*) is available since 1998 as an antiplatelet drug used in prevention or treatment of myocardial infarction and other diseases associated with atherosclerosis. Clopidogrel and ticlopidine are usually called antiplatelet thienopyridines. Their beneficial actions are linked with their ability to antagonise platelet ADP receptors. The difference between ticlopidine and clopidogrel chemical structure consists in the replacement of a CH_2 group by a $CH\text{-}COOCH_3$ group of atoms (Dupin *et al*., 2002).

Purpose of the Present Study

The tests were done to find the presence of the active chemical constituents such as alkaloids, terpenoids, steroids, flavonoids, reducing sugar, tannin**,** saponins, cardic glycoside and anthraquinones. The antioxidant property of fresh leaves of *Argemone mexicana* use in the management and treatment of various diseases. The present study investigates the antioxidant

and the total phenolic content property of the plant of *Argemone mexicana*. Herbs and their components can be use for thrombolysis and possessing antithrombotic activity has been reported before (Prasad *et al*., 2007). In this study, an attempt has been made to investigate whether *Argemone mexicana* leaves extracts possess thrombolytic activity or not.

CHAPTER 2:

LITERATURE REVIEW

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].

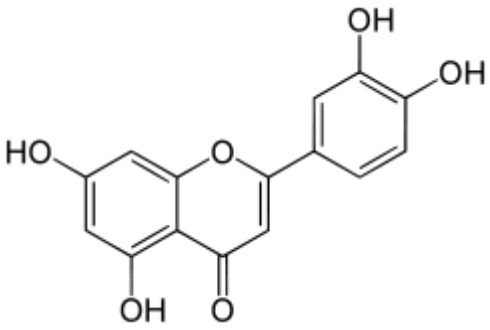


Figure2.1: Structure of luteolin

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].

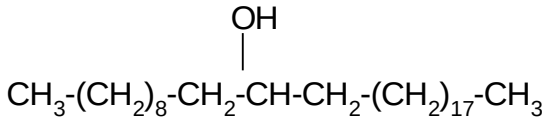


Figure2.2: Structure of triacontan-11-ol

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]

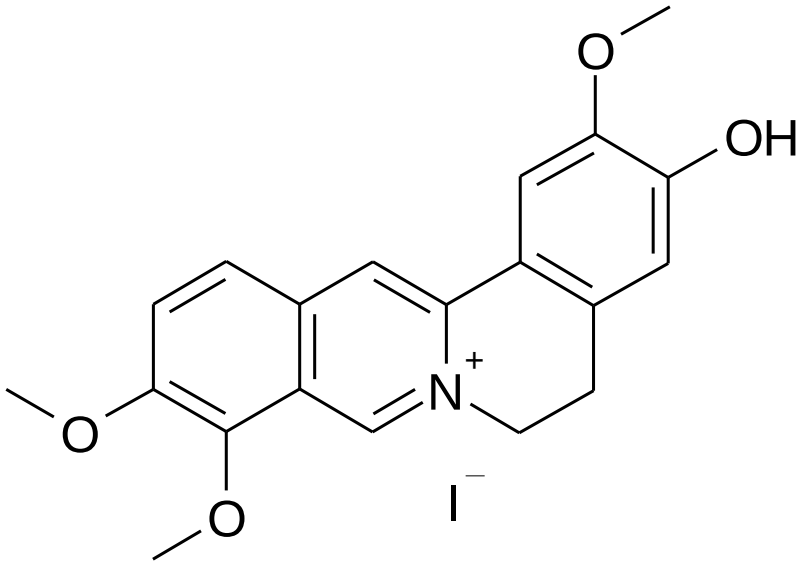


Figure2.3*:* Structure of jatrorrhizine

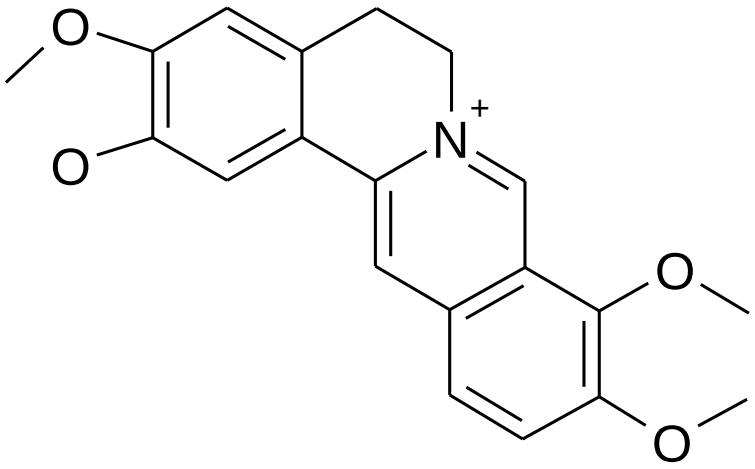


Figure2.4: Structure of columbamine

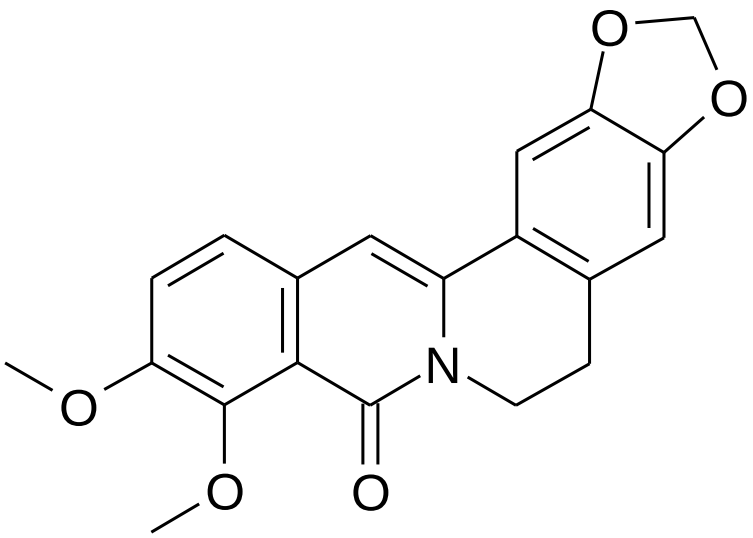


Figure2.5: Structure of oxyberberine

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] .

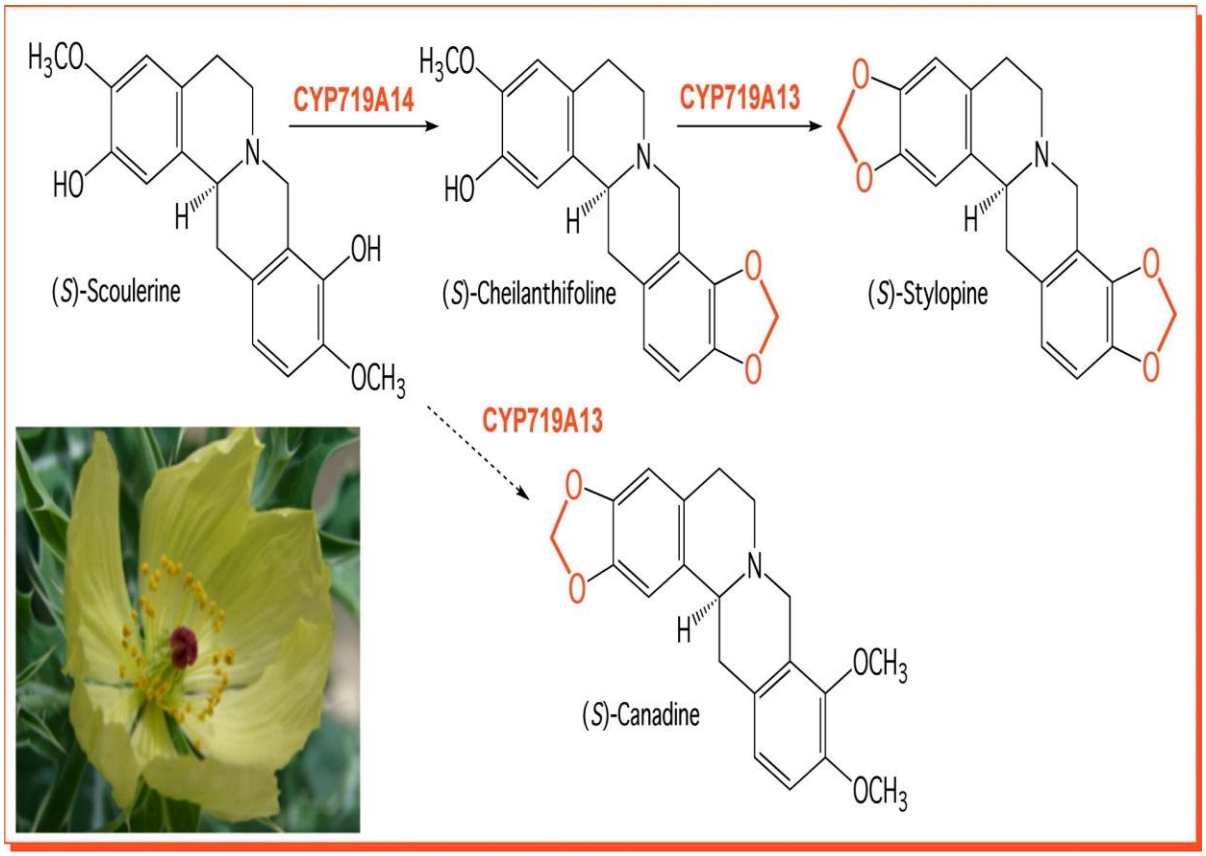


Figure2.6: Graphical view of CYP719A13 participates in both sanguinarine and berberine biosynthesis. Prepared from 2-month-old plantlets of the Mexican prickly poppy *Argemone Mexicana.*

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. mexicanawere 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. mexicanawere 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.

Table3.1: 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

Table 3.2: Time and temperature of extraction

Solvent	Temperature	Time
n-hexane	40°C	1 h 57 min
Ethyl acetate	50°C	3 h 31 min
Methanol	50°C	2 h 48 min

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

Figure 3.2: Soxhlet apparatus

Figure 3.3: Extracts (before evaporation)

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.

Figure3.4: 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.

Figure 3.5: 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)
- 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

- Glass rod
- Rotary evaporator (IKA RRV05 Basic, Biometra, Germany)
- Aluminum foil (covered the beaker and conical flask)
- Di-ethyl-ether (Merck, Germany)
- Electric Balance; SHIMADZU AY220 & SCALTEC SPB31
- Beaker; 25ml

Screening of Phytochemical Constituents

Phytochemical analysis of the extracts was conducted using the following procedures. The tests were carried out to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugars and tannins.

Test for alkaloids: Hager's test

Method

Preparation of 2% H_2SO_4 solution

2 ml of 1 N H_2SO_4 was taken in a volumetric flask. Volume was then adjusted to 100 ml using distilled water.

Preparation of Hager´s reagent

0.25 gm of picric acid was weighed in a volumetric flask. 25 ml of distilled water was added to prepare solution.

Procedure (Nobakht *et al*., 2010)

0.02 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. 10 ml of methanol was added in each test tube and test tubes were placed in vortex mixture to dissolve the extracts. The extract solutions were then filtered. 2 ml of filtrate were taken and mixed with 4 drops of 2% H_2SO_4. To 1 ml of this mixture 6 drops of Hager´s reagent was added. Yellow (turbid) color indicates presence of alkaloids (Figure 20).

Figure 3.6: Identification of alkaloids by Hager's reagent

Test for flavonoids: Ammonia test (modified)

Method

Preparation of 5% ammonia solution

5 ml of ammonia was transferred in a volumetric flask. Volume was then adjusted to 100 ml using distilled water.

Procedure (Edeoga *et al*., 2005)

0.25 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. 5 ml of ethyl acetate was added in each test tube. Then test tubes were heated at 40°C for 3 min in water bath. The mixtures were filtered and 2 ml of filtrate were taken. 0.5 ml of 5% ammonia solution was added. Yellow color shows the presence of flavonoid.

Test for steroids: Salkowski test

Method

Procedure (Nobakht *et al*., 2010)

0.02 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. The extracts were mixed with 2 ml of methanol and filtered. 1 ml chloroform and 1 ml concentrated H_2SO_4 were added into the filtrate. Yellow green fluorescent indicates the presence of steroids.

Test for terpenoids: Salkowski test (modified)

Procedure (Chhetri *et al*., 2008)

0.004 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. The extracts were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. 3 ml concentrated H_2SO_4 were added slowly. Red violet color represents the presence of terpenoid

Test for carbohydrates: Fehling's (Reducing sugar) test (modified)

Method

Preparation of Fehling I solution

3.436 gm copper II sulphate crystals (Merck, Germany) in water containing a few drops of dilute H_2SO_4 (5-10%) was dissolved in 20 ml distilled water. Final volume of solution was made upto 50 ml.

Preparation of Fehling II solution

6 gm of pure NaOH (Merck, Germany) and 17.1 gm of Na-K tartrate (BDH chemicals ltd., England) was dissolved in 20 ml of distilled water. Final volume of solution was made up to 50 ml.

Preparation of Fehling solution

5 ml of Fehling I solution and 5 ml of Fehling II solution was taken in a conical flask and mixed properly.

Procedure (Chhetri *et al*., 2008)

0.02 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. The extracts were dissolved in 0.5 ml of methanol. Then 1 ml of water was added in it. 5-8 drops of Fehling solution were added. The samples were heated. Carbohydrate present or absent depends on brick red precipitate..

Test for saponins: Frothing test

Procedure (Ayoola *et al*., 2008)

0.5 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed

in 3 different test tubes. 5 ml of distilled water was added. The solutions were shaken vigorously and observed for a stable persistent froth. The frothing were mixed with 3 drops of olive oil and shaken vigorously. An emulsion formation indicates the presence of saponin.

Test for tannins: $FeCl_3$ test

Method

Preparation of 0.1% ferric chloride solution

0.1 gm of ferric chloride was accurately weighed in a 100 ml volumetric flask and it was dissolved in 50 ml of distilled water. Final volume was made upto 100 ml.

Procedure (Edeoga *et al*., 2005)

0.125 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. 5 ml of distilled water was added and dissolved by vortex mixture. Then samples were boiled for 3 min. in water bath. The samples were filtered. 3 drops of 0.1% ferric chloride solution were added into the filtrate. Brownish green or blue-black colouration shows the presence of tannin (Figure 25).

Test for cardiac glycosides: Killer-Killani's test

Method

Procedure (Edeoga *et al*., 2005)

0.02 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. 5 ml of methanol was added in each test tube, and dissolved by vortex mixture. Then the extract solutions were treated with 2 ml of glacial acetic acid containing 3 drops of ferric chloride solution. They were underlayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

Test for anthraquinones: Chloroform layer test

Method

Procedure (Ayoola *et al*., 2008)

0.5 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different test tubes. 10 ml of H_2SO_4 were added in each test tube. The samples were kept in water bath for 3 min. to boil. Then they were filtered while hot. Filtrates were shaken with 5 ml of chloroform. Chloroform layer were pipette into another test tube. 1 ml dilute ammonia was added. Resulting solutions were observed for color changes.

DPPH Free Radical Scavenging 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.

Positive Control

After 24 hours the test tubes, in which positive control test was performed by using $KMnO_4$, were observed by using a magnifying glass to see any living nauplius was present in the test tube's solution. By counting the living nauplii, percent mortality for each of the dilution was calculated. Regression analysis of the data was done by using Microsoft Excel 2010, from which an equation was derived and these data were converted into a chart, from which LC_{50} value for the positive control was found out.

In here, LC_{50} or Median Lethal Dose represents the concentration of $KMnO_4$, in which half of the test population (brine shrimp nauplii) was suffered from fatality. The plot, by which LC_{50} value was determined by regression analysis, contains % mortality in the Y-axis and the concentration in the X-axis.

After taking absorbance of all test tubes including ascorbic acid which was used as positive control in DPPH free radical scavenging test. The gathered absorbance of each dilution of ascorbic acid in both cases, which is with DPPH and without DPPH, was used to prepare the % of Inhibition. The % of inhibition was calculated by using the following equation,

$$\%\text{inhibition} = 1 - \left[\frac{\text{Absorbance with DPPH} - \text{Absorbance without DPPH}}{\text{Absorbance of blank solvent} + \text{DPPH}}\right] \times 100$$

The efficacy of free radical (DPPH) scavenging was expressed by median Inhibitory Concentration (IC_{50}).

In table --, IC_{50} or concentration of Ascorbic Acid which inhibited or scavenged half of the free radical (DPPH) in all prepared dilutions of ascorbic acid, IC_{50} value was calculated by using regression equation. The regression equation was developed by the plot of % of inhibition in X axis against concentration in Y axis. By means of regression analysis the best-fit line was found from the curve data. The regression analysis was done by using Microsoft Office Excel 2007.

Nitric Oxide Scavenging Capacity Assay

Preparation of 5 mM solution of sodium nitroprusside

0.075 gm of Sodium nitroprusside was accurately weighed in 50 ml volumetric flask which was dissolved in distilled water. Then the volume was made upto 50 ml with distilled water. The absorbance of this solution was taken at 546 nm as a control solution absorbance against distilled water as a blank.

Preparation of Griss reagent

0.5 gm Sulfanilamide, 0.05 gm N-(1-Naphthyl ethylenediamine dihydrochloride), were accurately weighed and taken in a volumetric flask. 1 ml o-phosphoric acid was added into the volumetric flask and mixed properly. Then volume was adjusted by distilled water upto 50 ml.

Preparation of stock of extract solution

0.006 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighed in 3 different volumetric flasks and it was dissolved in 30 ml of ethanol by vortex mixture. Conc. of each extract solution was 200 µg / ml.

The recorded absorbances of different concentration of *Argemone Mexicana* extract solution those were 10 to 200µgm/ml and ascorbic acid as positive control were placed in a Microsoft office excel sheet. The absorbances were taken in two step, in first step absorbances were taken without introducing sodium nitroprusside and Griss reagent and in second step absorbances were taken after incorporating sodium nitroprusside and Griss reagent, subtraction first one from second yielded the net absorbance of different dilutions of n-hexane, ethylacetate and methanolic extract of *Argemone Mexicana*. The percent of scavenging was calculated by using the gathered absorbance data according to the previously mentioned equation.

Value of median inhibitory concentration or IC_{50} represented the effectiveness of an extract solution to scavenge free radical molecules or reactive oxygen species (NO).

Total Phenolic Content

Method (Ainsworth & Gillespie, 2007)

Preparation of solutions of plant extracts

0.02 gm of *n*-hexane, ethyl acetate and methanol extracts of *Argemone mexicana* leaves were weighe

in 3 different centrifuge test tubes. 2 ml of methanol was added in each test tube. The extracts were dissolved using vortex mixture. It was kept at room temperature for 48 h in dark.

Preparation of 10% Folin-Ciocalteu phenol reagent

5 ml of F-C reagent was transferred in a volumetric flask. Volume was then adjusted to 50 ml using distilled water.

Preparation of 700 mM Na_2CO_3 solution

7.416 gm of Na_2CO_3 was accurately weighed in a volumetric flask. 100 ml of distilled water was added and dissolved.

Procedure

After 48 h test tubes were centrifuge in 5000 rpm for 5 min. at room temperature. After centrifuge the sample supernatant were transferred in other test tubes. 300 µl sample supernatant was withdrawn from each plant extract solution to a separate test tube. 300 µl of methanol (blank) was taken as negative control to a separate test tube. 600 µl of 10% F-C reagent solution were added in each test tubes and vortex thoroughly. 2.4 ml of 700 mM Na_2CO_3 solution was added in each test tube (Figure 30) and it was kept at room temperature for 2 h. The absorbance of total volume (3.3 ml) was taken in UV-VIS spectrophotometer at 765 nm. Steps, from transferring 300 µl sample supernatant to taking the absorbance this were done for three times, accordance to constitutive interval. The total phenolic contents were determined from a standard curve prepared with gallic acid and the results were expressed as Mean±SD

***In Vitro* Thrombolytic Activity Test**

Blood sample

Whole blood (n=10) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 500µl of blood was transferred to the previously weighed eppendorf tubes and was allowed to form clots.

Preparation of stock solution:

5mL normal saline was added to streptokinase vial and was mixed properly.

Procedure:

The thrombolytic activity of the extract was evaluated by using streptokinase as the standard. 3mL venous blood was withdrawn from the human healthy volunteers who have not taken anticoagulant therapy or oral contraceptive for two weeks. Empty eppendorf tubes were weighed as W. 500µL of fresh blood were transferred to the pre weighed eppendorf tubes (500µL/tube) and incubated at 37˚C for 45 minutes for the formation of clot. After the clot has formed, the pale yellow plasma fluid or serum was completely removed by the syringe without the disruption of clot. The tube with the removed serum having the clot was again weighed as W'.

Clot weight= weight of clot containing tube – weight of tube alone

The eppendorf tubes containing clot was properly labeled. 100µL of the ethanolic extract solution was added to the tubes containing pre weighed clot. For positive control, 100µL of streptokinase (1500000 I.U/ml) and for negative control 100µL of saline were added to the tubes. All the tubes were incubated at 37˚C for 90 minutes and observed for the lysis of clot. The tubes were taken out from the incubator after the incubation has completed and the fluid released were withdrawn or removed. Each tubes were again weighed as W" to see the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

Clot lysis was expressed as percentage. % of clot lysis can be calculated by a paired T-test analysis or ANOVA**.**

CHAPTER 4:

RESULTS & DISCUSSION

4. RESULT AND DISCUSSION

Screening of Phytochemical Constituents

After preparation of *n*-hexane, Ethylacetate and Methanol extracts of Argemone Mexicana. The extracts were run through a phytochemical screening. Among different phytoconstituent Alkaloid, Flavonoid, Steroid, Terpenoid, Reducing Sugar, Saponins, Tannins, Cardiac glycoside and Anthraquinones these phytoconstituents were tested according to a standard procedure for each.

Table 4.1: Data of phytochemical screening of *A. mexicana*

Phytoconstituent	**Name of the test**	**AMHE**	**AMEA**	**AMME**
Alkaloid	Hager's test	+	+++	+
Flavonoid	Ammonia test (modified)	++	+++	+++
Steroid	Salkowski test	-	-	-
Terpenoid	Salkowski test (modified)	+++	++	+++
Reducing sugar	Fehling's test	-	-	+
Saponins	Frothing test	+	+++	-
Tannins	FeCl3 test	-	+	+++
Cardiac glycoside	Killer-Killani's test	-	-	-
Anthraquinones	Chloroform layer test	+	-	-

Key: +++ = highly present; ++ = moderately present; + = slightly present; - = absent

DPPH Free Radical Scavenging Test

Table 4.2: IC_{50} value of ascorbic acid (AA)

Code	Conc.(µg/ml)	Ab. Without DPPH	Ab. With DPPH	Absorbance	Blank	%Inhibition	IC_{50}
	0			0.197	0.197	0.00	
AA1	20	0.00	0.132	0.132	0.197	32.99	
AA2	40	0.001	0.120	0.119	0.197	39.59	76.11
AA3	60	0.001	0.108	0.107	0.197	45.69	
AA4	80	0.002	0.101	0.099	0.197	49.75	
AA5	100	0.003	0.089	0.086	0.197	56.35	

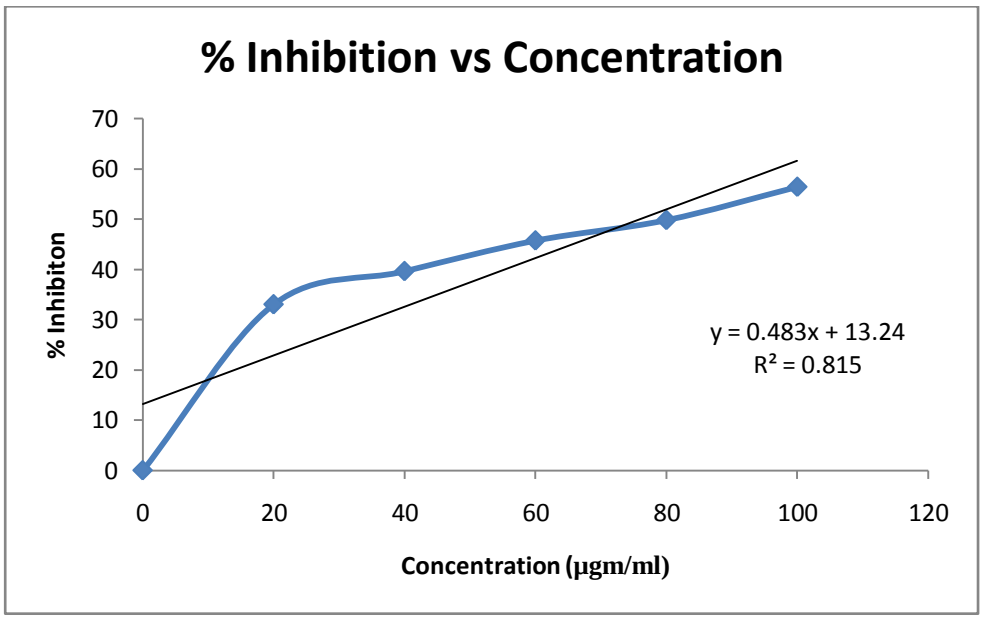


Figure: % inhibition curve of ascorbic acid (AA)

The degree of percent of inhibition or scavenging was being increasing respectively with the increasing of ascorbic acid ranging from highly significant highest concentration to significant lowest concentration. Maximum inhibition or scavenging of free radical took place at a concentration of 100µgm/ml. The obtained IC_{50} value was 76.11, which represents that

Table 4.3: IC_{50} value of the leaf of AMHE

	Concentration	Ab. Without DPPH	Ab. With DPPH	Absorbance	Blank	%Inhibition	IC_{50}
	0			0.197	0.197	0	
11	20	0.001	0.123	0.122	0.197	38.07	
12	40	0.003	0.113	0.110	0.197	44.16	73.73
13	60	0.005	0.107	0.102	0.197	48.22	
14	80	0.007	0.104	0.097	0.197	50.76	
15	100	0.009	0.098	0.089	0.197	54.82	

76.11µgm/ml of ascorbic acid was required to scavenge 50% of free radical present in the solution. This will be considered as positive control value for justifying the IC_{50} values of other three plant extract dilutions.

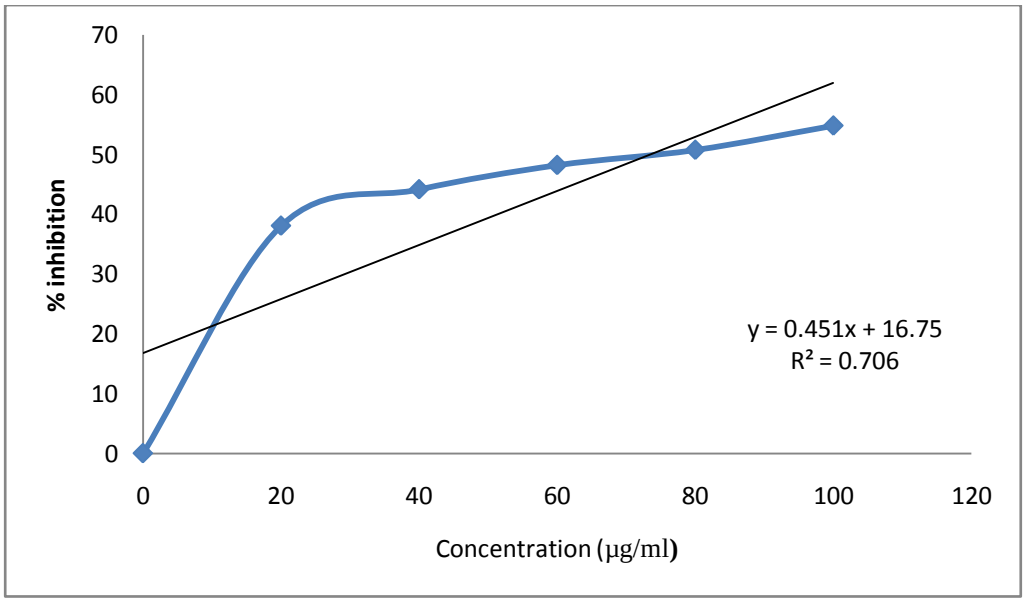


Figure: Scavenging effects of the leaf of AMHE

Similar to the positive control test, the absorbance of different concentration of n-hexane extract solution of *Argemone Mexicana* were taken, in both without and with DPPH and the percent of scavenges was calculated as it has been done in Ascorbic Acid.

In table --, The IC_{50} value of n-hexane extract dilutions was calculated by using regression equation. The regression equation was developed by the plot of % of inhibition or scavenge in X axis against representing concentration in Y axis. By means of regression analysis the best-fit line was found from the curve data. The regression analysis was done by using Microsoft Office Excel 2007.

Here, the extent of % of scavenging was directly proportional to given concentration, in other word the free radical scavenging was increasing with the increasing of concentration of extract from lowest significant concentration to highest significant concentration. IC_{50} value obtained from n-hexane dilutions was 73.73µgm/ml.

Table: IC_{50} value of the leaf of AMEA

Code	Concentration	Ab.without DPPH	Ab.with DPPH	Absorbance	Blank	%Inhibition	IC_{50}
	0			0.197	0.197	0	
21	20	0.009	0.096	0.087	0.197	55.84	
22	40	0.015	0.093	0.078	0.197	60.41	39.91
23	60	0.021	0.086	0.065	0.197	67.01	
24	80	0.027	0.074	0.047	0.197	76.14	
25	100	0.036	0.071	0.035	0.197	82.23	

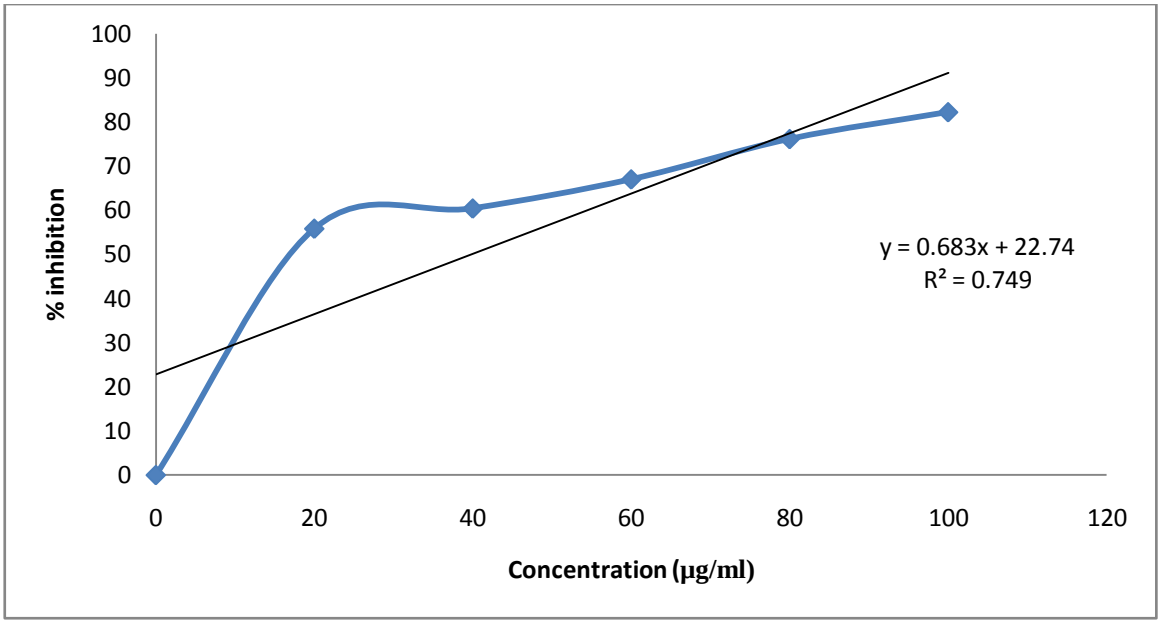


Figure: Scavenging effects of the leaf of AMEA

The percent of inhibition was calculated by using the gathered absorbance of both without and with DPPH in ethyl-acetate extract dilutions, as previous extract dilutions had been done.

The IC_{50} value of ethyl-acetate extract dilutions was calculated by using regression equation. The regression equation was developed by the plot of % of inhibition or scavenge in X axis against representing concentration in Y axis. By means of regression analysis the best-fit line was found from the curve data. The regression analysis was done by using Microsoft Office Excel 2007.

Here, the magnitude of % of inhibition or scavenging was directly proportional to given concentration, in other word the free radical scavenging was increasing with the increasing of concentration of extract from lowest significant concentration to highest significant concentration. IC_{50} value obtained from ethyl-acetate dilutions was 39.91µgm/ml.

Table 4.5: IC_{50} value of the leaf of AMME

Code	Concentration	Ab.without DPPH	Ab.with DPPH	Absorbance	Blank	%Inhibition	IC_{50}
	0			0.197	0.197	0	
31	20	0.002	0.160	0.158	0.197	19.80	
32	40	0.004	0.122	0.118	0.197	40.10	
33	60	0.005	0.069	0.064	0.197	67.51	54.32
34	80	0.007	0.061	0.054	0.197	72.59	
35	100	0.009	0.051	0.042	0.197	78.68	

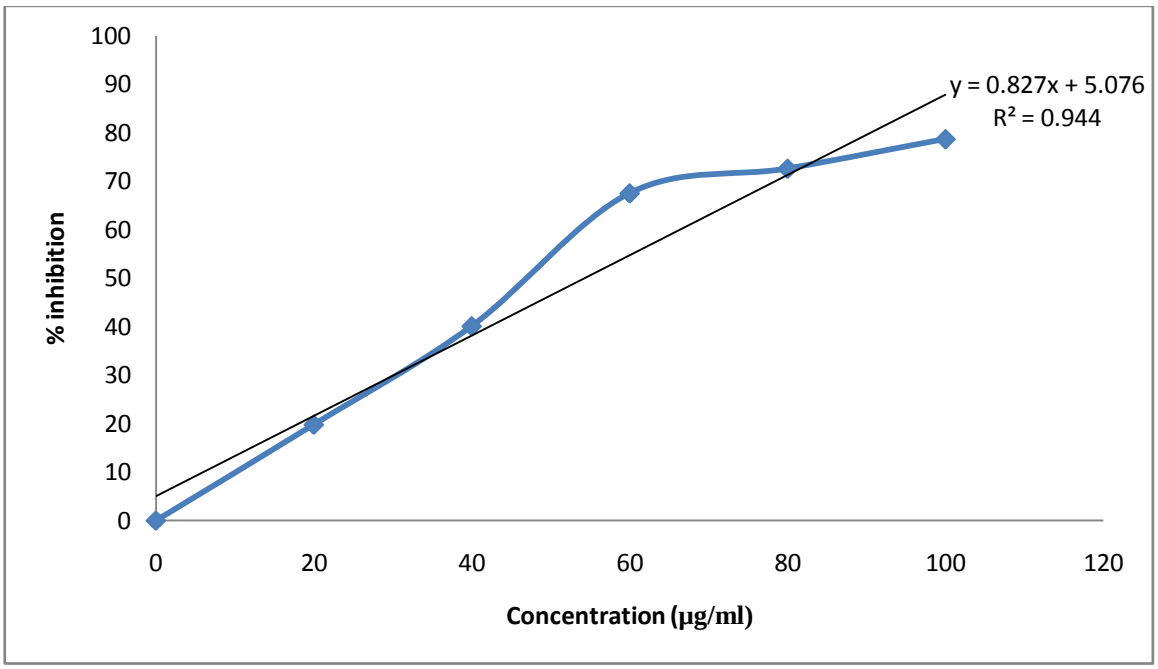


Figure: Scavenging effects of the leaf of AMME

Similar as previous extract dilutions the percent of inhibition was calculated by using the absorbance found from both without and with DPPH in methanol extract dilutions.

The IC_{50} value of methanol extract dilutions was calculated by using regression equation. The regression equation was developed by the plot of % of inhibition or scavenge in X axis against representing concentration in Y axis. By means of regression analysis the best-fit line was

found from the curve data. The regression analysis was done by using Microsoft Office Excel 2007.

In the table __, the level of % of inhibition was directly proportional to given concentration, in other word the free radical scavenging was increasing with the increasing of concentration of methanol extract from lowest significant concentration to highest significant concentration. IC_{50} value obtained from methanol extract dilutions was 54.32µgm/ml.

Table 4.6: % inhibition of AMHE, AMEA, AMME and AA

Concentration	AMHE	AMEA	AMME	AA
0	0.00	0.00	0.00	0.00
20	38.07	55.84	19.80	32.99
40	44.16	60.41	40.10	39.59
60	48.22	67.01	67.51	45.69
80	50.76	76.14	72.59	49.75
100	54.82	82.23	78.68	56.35

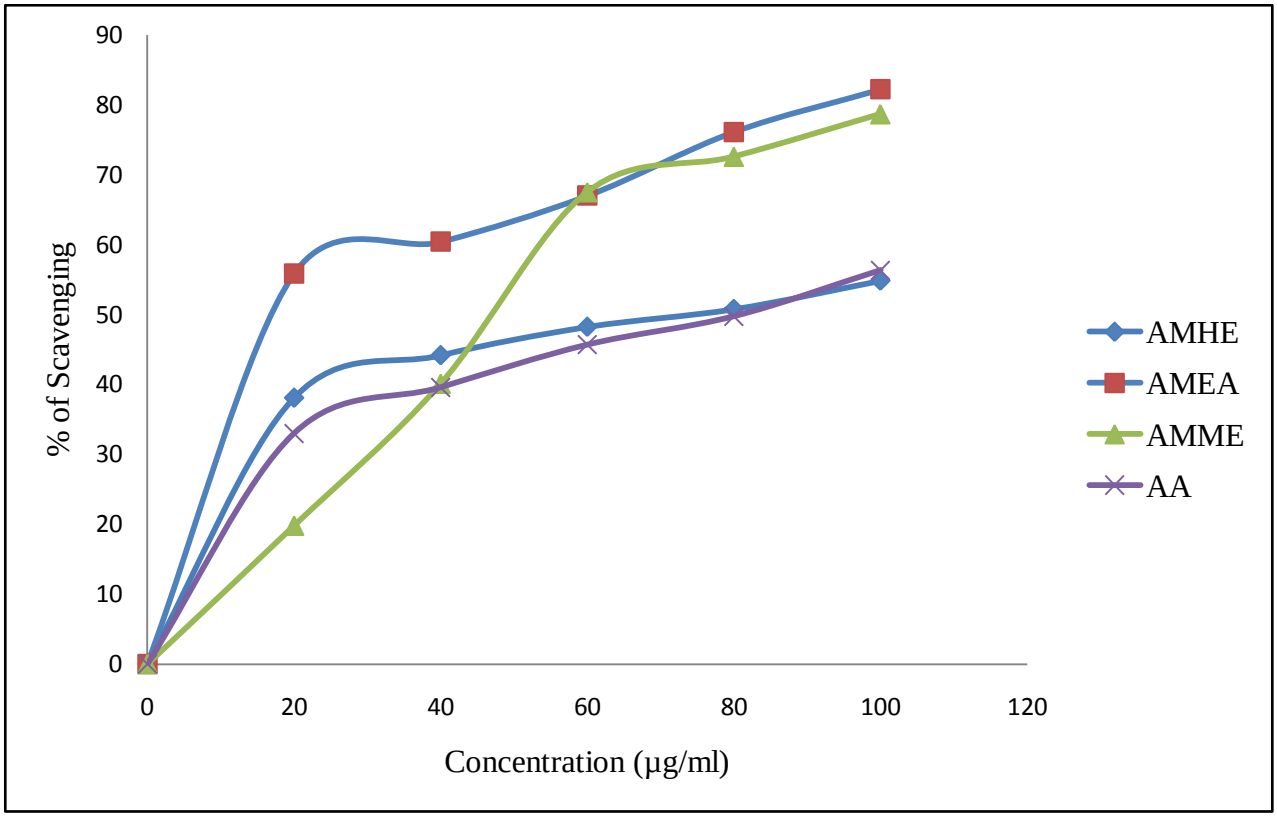


Figure: DPPH radical scavenging activity of AA and the APHE, APEA & APME extracts of *A. mexicana*

In table--, the % of scavenging of different extract were given in a plot against concentration the result of free radical scavenging method by using DPPH is given in following tables, along with representative IC_{50} values, regression equations with figure and value of R^2

Nitric Oxide Free Radical Scavenging of Leaf of *A. polystachya*

The recorded absorbances of different concentration of *Argemone Mexicana* extract solution those were 10 to 200µgm/ml and ascorbic acid as positive control were placed in a Microsoft office excel sheet. The absorbances were taken in two step, in first step absorbances were taken without introducing sodium nitroprusside and Griss reagent and in second step absorbances were taken after incorporating sodium nitroprusside and Griss reagent, subtraction first one from second yielded the net absorbance of different dilutions of n-hexane, ethylacetate and

methanolic extract of *Argemone Mexicana*. The percent of scavenging was calculated by using the gathered absorbance data according to the previously mentioned equation.

Value of median inhibitory concentration or IC_{50} represented the effectiveness of an extract solution to scavenge free radical molecules or reactive oxygen species (NO).

IC_{50} value was calculated by using regression equation developed from a plot where, % of scavenging in X axis versus concentration in Y axis. The best-fit line was obtained from curve data through regression analysis. The regression analysis was done by using Microsoft Office Excel 2007.

Table: IC_{50} value of ascorbic acid (AA)

Code	**Concentration**	**Absorbance**	**Abs of Blank**	**% inhibition**	**IC_{50}**
0	0	0.096	0.096	0.00	
AA1	5	0.049	0.096	48.96	
AA2	25	0.034	0.096	64.58	34.06
AA3	50	0.026	0.096	72.92	
AA4	100	0.021	0.096	78.13	
AA5	200	0.015	0.096	84.38	

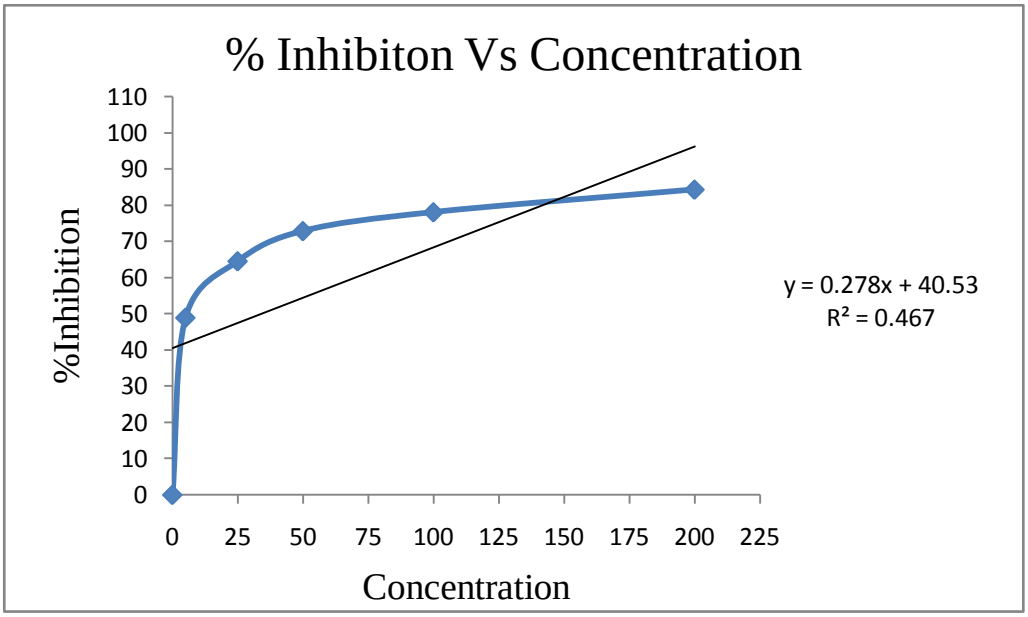


Figure: % inhibition curve of Ascorbic acid (AA)

The percent of free radical scavenging was increased respectively with the increasing of concentration of ascorbic acid ranging from highly significant highest concentration to significant lowest concentration. Maximum inhibition or scavenging of free radical took place at a concentration of 100µgm/ml. The obtained IC_{50} value was 76.11, which represents that 76.11µgm/ml of ascorbic acid was required to scavenge 50% of free radical present in the solution. This will be considered as positive control value for justifying the IC_{50} values of other three plant extract dilutions.

From fig—above, it is straightly distinguishable that the correlation among level of percent of free radical scavenging and concentration ranging from lowest to highest level of ascorbic acid is proportional. Maximum scavenging 84.3% of free radical occurred at a concentration of 200µg/ml. The IC_{50} value was recorded 34.06µg/ml. This value was regarded as positive control for comparing with IC_{50} values gathered from plant extract solutions.

Table 4.7: IC_{50} value of the leaf of AMHE

Code	Concentration	Abs without NO_2	Abs with NO_2	Absorbance	Abs of Blank	% inhibition	IC_{50}
0	0			0.096	0.096	0.00	
11	5	0.014	0.104	0.090	0.096	6.25	
12	25	0.044	0.126	0.082	0.096	14.58	152.73
13	50	0.014	0.085	0.071	0.096	26.04	
14	100	0.007	0.066	0.059	0.096	38.54	
15	200	0.001	0.039	0.038	0.096	60.42	

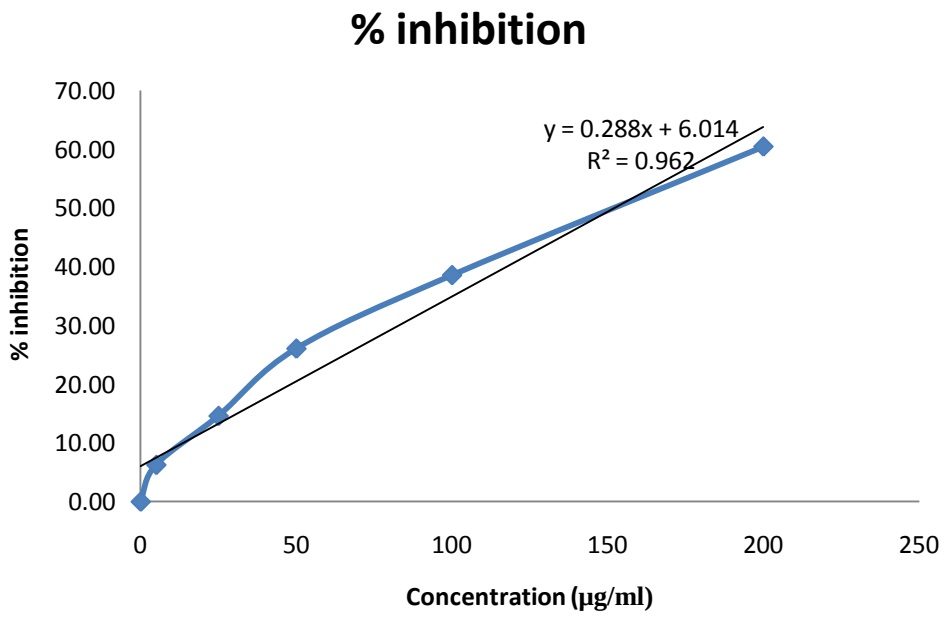


Figure: Scavenging effects of the leaf of AMHE

Alike previous one the value of IC_{50} was estimated by using regression equation from a % of scavenging versus concentration plot. By way of regression analysis the best-fit line was found from curve data. The regression analysis was done by using Microsoft Office Excel 2007. From fig—above, it was clearly observed that the level of percent of free radical scavenging was being increasing proportionally with concentration ranging from lowest to highest level of n-hexane extract. Maximum scavenging 60.42% of free radical occurred at a concentration of 200µg/ml. The calculated IC_{50} value was 152.73µg/ml.

Table 4.8: IC_{50} value of the leaf of AMEA

Code	**Concentration**	**Abs without NO_2 + Griss**	**Abs with NO_2 + Griss**	**Absorbance**	**Abs of Blank**	**% inhibition**	**IC_{50}**
00	0			0.096	0.096	0.00	
21	5	0.010	0.058	0.048	0.096	50.00	
22	25	0.008	0.052	0.044	0.096	54.17	69.61
23	50	0.003	0.044	0.041	0.096	57.29	
24	100	0.000	0.036	0.036	0.096	62.50	
25	200	0.001	0.031	0.03	0.096	68.75	

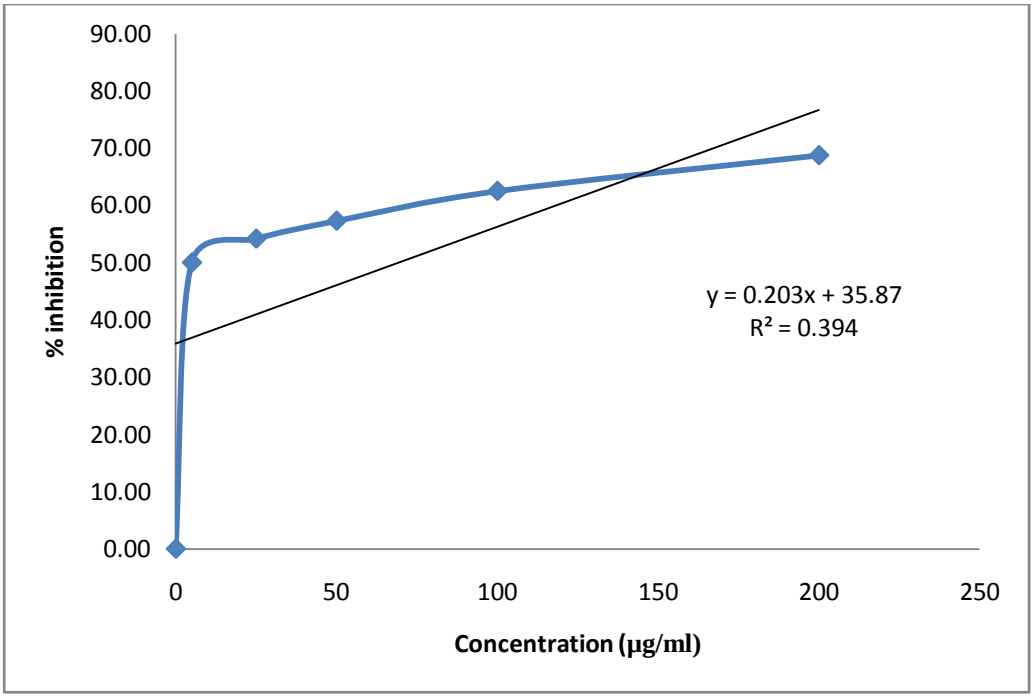


Figure: Scavenging effects of the leaf of AMEA

Here, IC_{50} value was calculated by using regression equation from a plot, in which % of scavenging placed in X axis against different concentrations of methanolic extract of *Argemone Mexicana* in Y axis. Through regression analysis the best-fit line was established from curve data. The regression analysis was done by using Microsoft Office Excel 2007.

From fig—above, it was observed that the level of percent of free radical scavenging directly proportional to concentration ranging from lowest to highest level of ethylacetate extract. Maximum scavenging 68.75% of free radical occurred at a concentration of 200µg/ml. The calculated IC_{50} value was 69.61µg/ml.

Table 4.9: IC_{50} value of the leaf of AMME

Code	Concentration	Abs without NO_2 + Griss	Abs with NO_2 + Griss	Absorbance	Abs of Blank	%inhibition	IC_{50}
0	0			0.096	0.096	0.00	
31	5	0.009	0.067	0.058	0.096	39.58	
32	25	0.007	0.047	0.040	0.096	58.33	65.56
33	50	0.002	0.039	0.037	0.096	61.46	
34	100	0.003	0.038	0.035	0.096	63.54	
35	200	0.001	0.026	0.025	0.096	73.96	

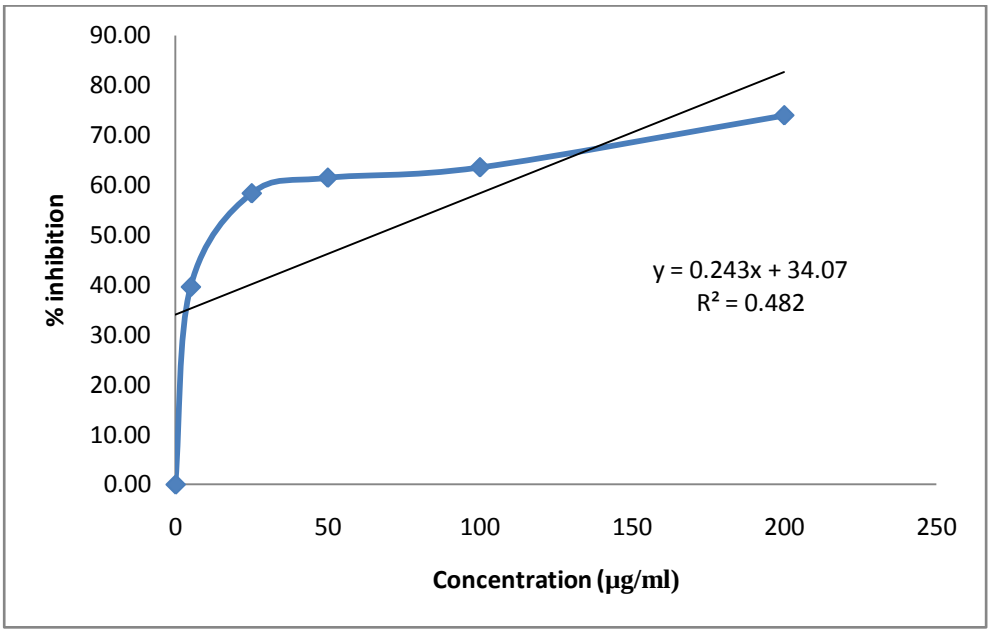


Figure: Scavenging effects of the leaf of AMME

In figure, the plot of percent of inhibition against concentration of given methanolic extract of *Argemone Mexicana* in ascending order yielded a regression equation which introduced the IC_{50} value. And from curve data the best fit line was obtained using regression analysis. The regression analysis was done by using Microsoft Office Excel 2007. From above diagram it was seen that the degree of percent of inhibition is directly proportional to concentration increasing in ascending order. More over according to the plot of methanolic extract of *Argemone Mexicana* the highest percent of scavenging of free radical 73.96% was recorded at the concentration of 200µg/ml. A sharp increase in %inhibition or scavenging was observed by the change of concentration from 0µg/ml to 5µg/ml. the calculated IC_{50} value was 65.56µg/ml.

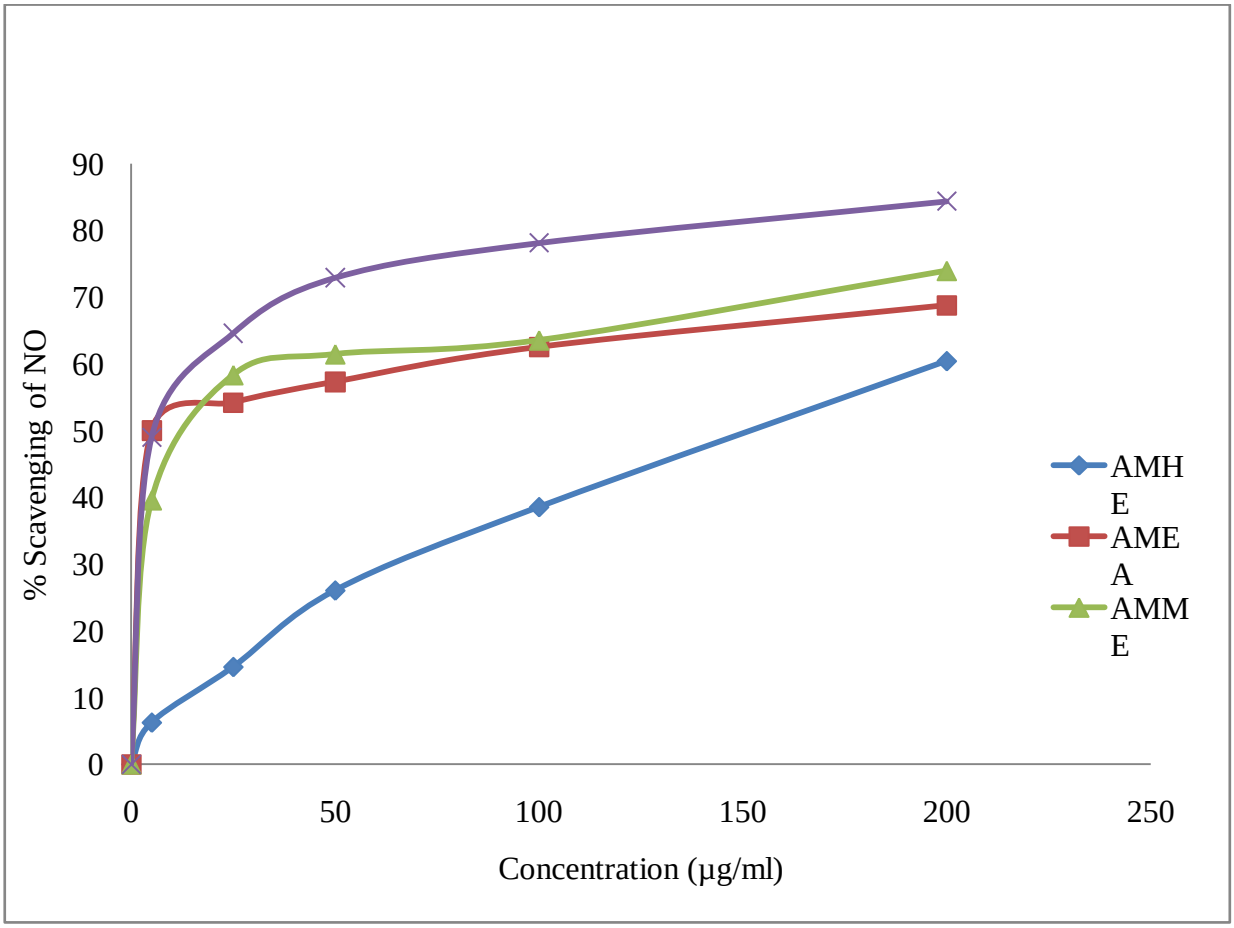


Figure: NO scavenging test

Total Phenolic Content

Total Phenolic Content of extract of *Argemone Mexicana*:

Table 4.10: Absorbance of gallic acid

SL. No.	Conc. Of the Standard (µg / ml)	Absorbance	Regression line	R^2
1	0	0		
2	10	0.234		
3	20	0.487		
4	30	0.718		
5	40	1.140		
6	50	1.521	y = 0.037x-0.209	0.984
7	60	2.294		
8	70	2.285		
9	80	2.711		
10	90	3.188		
11	100	3.665		

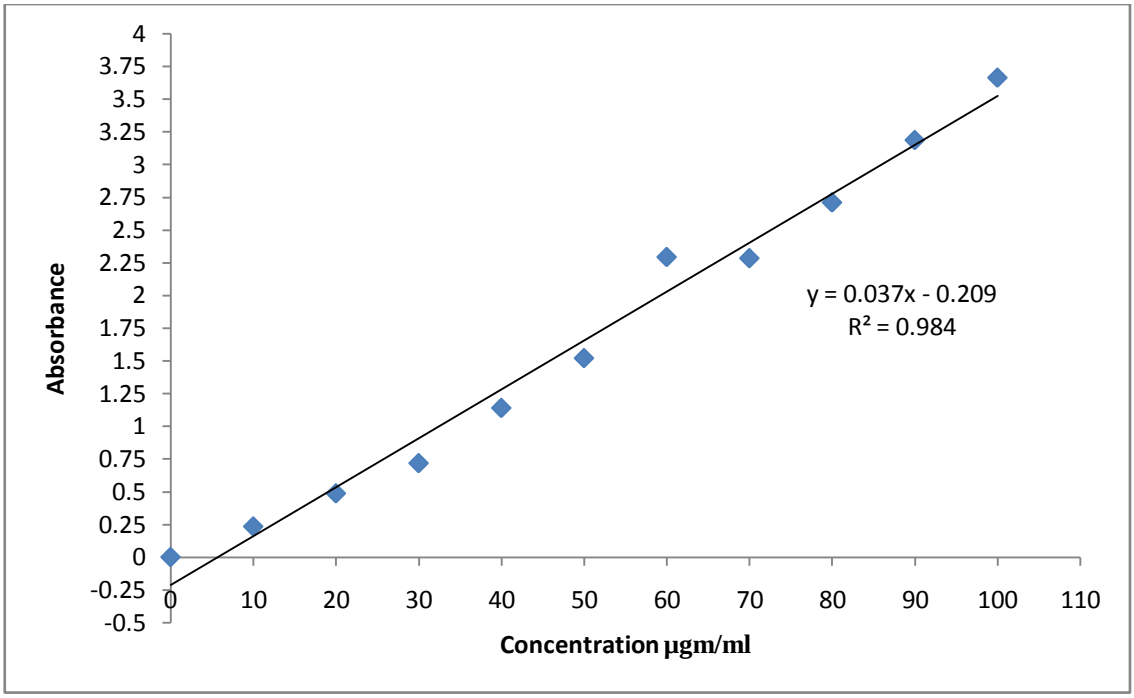


Figure: Standard curve of gallic acid

After absorbances were taken of different solution of Gallic acid, which formerly were prepared delivering a concentration ranging from 10 µg / ml to 100 µg / ml. A linear relationship was observed, when the absorbances in X axis was plotted against the concentration in Y axis. This linear curve was considered as a standard curve and to determine the total phenolic content of the test samples this standard curve was used. Regression analysis is calculated in Microsoft Office Excel 2007.

Extracts	Total Phenolic content (n=3) (in mg/g, Gallic acid equivalents)
AMHE	35.59±2.58
AMEA	106.65±2.42
AMME	70.19±3.64

After the absorbance was taken of the three extract samples of Argemone Mexicana for three times. The total phenols content of the samples were measured by using the standard curve

developed from different concentration of Gallic acid solution. The total phenolic contents of the plant Argemone Mexicana extract were expressed as mg of GAE (Gallic acid equivalent) / gm.

Thrombolytic Activity:

Table: Clot lysis of blood sample data of extracts of *A. mexicana*fruit and streptokinase.

Extracts/Positive control	**Mean ± S.D. (% Clot Lysis)**
AMHE	10.397±3.493[x]
AMEA	13.861±5.519[x]
AMME	3.163±1.764[y]
Streptokinase	40.755±5.141[x]
Normal Saline	4.712±0.889[y]

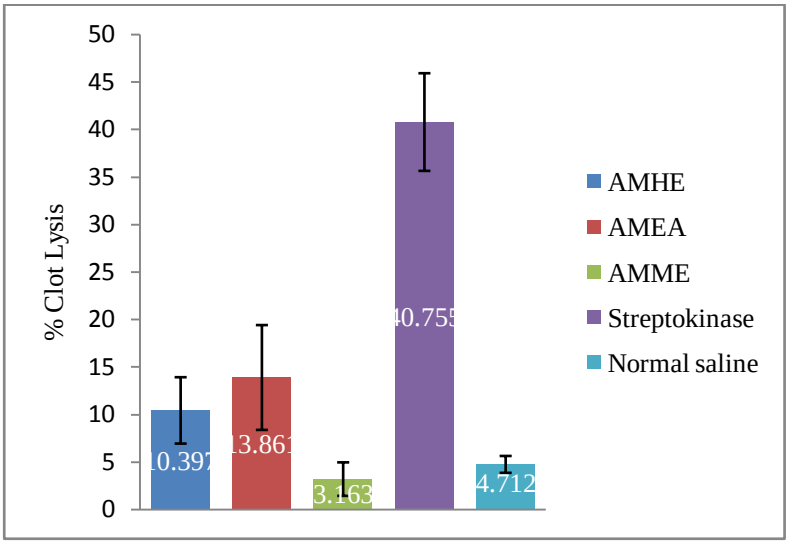


Figure 4.1: Clot lysis of blood samples of normal subjects by APHE, APEA, and APME extracts of *A. mexicana*leaves, streptokinase and normal saline.

After removing serum the colt of collected blood samples was treated with N-hexane, Ethyl acetate and Methanolic extract solution of Argemone Mexicana successively. In addition the

clots were also treated with streptokinase as positive control and normal saline as negative control. The clot lysis was observed after 90 minutes. The colt lysis was calculated in percentage. Values of colt lysis by different extract solution were expressed in Mean of percent value ± Standard Deviations (SD).

In table, Mean Percent values ± SD marked by ^{x} or ^{y} specify significant p values while compared with negative control (normal saline) those were $p<0.001$ and $p<0.05$ respectively.

CHAPTER 5:

CONCLUSION

5. CONCLUSION

The presence of the identified phytochemicals makes the leaves pharmacologically active. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. The medicinal values of the plant leaves may be related to their constituent phytochemicals. Due to its natural origin and potent free-radical scavenging ability *A. mexicana* could be used as a potential preventive intervention for free radical-mediated diseases. The presented data for total phenolic content are a basis of assessment of the preventive role of *A. mexicana* against free radicals effect. From *in vitro* clot lysis study, we demonstrated that *A. mexicana* have clot lysis activity. So that, we may assume that these extracts can be considered as a potential source of natural thrombolytic agent.

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