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Pharmacognostic and Phytochemical Investigation and Antibacterial Activity of Medicinal Plant *Argemone Mexicana Linn*

Running Title: Pharmacognostic and antibacterial activity of *Argemona Maxicana Linn*

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Abstract: The present studies aimed to the investigation of phytochemical, pharmacognostic characteristics and antimicrobial activity of *Argemona Maxicana Linn* leaves, stem, and root. All the parts of extracts possess antibacterial activity against bacterial strains. The preliminary phytochemical screening of these plants shows alkaloids, tannins, steroids, flavonoids, carbohydrates, glycosides, and saponins.

Materials and Methods: *Argemona Maxicana Linn* was identified, authenticated, and processed. Microscopic study: the fresh leaves, stem, and root of *Argemona Maxicana Linn* were studied for microscopical Characterization. The coarsely powdered materials were extracted using different solvents by a Soxhlet extraction process. The concentrated extracts were subjected to phytochemical screening, chromatographic, IR, and antimicrobial studies. The antimicrobial activities of the concentrated extracts were evaluated by determination of the diameter of the zone of inhibition against bacterial strains using the cup plate agar disc diffusion method.

Results: The microscopic images of cross section and powdered leaves revealed the presence of midrib, epidermis, trichomes, stomata, and mesophyll. In stem revealed the presence of the epidermis, cortex, pericyclic fibers, vascular bundle, and Pitt. In root shows the presence of periderm, secondary phloem, and secondary xylem. Phytochemical testing confirmed the presence of alkaloids, tannins, steroids, flavonoids, carbohydrates, glycosides, and saponin. Physicochemical parameters such as moisture content, ash value, extraction value, loss on drying, and fluorescent behavior of all parts of powder have also been established. TLC analyses were also performed and the extracts showed antimicrobial activity against different bacterial strains.

Conclusion: The results obtained from the antibacterial studies reveal that methanolic extracts show significant results compared to aqueous extracts.

Keywords: *Argemona Maxicana Linn*, microscopy, phytochemical screening, antibacterial activity.

INTRODUCTION:

Argemona Mexicana Linn is commonly found everywhere by roadsides and fields in India, especially from Bengal to Punjab, and in Shimla 5000 feet, (originally brought from Mexico) and appears in the cold season. It is a well-known weed in agricultural and wastelands. It is an erect, prickly annual herb, up to 1.2 meters in height, naturalized throughout India up to an altitude of 1,500 meters. Synonyms like Shailkanta, Bharbbhauda, Pivaladhatura, Satynashi, Daturi firangi kote, Yellow thistle, Prickly or Mexican poppy. It consists of dried, leaves, stem, and root of *Argemona Mexicana Linn* belonging to the family Papaveraceae.

The various part of the plant reported to possess potent emetic and narcotic activities have traditionally been used to treat, syphilis and skin diseases [1]. The plant *Argemone mexicana* is traditionally used as a potent diuretic agent the plant shows anti-anthelmintics, anti-inflammatory, wound healing, anti-bacterial, antifungal, anti-anxiety, and anti-anxiety anticancer [2-7]. In Ayurveda whole plant is used for Guinea worm infestations, Purgative, Diuretic, leprosy, skin – diseases, inflammation, and bilious fevers, seeds are used as an Antidote in Snake poisoning, Emetic, Expectorant, Demulcent, Laxative, curing warts, Cold sores, cutaneous infections, itches, jaundice & Dropsy, Juice of plant used as Ophthalmic, Opacity of Cornea, dropsy, jaundice, skin diseases, leprosy, blisters, conjunctivitis, Roots are Used in Leprosy, Inflammations, pruritus, blennorrhagia. In Homeopathy whole plants are used for Tapeworm infections, Whooping cough & Bronchitis. Its phytochemical composition includes several useful alkaloids and antioxidants of pharmaceutical importance [8-9]. Flavonoids have an inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, antimicrobial, and anticancer activity which can be used for different diseases that are generally found in the bark. Tannins have general antimicrobial and antioxidant activities [10].

MATERIAL AND METHOD:

Collection of Plant Material:

Argemona Mexicana Linn plant material was collected from the roadside area from Morochi, Solapur, Maharashtra, and authenticated by the Dept. of Botany, Y. C. I. S. Satara, Maharashtra, India. Specimen voucher was deposited in the college herbarium for future reference. After due authentication, fresh drugs obtained were shade dried, coarsely powdered, passed through sieve 100 mesh sizes, and stored in air-tight containers for further study.

Preparation of Extract:

The pulverized dried, leaves, stem, root, and powder of *Argemone Mexicana Linn* were reduced to a coarse powder, and around 25 gm was subjected to hot continuous extraction (Soxhlet) with methanol and water. After the effective extraction, the solvents were evaporated to dryness [11]. The extracts obtained from *Argemona Mexicana* Linn were stored in a refrigerator at 4ºC for further use for phytochemical screening and pharmacognostic investigation.

Macroscopic Characteristic:

The macroscopic of the fresh leaves, stems, and roots of *Argemona Maxicana Linn* were studied according to standard methods [12].

Microscopic characteristic:

For microscopy hand section of the leaf was taken, stained, and mounted following usual micro-techniques [13].

Physical Evaluation:

The ash values, extractive values, and loss on drying were performed according to the Indian pharmacopeia [14].

Fluorescence Analysis:

Many crude drugs show fluorescence when the sample is exposed to ultraviolet radiation. Evaluation of crude drugs based on fluorescence in daylight is not much used, as it is usually unreliable due to the weakness of the fluorescence effect. The fluorescence characteristics of the powdered drug were studied under UV light after treatment with different chemical reagents. Fluorescence analysis was carried out

according to the method of Chase and Pratt [15].

Phytochemical Screening:

The dried, leaves, stem, root, and powder of *Argemone Mexicana Linn* were extracted with methanol and water. The performance of powder with various chemical reagents and preliminary chemical tests for various extracts were also carried out according to the standard procedures described by Kokate [16].

Antimicrobial Activity: [17, 18]

Collection of microbes:

Stock cultures of Bacterial strains such as *Staphylococcus aureus* and *Bacillus subtilis* were used in this study. The collected microbes were maintained in Nutrient agar broth and cultured in Nutrient Agar media. (Hi Media (P) Ltd Mumbai).

Preparation of medium:

Nutrient agar medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. The solution was sterilized in an autoclave at 121°C for 15 min. It was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was measured at 4 cm.

Determination of antimicrobial activity:

The antibacterial activity of aqueous and solvent extracts of leaves, stem, root, and powder of *Argemone Mexicana Linn* had been seen by using the cup plate method. The extracts were dissolved in 1% dimethyl sulphoxide and the concentration used of the extract was 100mg/ml. The antibacterial activity was compared with ciprofloxacin in a nutrient agar medium. Then introduce 0.1 ml of bacterial inoculum on the prepared cultured media. Then spread with the help of a glass spreader. Create the bore on culture media with the help of a cork borer. Introduce the extract and reference sample on that bore. Keep this plate on the deep refrigerator for 1 h. for penetration. Keep this plate on an incubator for incubation for 48 hrs. at 37^0C. Observed and measured the zone of inhibition.

Chromatographic studies:

Thin Layer Chromatography studies were carried out for various extracts to confirm the presence of different phytoconstituents in these extracts. TLC is a mode of liquid chromatography, in which, the extract is applied as a small spot or band at the origin of a thin sorbent layer supported on a glass/plastic/metal plate. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential absorption/ partition coefficient with respect to both mobile and stationary phases. Each separated component has the same migration time but a different migration distance. The mobile phase consists of a single solvent or a mixture of solvents. Although a number of sorbents like silica gel, cellulose, polyamide, alumina, chemically modified silica gel, etc. are used, silica gel (type 60) is the most commonly used sorbent. Handmade plates are prepared by using techniques like pouring, dipping, or spraying. Nowadays, readymade precoated plates are also available. The plates need to be activated at 110^0c for 1h. This removes water/ moisture loosely bound to the silica gel surface [19, 20].

The retardation factor (R_f) is calculated using the following formula,

$$Rf = \frac{\text{Distance traveled by sample from base line}}{\text{Distance traveled by solvent from base line}}$$

Thin Layer Chromatography:

The three extracts were subjected to thin-layer chromatography for the presence of phytoconstituents. In this

technique, the Silica gel-GF_{254} (for TLC) was used as an adsorbent, and plates were prepared by spreading technique, then air dried overnight and activated for one hour at 110°C and used [21].

Thin Layer Chromatography of Flavonoid:

Extracts are spotted in Silica gel G plates and developed using Chloroform: Acetone: Formic acid (75:16.5:8.5) as solvent system[18], the yellowish-orange colored spot will appear when the plates are kept in the iodine-containing beaker after drying the slide.

The results were shown in (Table 7).

IR of isolated compound:

IR spectrum was recorded in IR- spectrometer in 400- 4000 frequency in cm^{-1} for isolated moiety. IR spectrum of compounds was carried in KBR pellet and reproduced in (fig.7). The important absorption can be correlated.

RESULTS AND DISCUSSION:

In the present study extracts obtained from *Argemone Mexicana* Linn (Table 1), the fresh leaves, stem, and roots of plants were investigated for their macroscopic characteristics shown (fig1.). (Table 2).

Microscopic study of the transverse section of leaves shows that it consists of midrib and lamina. Midrib consists of epidermis layers that are continuous, and contain rectangular cells present. Collenchyma presents below the upper and lower epidermis, containing groups of thick-walled cellulosic cells. Cortical parenchyma is loosely arranged. Two vascular bundles are arranged in a ring. Xylem vessels are lignified and phloem vessels are non-lignified. Lamina contains dorsiventral nature with straight walls and single-layered, rectangular parenchymatous cells covered with a thin white cuticle on the upper and lower epidermis. The epidermis shows the presence of uniseriate, multicellular straight, blunt tip-covering trichomes and a unicellular stalk with unicellular head glandular trichomes. Anomocytic (Ranunoculaceous or irregular-celled) stomata are present on the epidermal surface. Below the upper epidermis mesophyll consists of single-layered, compact cells, radially elongated palisade, covering lamina portions followed by 6-8 layers, loosely arranged Spongy parenchyma which is rounded in shape and devoid of intracellular space. Starch grains are present in the endodermis. Staining/Microchemical tests like phloroglucinol and conc. HCL (1:1) shows a pink-colored stain lignified xylem of vascular bundles. The second test shows blue-colored starch grains after being treated with dil. Iodine solution (fig. 2). Microscopic study of the transverse section of the stem shows that the epidermis, cortex, pericyclic fibers, vascular bundle, phloem, xylem, and pith. The Epidermis of the stem constitutes two-layered, quadrangular cells with thick and smooth cuticles, which are arranged in serially. The cortex shows 3 to 4 layers of thin-walled cellulosic parenchyma. Below the epidermis which contains 1 to 2 layers of loosely arranged cells are present. (Containing chloroplast). Vascular bundle around 6-8 collateral conjoint, open, and arranged in a ring. This vascular system consists of the xylem and phloem, xylems are well developed, lignified, and present below the phloem. Phloems are non-lignified and companion cells. Pith is Large, thin-walled, lignified big, polygonal parenchyma present with intercellular space. Staining/Microchemical tests like phloroglucinol and conc. HCL (1:1) shows a pink-colored stain lignified xylem of vascular bundles. The second test shows blue-colored starch grains after being treated with dil. Iodine solution (fig. 3). The transverse section of the stem shows periderm, secondary phloem, and secondary xylem. Periderm contains cork is made up of 9-10 layers or more of tabular cells. Outer layers contain reddish brown matter. Phellogen is indistinct. Phelloderm consists of 1-2 layers of radially arranged parenchymatous cells, containing starch grains. The secondary phloem contains phloem fibers with thickened walls, lignified in the outer part and these are present in isolated form and surrounded by a parenchymatous sheath. Phloem parenchyma shows thin-

walled cells. Also contain medullary rays are distinct, bi to multiseriate parenchymatous cells, narrow in the xylem region and wider in the phloem region, starch grains are present in few cells. Cambium shows Thin-walled cells with 1-2 layers. The secondary xylem consists of vessels, xylem fibers, xylem parenchyma, and medullary rays**.** These vessels are pitted thickened walls, lignified present in isolated as well as groups of two. Xylem fibres are lignified these are surrounded by parenchyma containing calcium oxalate crystals called crystal sheaths. Xylem parenchyma is this are lignified parenchymatous cells which contain few starch grains. Staining/Microchemical tests like phloroglucinol and conc. HCL (1:1) shows a pink-colored stain lignified xylem of vascular bundles. The second test shows blue-colored starch grains after being treated with dil. Iodine solution (fig.4).

In the present study the result of physical evaluation of the loss on drying, ash values like (Total ash, acid insoluble ash and water-soluble ash), Methanol soluble extractive, and aqueous soluble extractive of leaves, stem, and root powders (Table 3).

The result of fluorescence characteristics of the powdered drug was studied under U.V. light after treatment with different chemical reagents is reported (Table 4). The powders of all parts of the plant show fluorescence at 254 and 366 nm.

The leaves, stem, and root of the plant were subjected to hot continuous extraction (Soxhlet) with methanol and water. These extracts were subjected to phytochemical investigation. Phytochemical investigation of plant extracts shows that aqueous extract contains alkaloids, tannins, flavonoids, carbohydrates, glycosides, and saponins. While methanolic extracts show the presence of alkaloids, tannins, steroids, flavonoids, carbohydrates, glycosides, and saponins (Table 5).

The result of the antibacterial activity of methanolic extracts and aqueous extracts of all the parts of the *Argemone Mexicana Linn* have been summarized in (Table 6). From the results obtained in (Table), it was found that methanolic extract was more effective and shows the high zone of inhibition for *Staphylococcus aureus* and *Bacillus subtilis* than aqueous extracts (fig. 5). The results of the present study prove the traditional use of *Argemone Mexicana Linn* leaves, stem, and root in various infectious diseases. This activity may be due to the presence of flavonoids, alkaloids, glycosides, and tannin as active phenolic phytoconstituents in the herb as observed in earlier phytochemical screening.

In the present chromatographic study of the extracts of herbs was carried out. Where the Thin layer chromatography was carried out for flavonoid by using stationary phase silica gel GF- $_{254}$, mobile phase Chloroform: Acetone: Formic acid (75:16.5:8.5). which shows R_f values 0.358, 0.50, and 0.55 for flavonoid for leaves, stem, and root methanolic extract respectively (fig.6) (Table 7).

For these isolated compounds of methanolic extracts Infrared spectroscopy was carried out which shows that all the methanolic extracts of *Argemone mexicana linn* contain Alkanes, Ether, Phenolic, Aromatic, and Alcoholic group, etc. (fig.7) (Table 8a, 8b, 8c).

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Conflict of Interest:
The authors declared no conflict of interest

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TABLE 1: NATURE, COLOUR, YIELD OF *ARGEMONE MEXICANA* LINN EXTRACTS.

Extracts	**Plant part**	**Nature of Extract**	**Color**	**Weight (g)**	**%Yield (w/w)**
Methanol	Leave	Semi-solid	Brown	5	20
	Stem	Semi-solid	Brown	4.2	16.8
	Root	semisolid	Brown	2.3	9.2
Aqueous	Leave	Solid	Brown	4.6	18.4
	Stem	Solid	Brown	4.5	18
	Root	solid	Brown	2	8

TABLE 2: MACROSCOPIC CHARACTERISTICS OF FRESH LEAVES STEMS, AND ROOTS OF *ARGEMONA MAXICANA LINN:*

Parameter	**Leave**	**Stem**	**Root**
Color	Green	Brown	Brown
Odor	Characteristics	Characteristics	Characteristics
Taste	Characteristics	Characteristics	Characteristics

TABLE 3: PHYSICAL CONSTANTS OF PLANTS

Physical Constants	***Argemone mexicana Linn***		
	Leave	**Stem**	**Root**
Ash Value (% w/w)			
Total Ash	20.8	10	6.4
Acid Insoluble Ash	6	2.8	0.8
Water Soluble Ash	2.4	0.8	2.12
Loss on Drying (% w/w)	13.4	13.4	12
Extractive Values (% w/w)			
Methanol soluble extractive	1.32	0.76	1.2
Aqueous soluble extractive	3.72	1	0.8

TABLE 4: FLUORESCENCE ANALYSIS OF PLANT

Reagent	**Fluorescence Analysis of *Argemome maxicana* Linn**					
	At 254 nm			**At 366 nm**		
	Leave	**Stem**	**Root**	**Leave**	**Stem**	**Root**
Powder + 1N NaoH in methanol.	Light Green	Yellow	Dark brown	Light green	Light green	Brown
Powder + 1N NaoH + water	Dark Brown	Light yellow	Light brown	Dark green	Light green	Black
Powder + 50% HCL	Faint green	Light green	Light yellow	Light green	Yellow-green	Black
Powder + 50% H_2SO_4	Light green	Light brown	Light brown	Light green	Brown	Black
Powder + 50% HNO_3	Light green	Light brown	Light brown	Light green	Light brown	Greenish black
Powder + petroleum ether	Dark brown	Yellowish brown	Light brown	Faint green	Faint yellow	Green
Powder + chloroform+ picric acid	Dark brown	Dark brown	Black	Light green	Dark brown	Light yellow
Powder + 5% $Fecl_3$	Light green	Light brown	Black	Light brown	Light brown	Greenish black
Powder + 5% Iodine	Light brown	Brown	Light brown	Brown	Brown	Light brown
Powder + methanol	Light green	Light brown	Light brown	Green	Light brown	Light green
Powder + (HNO_3 + NH_3)	Green	Light brown	Light brown	Green	Light brown	Light grey

TABLE 5: QUALITATIVE CHEMICAL INVESTIGATION OF *ARGEMONE MEXICANA LINN* EXTRACT.

Name of the test	***Argemone mexicana* Linn**					
	Leave		**Stem**		**Root**	
	Methanol	**Aqueous**	**Methanol**	**Aqueous**	**Methanol**	**Aqueous**
Test for sterols	+	_	+		_	+
Test for glycosides	+	+	+	_	+	+
Test for carbohydrates	+	+	+	+	+	+
Test for alkaloids	+	+	+	+	+	+
Test for flavonoids	+	_	+	_	+	_
Test for tannins and phenolic	+	_	+	+	_	_
Tests for proteins	+	_	+	+	_	_
Test for amino acid	_	_	_	_	_	_
Test for saponins	+	_	+	+	+	+

(+) for presence of phytochemical and (-) for absence of phytochemical.

TABLE 6: OBSERVATIONS OF ANTIMICROBIAL ACTIVITY

Organism	Nutrient media	Extracts	Inhibition zone		
			Standard	Methanol	Water
Staphylococcus aureus	Nutrient agar	Leave	28mm	18mm	8mm
		Stem	24mm	14mm	10mm
		Root	28mm	14mm	8mm
Bacillus subtilis	Nutrient agar	Leave	30mm	16mm	10mm
		Stem	32mm	14mm	10mm
		Root	32mm	14mm	8mm

TABLE 7: OBSERVATIONS OF THIN-LAYER CHROMATOGRAPHY

Extract	Observation		R_f values
	No. of spots	Color of spots	
Leave	1	Yellowish Orange	0.358
Stem	1	Faint Yellow	0.50
Root	1	Yellow	0.55

TABLE 8 a: I.R. SPECTRAL PEAKS (LEAVES)

Spectral peak(cm -1)	Molecular Nature	Standard value
3300.20	O-H Stretching (H-Bonded)	3400-3200
2920.23	C-H Stretching (Aromatic)	3150-3050
1583.56	C=C bending (Aromatic) medium to strong	1600-1475
1396.46	CH_3 Bending (Alkanes)	1475-1365
1041.56	C-O Bending (aromatic)	1300-1000

TABLE 8b: I.R. SPECTRAL PEAKS (STEM)

Spectral peak(cm -1)	Molecular Nature	Standard value
3323.35	H- Bonded	3400-3200
3288.63	C-H Stretching	3300-2700
2922.16	C-H Stretching (Hydrogen)	3300-2700
1039.63	C-O Stretching (alcohol and phenol)	1260-1000

TABLE 8c: I.R. SPECTRAL PEAKS (ROOT)

Spectral peak(cm -1)	Molecular Nature	Standard value
3338.78	O-H Stretching	3400-3200
3282.84	C-H Stretching	3300-2700
1591.27	N-H Bending	1700-1500
1037.70	C-O Bonding (ether)	1300-1000

Fig. 1: Plant of Argemone mexicana linn.

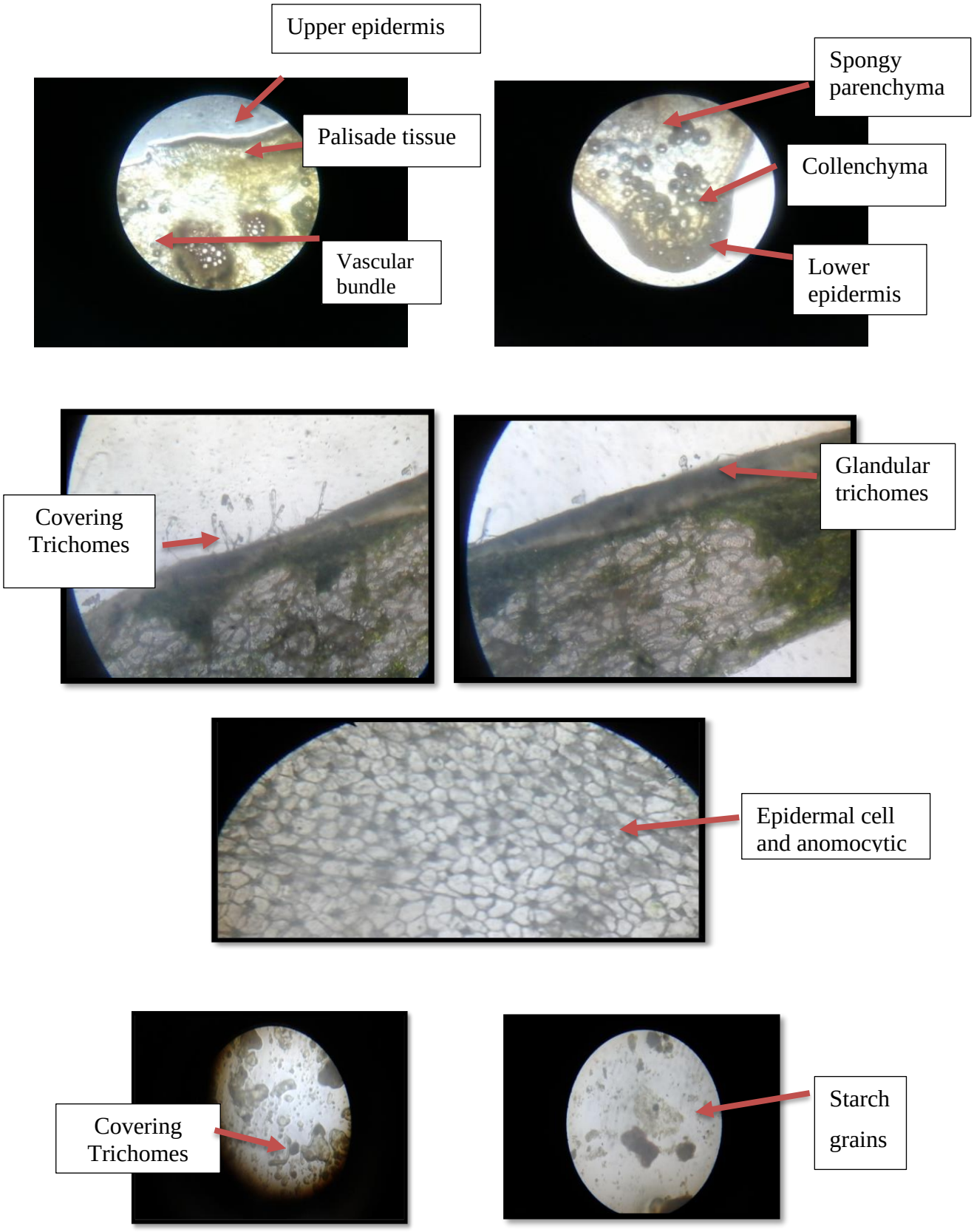


Fig. 2: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Leaves

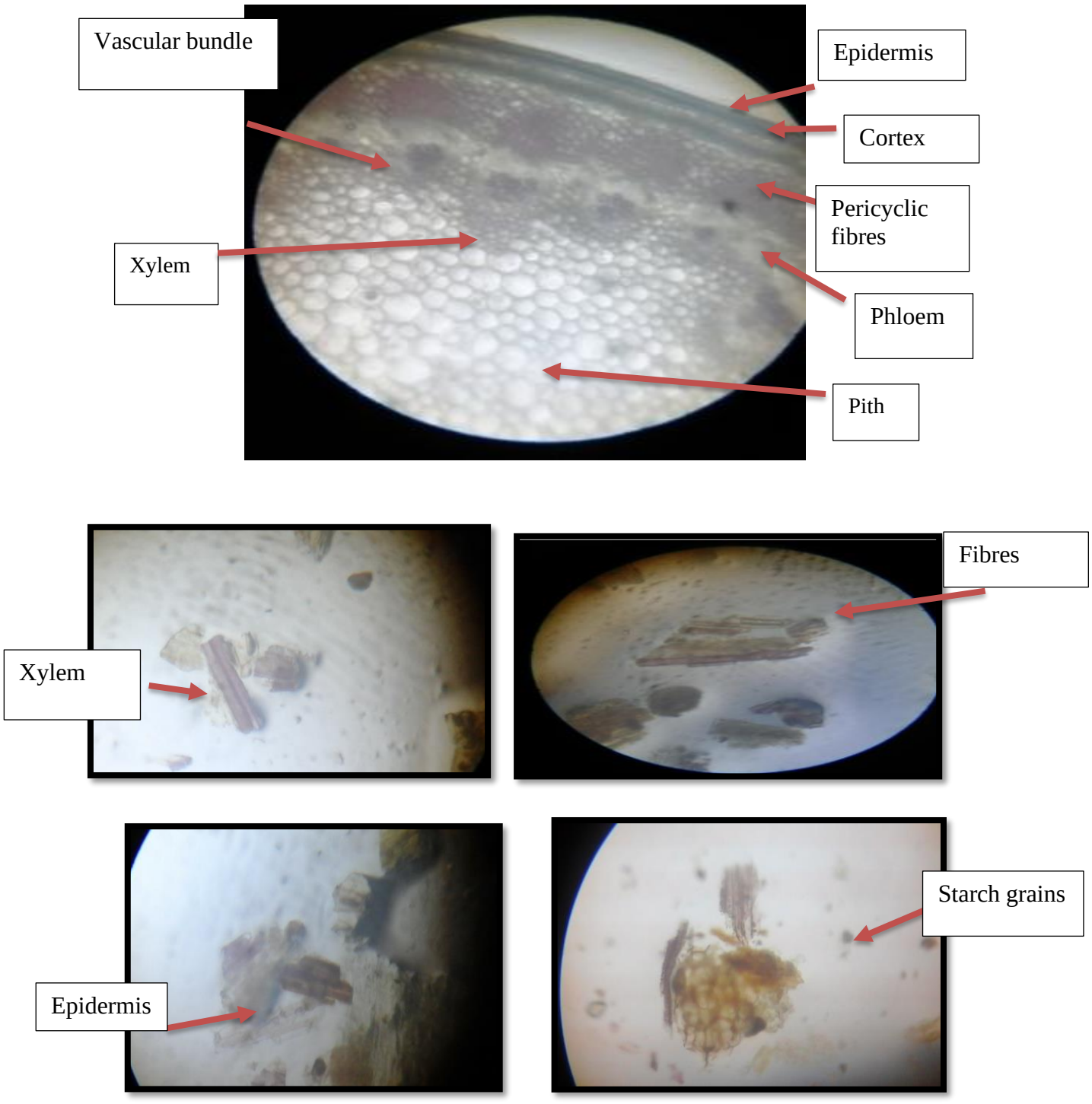


Fig. 3: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Stem.

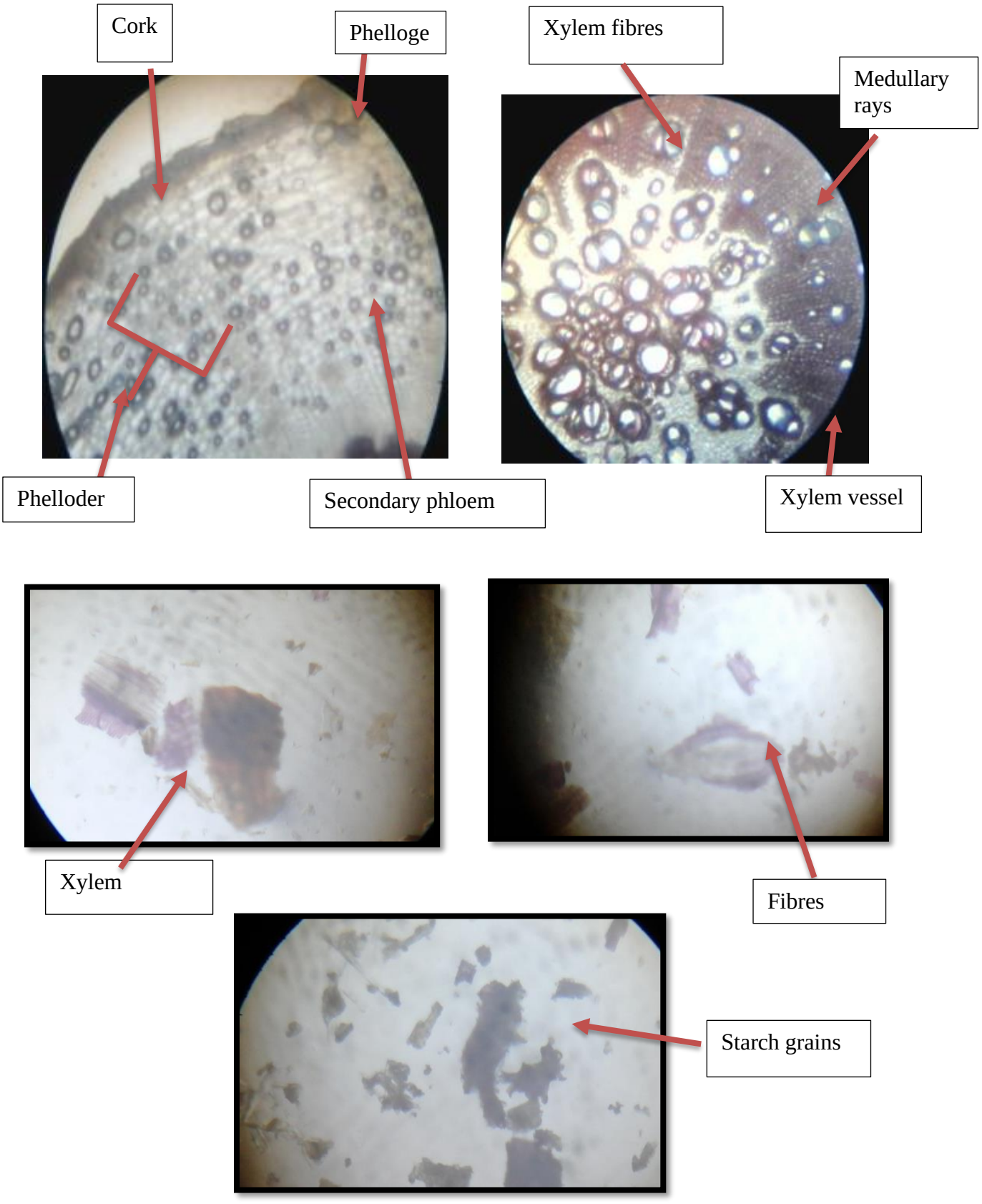
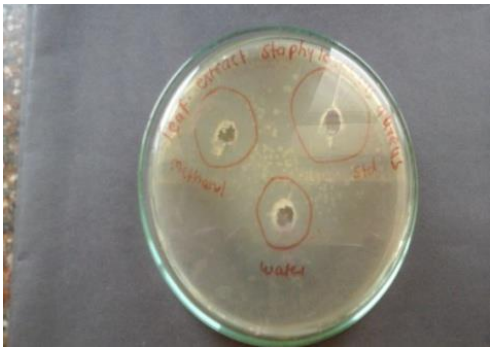
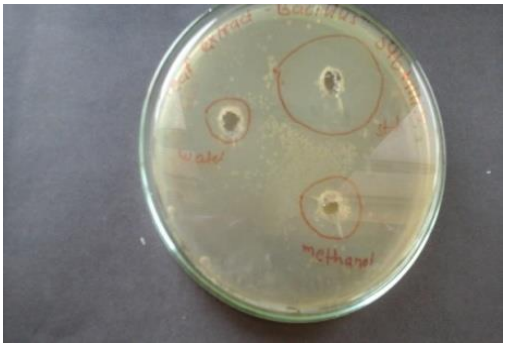
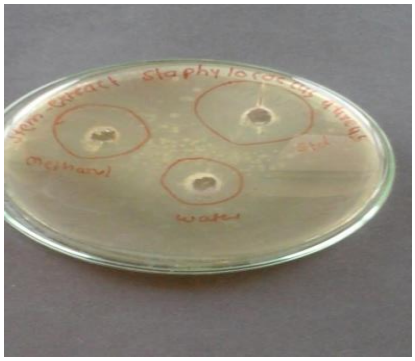


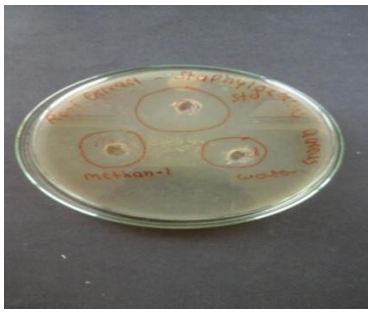
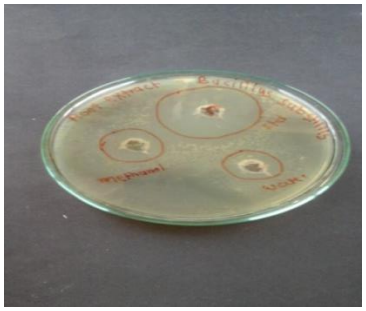
Fig. 4: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Root.

Leaves extracts: Staphylococcus aureus **Bacillus subtilis**




Stem extracts: Staphylococcus aureus **Bacillus subtilis**

Root extracts: Staphylococcus aureus **Bacillus subtilis**

Fig. 5: Observations of a zone of inhibition of *Argemone Mexicana linn* extracts

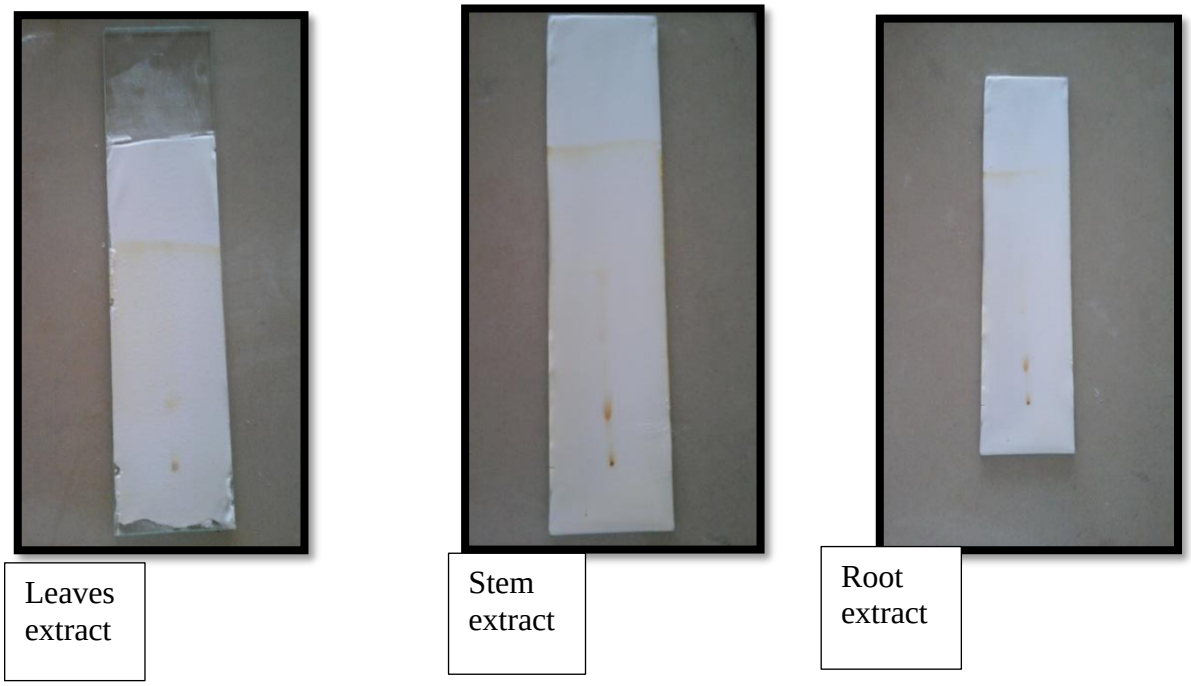
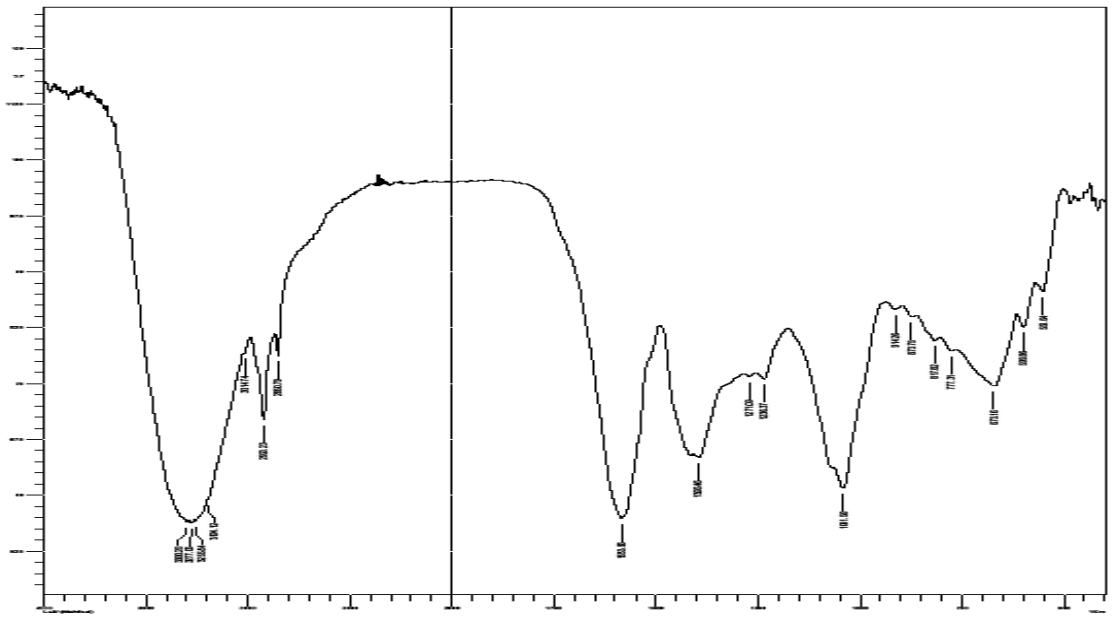
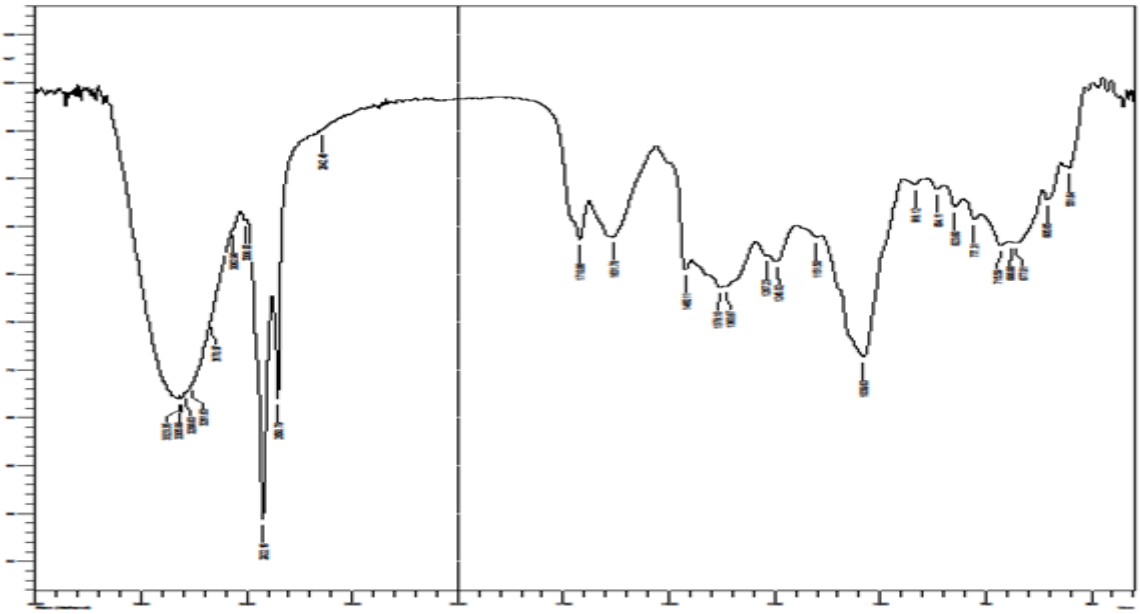


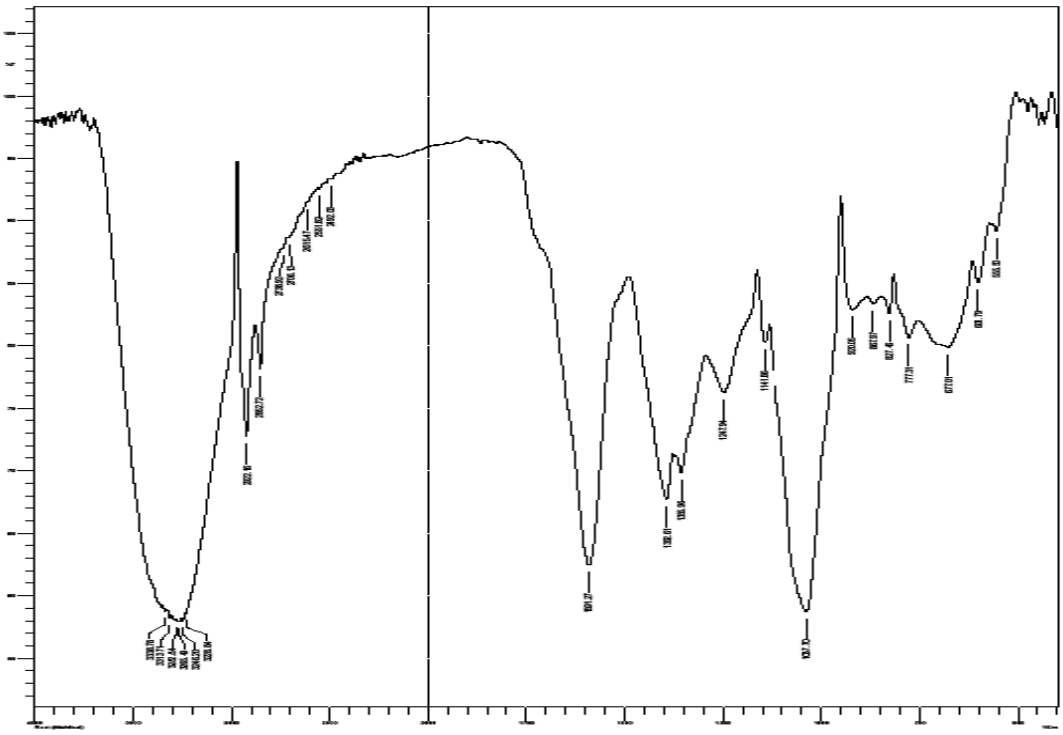
Fig.6: Observations of TLC of Methanolic extracts of *Argemone Mexicana linn*



Spectrum of Leaves



Spectrum of Stem



Spectrum of Root

Fig. 7: IR Spectrum of isolated compound from Methanolic extracts leaves, stem, and root.

Tables and Figure title and legend:

TABLE 1: NATURE, COLOUR, YIELD OF *ARGEMONE MEXICANA* LINN EXTRACTS.

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TABLE 3: PHYSICAL CONSTANTS OF PLANTS

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TABLE 5: QUALITATIVE CHEMICAL INVESTIGATION OF *ARGEMONE MEXICANA LINN* EXTRACT.

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Fig. 1: Plant of *Argemone Mexicana linn.*

Fig. 2: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Leaves

Fig. 3: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Stem.

Fig. 4: T.S, Microscopic and surface preparation study of *Argemone mexicana linn* Root.

Fig. 5: Observations of a zone of inhibition of *Argemone Mexicana linn* extracts

Fig.6: Observations of TLC of Methanolic extracts of *Argemone Mexicana linn*

Fig. 7: IR Spectrum of isolated compound from Methanolic extracts leaves, stem, and root.