2.1. Introduction

Standardization is the method of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and natural observations (Agarwal and Paridhavi, 2007). Herbal standardization is of more importance because plant extract is the complex mixture of several compounds and in case of pharmaceutical medicines a very high quality is required (Mukherjee, 2002).

WHO has recognized the need to ensure quality control of medicinal plants products by using modern techniques and by applying suitable standards. For pharmaceutical purpose the quality of medicinal plant materials must be as high as that of other medicinal preparations. However, it is impossible to assay for specific chemical entity when the bioactive ingredient is not known. In herbal drugs there is a great scope of variation of active ingredients and this may occur due to variation in place of cultivation, time of collection and method of storage. To eliminate some of the causes of inconsistency, one should use cultivated rather than wild plant which are often heterogeneous in respect of the factors and consequently in their content of active principles. The purpose of standardization of medicinal plant products is to ensure therapeutic efficacy and to check any adulteration or non-deliberate mixing in commercial batches (Reskin et al., 2002).

The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its

morphological, anatomical and biochemical characteristics (Thomas et al., 2008). Herbal drug development is possible only through the development of standardized herbal products with reference to their active phytoconstituents present for commercialization, correct identification and supply of raw material and to avoid adulteration. It becomes extremely important to make an effort towards standardization of the plant material to be used as medicine.

2.2. Material and Methods

Table 2.1 List of equipments

S.No.	Equipments	Manufacturer
1.	Heating mantle	Ambassador
2.	Muffel furnace	Ambassador
3.	UV chamber	Ambasssador
4.	Electronic Balance	Contech
5.	Incubator	Science-tech
6.	Oven	Wirso Instruments, Delhi
7.	pH-meter	MP 220 Mettler Toledo,
8.	Dryer	Decibel, India
9.	Vacuum Filter	Remi scientific, India
10.	Orbital shaker	Nulife,Nucleus biological

Table 2.2. List of Chemicals

S.No.	Chemicals	Manufacturer
1.	Petroleum ether	SD fine chem. Limited
2.	Chloroform	Fisher scientific
3.	Ethanol	Jiangsu Huaxi International trade
4.	Benzene	Fisher scientific
5.	Methanol	Fisher scientific
6.	Nitric acid	Fisher scientific
7.	Acetone	Fisher scientific
8.	Ethyl acetate	Fisher scientific
9.	Conc. Sulphuric acid	Fisher scientific
10.	Glacial acetic acid	Merck
11.	n- butanol	Merck
12.	Ferric chloride	SD fine chemicals
13.	Pyridine	SD fine chemicals
14.	Sodium nitroprusside	SD fine chemicals
15.	Sodium picrate	SD fine chemicals
16.	Bismuth nitrate	SD fine chemicals
17.	Tartaric acid	Qualigens
18.	Potassium iodide	Qualigens

2.2.1. Collection and Authentication of the plant parts

The root of *Asparagus racemosus* Willd. were obtained from Hamdard dawakhana, Aminabad, Lucknow, and the whole plant of *Euphorbia hirta* Linn. were purchased from garden of National Botanical Research Institute (NBRI), Lucknow, India. For identification and taxonomic authentication, samples were given to National Botanical Research Institute (NBRI) Lucknow, India. The plant material was confirmed and authenticated by Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute Lucknow, India with the receipt no. CIF-RB-2-129 and CIF-RB-2-153 respectively.

2.2.2. Morphological Characters

The morphology of a drug includes its visual appearance from naked eye. Morphological evaluation is of primary importance before any further study can be carried out. This one is the very first and quickest step towards establishment of identity and degree of purity and there by ensures quality of a particular sample. The variations in sources of crude drugs and their chemical nature, herbal drugs are standardized by using different techniques including the method of estimation of chief active constituent. Macroscopy of a crude drug involves evaluation of a drug by colour, odour, size, shape, taste and special features such as touch, textures etc (Ansari, 2008).

Quality control of herbal drugs and their bioconstituents is of paramount importance in justifying their acceptability in modern system of medicine. Macroscopy depends to a large extent on the part of the plant from which the drug is obtained. For each particular morphological group a particular systemic examination can be carried out. For authentication, characters of an unknown sample are compared with the authentic monographs written in the pharmacopoeia (Thomas et al., 2008).

2.2.3. Microscopical characters

Microscopical technique provides very detailed information about the crude drug by virtue of its two main analytical uses. Firstly its property to magnify permits the fine structure of minute objects to visualized and there by confirm the structural details of the plant drug under evaluations. Secondly, these techniques can be used in the determination of the optical as well as micro-chemical properties of the crud drug specimen (Ali, 2004).

The microscopical examination of crude drug aims at determination of the chemical nature of the cell wall along with the determination of the form and chemical nature of the cell contents. Thus determine the size shape and relative structure of the different cells and tissues in a plant drug, inspite of these facts microscopy is of great necessity in the identification of powdered drugs because in powdered drugs the cells are mostly broken expect lignified cells. The cell content such as starch, calcium oxalate crystal aleurone, etc, are scattered in the powder.

Microscopic evaluation also covers study of the constituents by application of chemical methods to small quantities of drugs in powdered form or to histological section of the drug (microchemistry). Microscopic linear measurements and quantitative microscopy are also covered under this technique of evaluation. The various parameters studied here are stomatal number and index, palisade ratio, vein islet number, vain termination number, size of starch grain and length of fibers etc (Ali, 2004).

2.2.4. Extractive value

The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount of certain constituents that the drug contains. The drug should be extracted with different solvents in order of their increasing polarity to get the correct and

dependable values. Generally petroleum ether, alcohol and water extractives are taken into consideration for fixing the standard of a drug. The petroleum ether extracts contains fixed oil, resins and volatile substances, but when the extract is heated at 105°C until constant weight, the volatile contain substances are volatilized leaving only resin, colouring matter and fixed oil. Alcohol can dissolve almost all the substances, but is generally used for determining the extractive index for those drugs, which contain glycosides, resins, alkaloid etc. Water is used for the drugs containing water soluble substances as chief constituents. The extractives values were determined according to the method described in Pharmacopoeia [(Ali, 2002).

2.2.4.1. Cold extraction

2.2.4.1.1. Petroleum Ether Extractives

The air dried coarse drug powder (5g) was macerated with petroleum ether (100ml) in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It was filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tared flat bottom dish and dried at 105°C, to constant weight and weighed (Indian Pharmacopoeia, 1996).

2.2.4.1.2. Chloroform Extractives

The air dried coarse drug powder (5g) was macerated with chloroform (100ml) in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It was filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tared flat bottom dish and dried at 105°C, to constant weight and weighed.

2.2.4.1.3. Alcoholic Extractives

The air dried coarse drug powder (5g) was macerated with alcohol (100ml) in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It was

filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tared flat bottom dish and dried at 105°C, to constant weight and weighed.

2.2.4.1.4. Water Extractives

The air dried coarse drug powder (5g) was macerated with water (100ml) in a closed flask for 24 hours, shaking frequently during six hours and allowing to stand for 24 hours. It was filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tared flat bottom dish and dried at 105°C, to constant weight and weighed.

2.2.4.2. Hot extraction

The powdered material of the drug (20g) was packed in a soxhlet apparatus separately for each solvent like petroleum ether, chloroform, alcohol and water. Each extract is evaporated to dryness and constant extractive value was recorded (Ansari, 2008).

2.2.4.3. Successive extraction

The dried and coarsely powdered material (20g) was subjected to successive extraction in a soxhlet apparatus with different solvents like petroleum ether, chloroform, alcohol and it was aqueous. The extracts are evaporated to dryness and their constant extractive values were recorded (Ansari, 2008).

2.2.5. Ash value

This parameter can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same

drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly material. The water soluble ash is used to estimate the amount of inorganic elements. The ash values were determined according to the method described in Pharmacopoeia of India

2.2.5.1. Total ash:

The ground drug (2g) was incinerated in a silica crucible at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content (Indian Pharmacopoeia, 1996).

2.2.5.2. Acid insoluble ash:

Ash was boiled with 25 ml dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight (Indian Pharmacopoeia, 1996).

2.2.5. 3. Water soluble ash:

Ash was dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450°C to constant weight. By substracting the weight of insoluble part from that of the total ash, the weight of soluble part of ash was obtained (Indian Pharmacopoeia, 1996).

2.2.5. Foreign matter

Foreign organic matter is the material consisting of parts of the organ or organs from which the drug is derived other than the parts named in the definition and description or for which the limit is prescribed in the individual monograph, or matter not coming from source plant and moulds, insects or other animal contamination.

2.2.6. Fluorescent analysis

The powder drug was treated with different solvents and was examined under UV light (Ansari, 2008).

2.2.7. Powdered drug reaction with different reagents

The powder drug was treated with different reagents and the colour shown by that treatment was noted (Ansari, 2008).

2.2.8. Determination of pH

pH 1% solution: 1 gm of the accurately weighed drug was dissolved in water and filtered. pH of filtrate was checked with a standardized glass electrode.

pH 10% solution: 10 gm of the accurately weighed drug was dissolved in water and filtered. pH of filtrate was checked with a standardized glass electrode (Ansari, 2008).

2.2.9. Loss on drying

This parameter determines the amount of moisture as well as volatile components present in a particular sample.

The powdered drug sample (10gm) was placed on a tarred evaporating dish and dried at 105°C for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25% of constant weight (Ansari, 2008).

2.2.10. Thin layer chromatography

In 1958, Stahl demonstrated application of TLC in analysis, a method based on adsorption chromatography. TLC is an important analytical tool for qualitative and quantitative analysis of a number of natural products.

TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass plate. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

The special advantages of TLC include versatility, speed and sensitivity. Versatility is due to the fact that a no of adsorbents may be spread on glass plate. The greater speed of TLC is due to the more compact nature of the adsorbent. Finally the sensitivity of TLC is such that separations on less than micro gram amount of material can be achieved if necessary (Stahl, 2007).

Methanol and aqueous extract of plants were subjected to thin layer chromatography to find out the number of compounds present. The detail of the procedure is given below

Procedure

Step 1: Preparation of the plates:

The adsorbent used for thin layer chromatography was silica gel G. Silica gel G was taken in a glass mortar and distilled water was added to it. The mixture was stirred with glass rod until it becomes homogeneous. This mixture was then allowed to swell for few minutes. Then additional distilled water was added with continuous stirring to make slurry. This suspension was then spread immediately on thin layer chromatographic plates.

Step 2: Drying and storage of plates

The freshly coated plates were then air dried and was placed in an oven for 30 minutes at 110°C. Activated plates were kept in a desiccators till required for further use.

Step 3: Application of the sample

For applying test samples on TLC plates, glass capillaries were used. The spots were applied with the help of a capillary on the base line.

Step 3: Chromatographic chamber

Chromatographic rectangular glass chamber was used in the experiments. To avoid insufficient chamber saturation, a smooth sheet of filter paper was placed in the chromatographic chamber. After being thus moistened, the paper was then pressed against the walls of the chamber, so that it adhered to the walls. The experiments were carried out at room temperature in diffused day light.

Step 4: Developing solvent system

A number of developing solvent system was tried, but the good result and the maximum numbers of spots were obtained in the solvent system mentioned. The plates were air dried. The visualizing reagent employed was exposer to iodine vapors to effect visualization of the resolved spots and number of spots were noted and R_f value were calculated.

2.2.11. High Performance Thin Layer Chromatography (HPTLC)

High performance thin layer chromatography is very useful in analysis of phytopharmaceuticals as it combines the art of chromatography with quickness. HPTLC is a major advancement of TLC principle requiring shorter time and better resolution. Silica gel of very fine particle size is widely used as sorbent in HPTLC. The use of smaller particle size helps in greater resolution and sensitivity. Sample preparation in HPTLC needs a high concentrated solution, as very less amount of sample is needed for application. The analytical profiles for carotenoids, tropane alkaloids, flavanoids, steroidal compounds, anthracene aglycones, lipids etc, have been developed by using this technique. Now a day, it is applied to

obtain “finger-print” patterns of herbal formulations, quantification of active ingredients and also detection of adulteration.

In the last two decades (HPTLC) has emerged as an important tool for the qualitative semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing TLC fingerprint profiles and estimation of chemical markers and biomarkers. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase (Mukherjee, 2007).

2.2.11.1. Steps involved in HPTLC

- **Selection of chromatographic layer**

  Precoated silica gel plate 60 F 254 (Merck), were selected for the chromatographic separation.

- **Sample preparation**

  Sample was prepared by redissolving a small quantity of methanol residue in methanol.

- **Application of the sample**

  Sample was applied on precoated plate with the help of CAMAG LANOMAT-V sample applicator.

- **Chromatographic development**

  A twin trough chamber was used for the development of chromatogram. The chamber was allowed to saturate with solvent system before use. Then pre coated plate was dipped in saturated chromatographic chamber containing the solvent system and was allowed to elute up to few cm. Plate was air dried after resolution. The experiment was carried out at room temperature in diffused day light.

- **Detection of the spots**

  Spots on the plate were detected with the help of U.V.

- **Scanning**

  CAMAG TLC Scanner 3 was used for scanning the HPTLC plate and scan was done at 366nm wavelength.

- **Documentation of chromatoplates**

  Documentation of chromatoplates was done with the help of computer and simultaneously photograph of HPTLC was taken.

2.3. Results

2.3.1. *Asparagus racemosus*

2.3.1.1. Macroscopical characteristics

Fig 2.1. Roots of *A. racemosus*

Dried tuberous root commonly 30 to 100 cm long and 1-2 cm thick, it is fairly smooth but there is a presence of few rootlets. Shape consists of tapering towards the basal as well as distal ends. Color is cremish white to light yellow. Surface rough, mark of shrinkage after drying. Fracture short and fibrous and no specific odour.

2.3.1.2. Microscopical characters

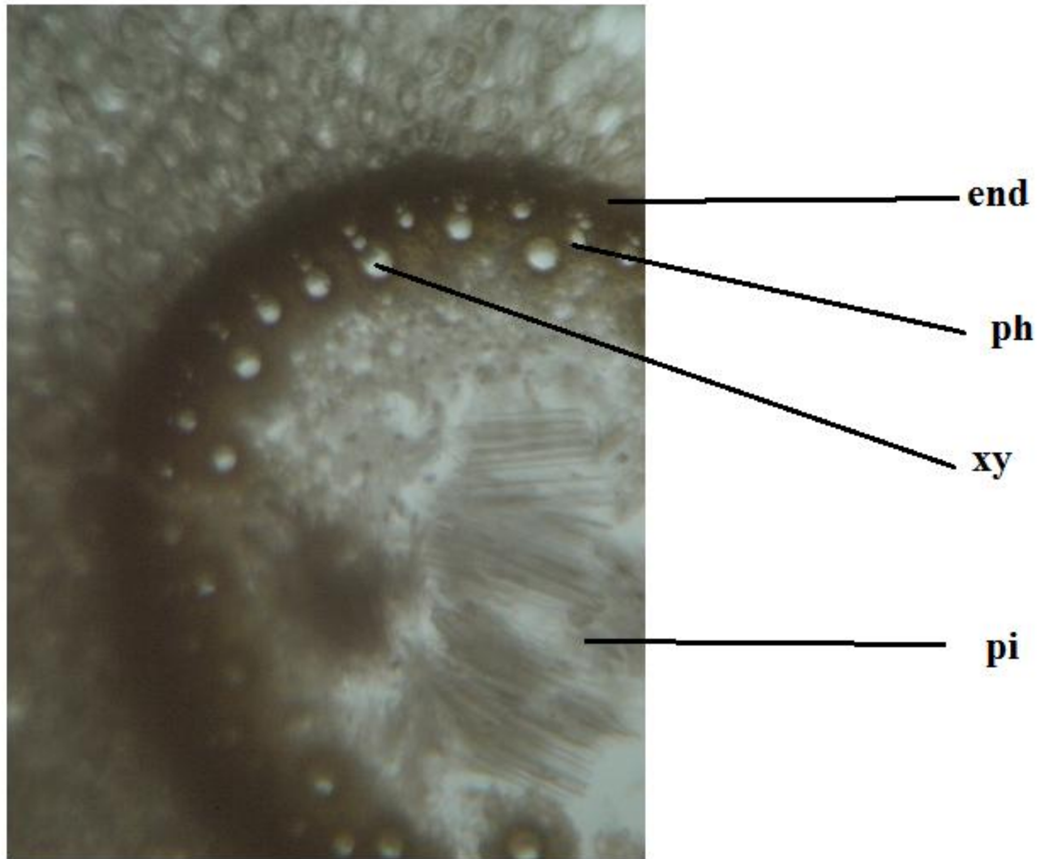


Fig-2.2. TS of root of *Asparagus racemosus*- Inner cortex part of vascular ring and pith; end-endodermis, ph-Phloem, xy-Xylem, pi- Pith.

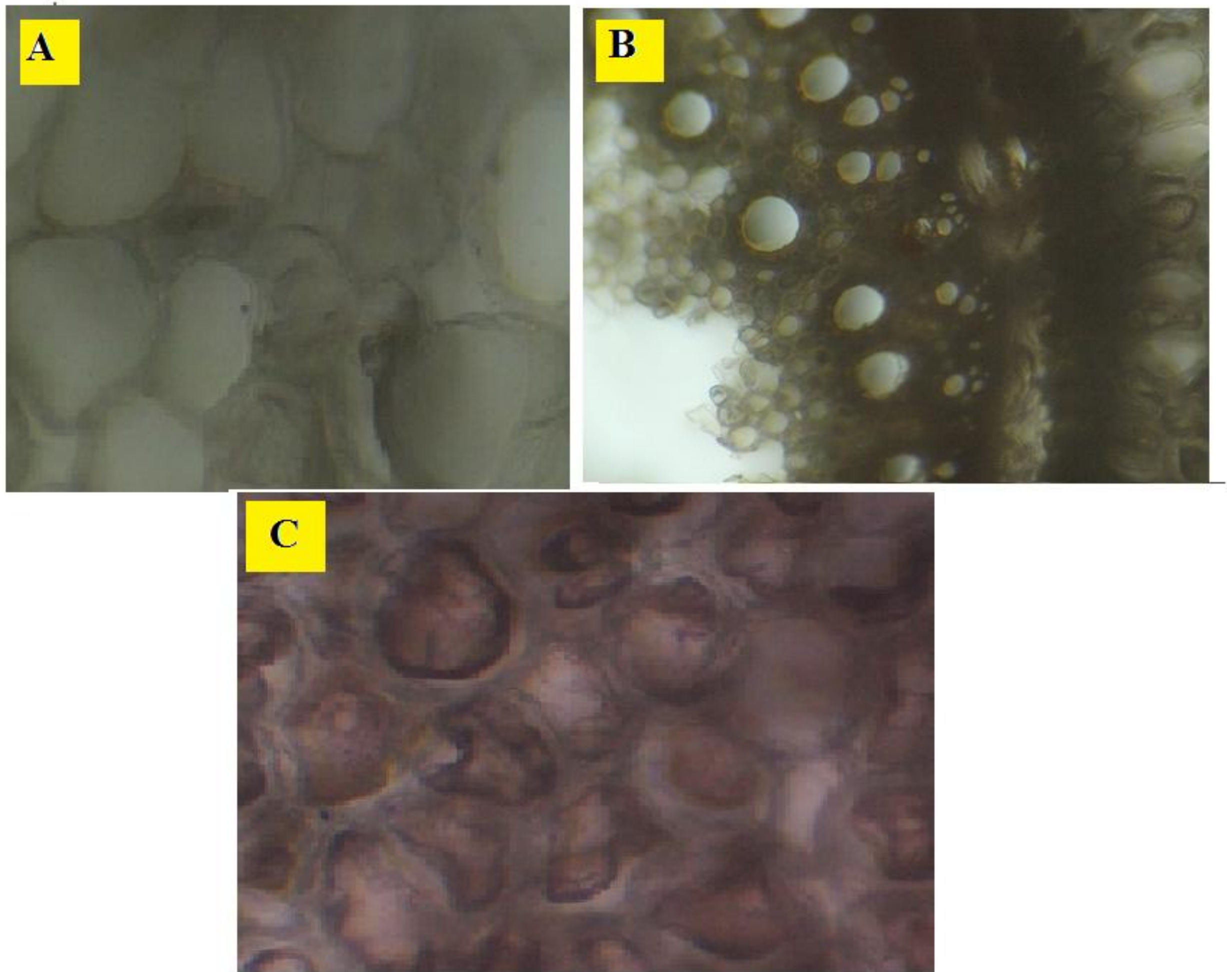


Fig-2.3. TS of root of *A. racemosus* , A-TS showing part of outer cortex at higher magnification, B-TS showing part of vascular ring at higher magnification, C- part of inner cortex at higher magnifications.

TS of root shows lignified cortex. The innermost 1 or 2 layer of cortex immediately outside the endodermis comprise thick walled cells, with numerous circular or oval pits on their walls. Endodermis is composed of a single layer of compactly arranged, barrel shaped, parenchymatous cell. Phloem and xylem present many in number, are arranged on alternate radii and form ring.

2.3.1.3. Extractive value

2.3.1.3.1. Cold Extraction

Table: 2.3. Petroleum ether extractive

Wt. of drug (gm)	Wt. of empty petri plate (gm)	Wt. of petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive Value (%)
5.000	43.418	43.443	0.025	0.50
5.000	42.621	42.649	0.028	0.56
5.000	44.482	44.712	0.023	0.46
Mean	-	-	-	0.50

Table 2.4. Chloroform extractive

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive Value (%)
5.000	42.541	42.712	0.171	3.42
5.000	44.356	44.528	0.172	3.44
5.000	41.632	41.801	0.169	3.38
Mean	-	-	-	3.41

Table 2.5. Methanol extractive

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
5.000	41.34	41.733	0.393	7.86
5.000	43.251	43.682	0.431	8.62
5.000	42.621	43.003	0.382	7.64
Mean	-	-	-	8.04

Table 2.6. Aqueous extractive

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
5.000	44.412	44.923	0.511	10.22
5.000	45.271	45.794	0.523	10.46
5.000	46.536	47.032	0.496	9.92
Mean	-	-	-	10.2

2.3.1.3.2. Hot Extraction

Table 2.7. Petroleum ether extractive:

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	35.351	35.533	0.182	0.91
20.000	37.262	37.441	0.179	0.895
20.000	40.436	40.617	0.181	0.905
Mean	-	-	-	0.901

Table 2.8. Chloroform Extractive:

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	41.527	42.448	0.921	4.605
20.000	42.853	43.784	0.931	4.655
20.000	43.571	44.482	0.911	4.555
Mean	-	-	-	4.605

Table 2.9. Methanol Extractive:

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter(gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	42.572	44.652	2.08	10.40
20.000	46.751	48.902	2.151	10.755
20.000	43.357	45.368	2.011	10.055
Mean	-	-	-	10.403

Table: 2.10 Aqueous Extractive:

Wt. of drug (gm)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	40.516	42.628	2.112	10.56
20.000	46.362	48.493	2.131	10.655
20.000	42.206	44.326	2.120	10.60
Mean	-	-	-	10.605

2.3.1.3.3. Successive Extraction

Table 2.11. Petroleum ether Extractive:

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	43.536	43.737	0.201	1.005
20.000	41.185	41.397	0.212	1.06
20.000	44.652	44.843	0.191	0.955
Mean	-	-	-	1.006

Table 2.12. Chloroform Extractive:

Wt. of drug (gm)	Wt. of empty petri plate (gm)	Wt. of petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	41.532	42.411	0.879	4.395
20.000	42.743	43.627	0.884	4.42
20.000	43.632	44.513	0.881	4.40
Mean	-	-	-	4.40

Table 2.13. Methanol Extractive:

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	43.573	45.584	2.011	10.055
20.000	41.364	43.345	1.981	9.905
20.000	44.294	46.306	2.012	10.060
Mean	-	-	-	10.00

Table 2.14. Aqueous Extractive:

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	wt. of extractive matter (g)	Extractive value (%)
20.000	44.362	46.482	2.12	10.6
20.000	42.847	44.978	2.131	10.655
20.000	41.683	43.748	2.065	10.325
Mean	-	-	-	10.526

Table 2.15. Total Ash

Wt. of crucible (gm)	Wt. of crucible + drug (gm)	Wt. of crucible after ignition (gm)	Wt of ash (gm)	Ash value (%)
13.53	15.53	15.359	0.171	8.55
14.67	16.67	16.513	0.157	7.9
15.35	17.35	17.187	0.163	8.15
Mean	-	-	-	8.2

Table 2.16. Acid insoluble Ash

Wt. of crucible (gm)	Wt. of crucible + drug (gm)	Wt. of crucible + acid treated ash after ignition (gm)	Wt of ash (gm)	Ash value (%)
15.34	17.34	17.281	0.059	2.96
14.36	16.36	16.302	0.058	2.93
13.48	15.48	15.421	0.059	2.95
Mean	-	-	-	2.946

Table: 2.17. Water soluble Ash

Wt. of crucible (gm)	Wt. of crucible + Wt. of crude drug (gm)	Wt. of crucible + Distilled water treated ash after ignition (gm)	Wt. of water insoluble ash (gm)	Wt of water soluble ash	Ash value (%)
14.23	16.23	16.172	0.058	0.113	5.65
13.61	15.61	15.565	0.045	0.112	5.64
15.41	17.41	17.36	0.050	0.113	5.66
Mean	-		-	-	5.65

Table 2.18. Foreign matter analysis:

Wt. of crude drug (gm)	Wt. of drug after removal of foreign matter (gm)	Wt. of foreign matter (gm)	Foreign matter (%)
10.00	9.890	0.110	1.10
10.00	9.880	0.120	1.20
10.00	9.900	0.100	1.00
Mean	-	-	1.10

Table 2.19. Loss on Drying

Wt. of drug + China dish (Before drying) (g) A	Wt. of drug + China dish (After drying) (g) B	A-B (g)	Loss on Drying (%) (w/w)
10.000 +102.121= 112.121 10.00+101.352=111.352 10.00+103.284=113.284	111.577 111.537 111.590	0.544 0.584 0.531	5.44 5.84 5.31
Mean			5.53

2.3.1.3.4. Fluorescence analysis

Table 2.20. Flourescence analysis of root of *A. racemosus*

Chemical Treatment	observation		
	Day Light	**UV 254nm**	**UV 366nm**
Drug powder as such	Yellowish brown	Light Brown	Gray white
Conc. H_2SO_4	Brown	Dark green	Black
Conc. H_2SO_4 + Distilled Water	Light cream	Dark green	Black
Conc. HCl	Dark red	Light red	Reddish
Conc. HCl + Distilled Water	Light Brown	Dark green	Black
Conc. HNO_3	Dark Brown	Dark green	Brown
Conc. HNO_3 + Distilled Water	Light Brown	Dark green	Brown
Methanol	Light Brown	Brown	Brown
Chloroform	Light brown	Black	Black
Petroleum ether	Light brown	Dark green	Black
$FeCl_3$	Black	Black	Black
Picric acid	Light grey	grey	Yellowish brown
Sodium Hydroxide	Brown	Dark green	Brownish green
Iodine	Dark brown	Dark green	Black
Distilled Water	Brown	Dark green	Black

Table 2.21. Powdered drug reaction with different reagent

Treatment	Observation
Conc. HCL	Reddish brown
Conc. HNO3	Brownish
Conc. H2SO4	Black
Glacial Acetic acid	Yellowish brown
Iodine solution	Redish
NaOH in Methanol	Brown

2.3.1.3.5. Determination of pH of the drug

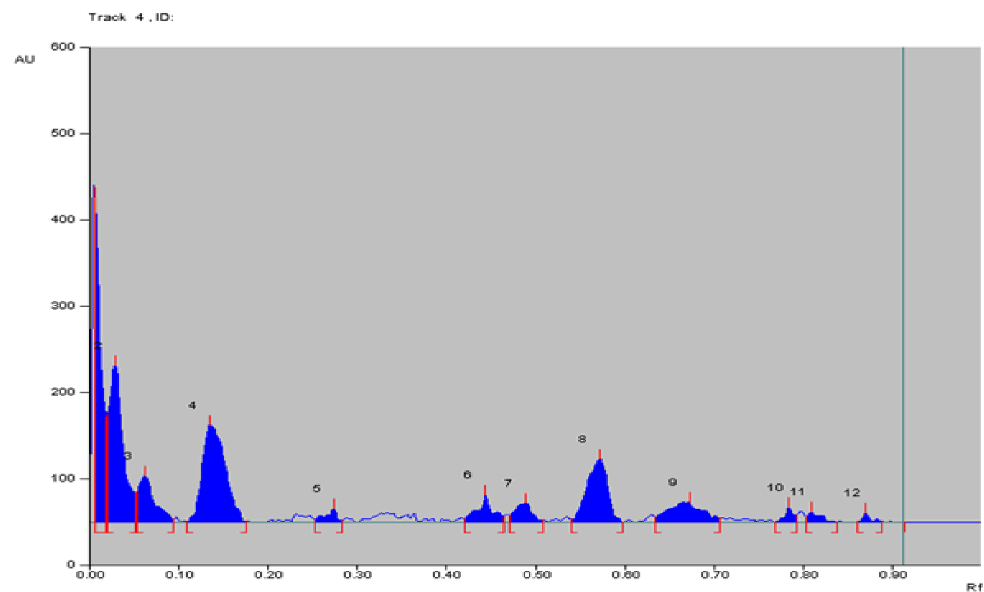
Table 2.22. pH of the drug

Sample	pH of 1 % solution	pH of 10 % solution
A. racemosus	4.87	5.66
	4.62	5.51
	4.64	5.52
Mean	4.71	5.56

Table 2.23. Thin layer chromatography of root extracts.

S.no.	**Root extracts**	**Solvent system**	**Number of spots**	**R_f value**
2.	Methanolic extract	Chloroform : methanol (6:4)	5	0.28,0.45,0.63, 0.74,0.82

2.3.1.2. HPTLC profile of *A. racemosus*



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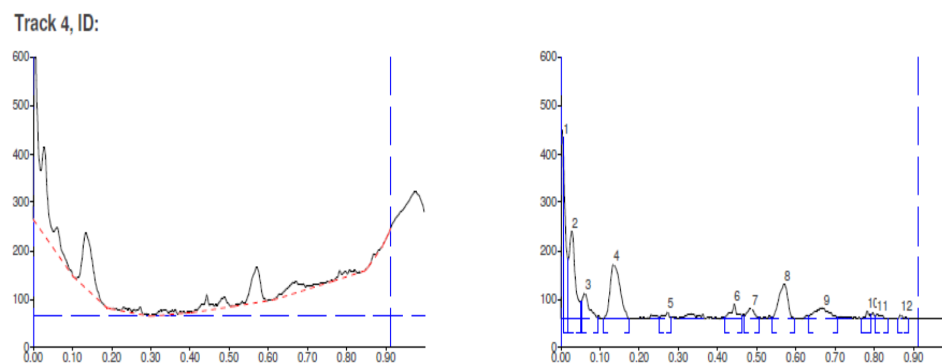


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	376.5	0.01	376.5	40.47	0.02	122.5	2367.1	19.04	unknown *
2	0.02	124.1	0.03	182.2	19.59	0.05	34.5	2675.9	21.52	unknown *
3	0.05	35.2	0.06	54.1	5.81	0.09	3.2	912.9	7.34	unknown *
4	0.11	1.1	0.14	112.3	12.07	0.17	0.9	2666.3	21.44	unknown *
5	0.25	3.8	0.27	15.3	1.64	0.28	2.4	185.3	1.49	unknown *
6	0.42	4.0	0.44	31.7	3.41	0.46	6.6	478.0	3.84	unknown *
7	0.47	7.7	0.49	22.1	2.37	0.51	2.3	391.5	3.15	unknown *
8	0.54	3.2	0.57	73.0	7.85	0.60	0.0	1538.9	12.38	unknown *
9	0.63	6.1	0.67	23.6	2.53	0.71	4.6	825.1	6.64	unknown *
10	0.77	0.1	0.78	17.2	1.85	0.79	9.0	151.8	1.22	unknown *
11	0.80	5.8	0.81	11.7	1.26	0.84	0.3	156.7	1.26	unknown *
12	0.86	0.8	0.87	10.6	1.14	0.89	0.8	83.8	0.67	unknown *

Fig: 2.4. HPTLC Finger printing of methanol extract of *A. racemosus*

2.3.2. *Euphorbia hirta*

2.3.2.1. Macroscopic characters

It is a slender- stemmed, annual hairy plant with many branches from the base to top, spreading upto 40 cm in height, reddish or purplish in color. Leaves are opposite, elliptic - oblong to oblong-lanceolate, acute or subacute, dark green above; pale beneath, 1- 2.5 cm long, blotched with purple in the middle, and toothed at the edge. The fruits are yellow, three- celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds**.**

2.3.2.2. Morphological and Microscopy of *Euphorbia hirta*

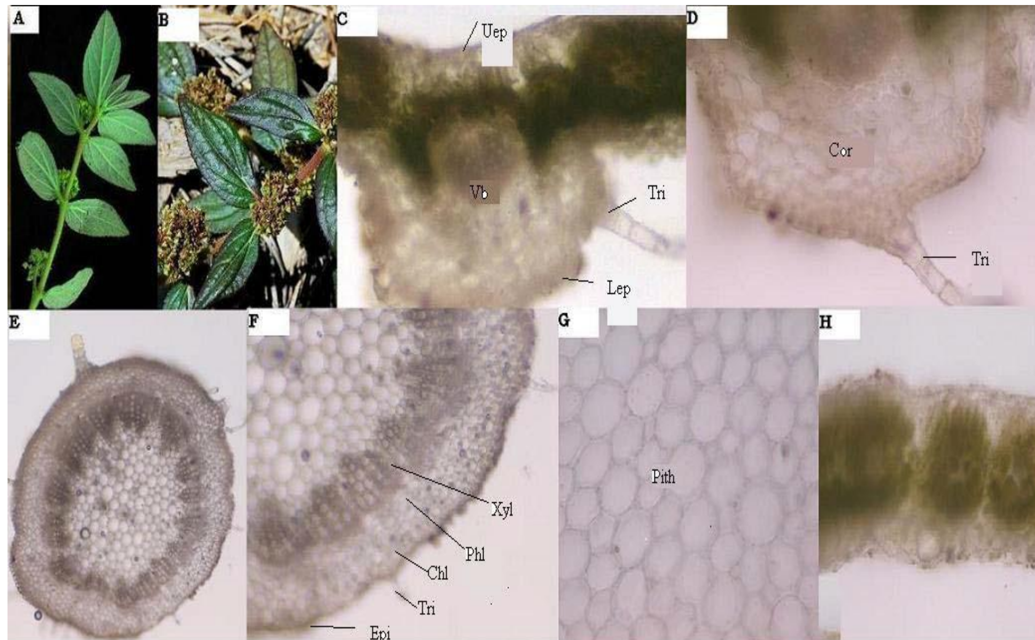


Plate 1: Figs.A-I. Macro- and Microscopical character of the leaves A. Leaf - ventral surface; B. Leaf- dorsal surface; C. TS of mid rib with lamina; D. region of mid rib from epidermis to vascular bundle enlarged; E.TS of stem; F. TS of stem in high magnification; G. TS of stem showing large pith; H. TS of leaf with lamina (Abbreviation: VB-vascular bundle; COL-collenchyma; COR- cortex;; PH-Phloem; XY-xylem; TRI-trichrome; Lep- Lower epidermis; Uep- upper epidermis

Fig 2.5. & 2.6.

Transverse section of leaf passing through mid-rib region shows single layered upper and lower epidermis, multicellular trichomes 4-6 layered collenchymatous cell are present below lower epidermis. The single arch shaped collateral meristele is located in the centre of mid rib.

2.3.2.3. Extractive value

2.3.2.3.1. Cold Extraction

Table: 2.24. Petroleum ether extractive

Wt. of drug (gm)	Wt. of empty petri plate (gm)	Wt. of petri plate + wt. of extractive matter (gm)	wt. of extractive matter (gm)	Extractive Value (%)
5.000	43.342	43.402	0.060	1.20
5.000	42.674	42.744	0.070	1.40
5.000	40.374	40.425	0.051	1.020
Mean	-	-	-	1.206

Table 2.25. Chloroform extractive

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive Value (%)
5.000	42.629	42.755	0.126	2.52
5.000	43.428	43.555	0.127	2.54
5.000	41.281	41.404	0.123	2.46
Mean	-	-	-	2.506

Table 2.26. Methanol extractive

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
5.000	41.753	41.397	0.485	9.70
5.000	43.608	44.107	0.499	9.98
5.000	44.864	45.35	0.486	9.72
Mean	-	-	-	9.80

Table 2.27. Aqueous extractive

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
5.000	42.684	43.354	0.670	13.40
5.000	41.795	42.446	0.651	13.02
5.000	43.364	44.055	0.691	13.82
Mean	-	-	-	13.41

2.3.2.3.2. Hot Extraction

Table 2.28. Petroleum ether extractive

Wt. of drug (gm)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	44.758	45.158	0.40	2.00
20.000	43.623	44.033	0.410	2.05
20.000	41.831	42.226	0.395	1.975
Mean	-	-	-	2.00

Table 2.29. Chloroform Extractive:

Wt. of drug (gm)	Wt. of empty Petri plate (gm)	Wt. of Petri plate + wt. of extractive matter (gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	41.683	42.173	0.490	2.45
20.000	41.759	42.230	0.471	2.355
20.000	43.631	44.141	0.510	2.55
Mean	-	-		2.451

Table 2.30. Methanol Extractive

Wt. of drug (gm)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter(gm)	Wt. of extractive matter (gm)	Extractive value (%)
20.000	43.374	45.814	2.44	12.20
20.000	42.831	45.282	2.451	12.255
20.000	41.429	43.861	2.432	12.160
Mean	-	-		12.205

Table 2.31. Aqueous Extractive:

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	42.173	45.484	3.311	16.555
20.000	43.411	46.762	3.351	16.755
20.000	40.752	44.062	3.310	16.550
Mean	-	-	-	16.620

2.3.2.3.3. Successive Extraction

Table 2.32. Petroleum ether Extractive

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	43.532	43.916	0.384	1.920
20.000	41.175	41.536	0.361	1.805
20.000	44.573	44.971	0.398	1.990
Mean	-	-	-	1.905

Table 2.33. Chloroform Extractive

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	42.438	42.899	0.461	2.305
20.000	41.643	42.114	0.471	2.355
20.000	44.431	44.884	0.453	2.265
Mean	-	-	-	2.308

Table 2.34. Methanol Extractive

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	Wt. of extractive matter (g)	Extractive value (%)
20.000	43.431	45.671	2.240	11.20
20.000	41.528	43.799	2.271	11.355
20.000	44.537	46.750	2.213	11.065
Mean	-	-	-	11.065

Table 2.35. Aqueous Extractive

Wt. of drug (g)	Wt. of empty Petri plate (g)	Wt. of Petri plate + wt. of extractive matter (g)	wt. of extractive matter (g)	Extractive value (%)
20.000	44.852	48.144	3.292	16.460
20.000	45.731	49.014	3.283	16.415
20.000	43.854	47.156	3.302	16.510
Mean	-	-	-	16.461

Table 2.36. Total Ash

Wt. of crucible (gm)	Wt. of crucible + drug (gm)	Wt. of crucible after ignition (gm)	Wt of ash (gm)	Ash value (%)
12.35	14.35	14.238	0.112	5.60
13.23	15.23	15.117	0.113	5.65
14.24	16.24	16.128	0.105	5.25
Mean	-	-	-	5.50

Table: 2.37. Acid insoluble Ash

Wt. of crucible (gm)	Wt. of crucible + drug (gm)	Wt. of crucible + acid treated ash after ignition (gm)	Wt of ash (gm)	Ash value (%)
13.34	15.34	15.299	0.041	2.05
12.46	14.46	14.415	0.045	2.25
14.25	16.25	16.204	0.046	2.30
Mean	-	-	-	2.20

Table: 2.38. Water soluble Ash

Wt. of crucible (g)	Wt. of crucible + Wt. of crude drug (g)	Wt. of crucible + Distilled water treated ash after ignition (g)	Wt. of Water Insoluble ash (g)	Wt of Water soluble ash (Total ash – water insoluble ash)	Ash value (%)
12.64	14.64	14.606	0.034	0.078	3.90
14.28	16.28	16.25	0.030	0.083	4.15
13.57	15.57	15.546	0.024	0.081	4.05
Mean	-		-	-	4.03

Table 2.39. Foreign matter analysis

Wt. of crude drug (g)	Wt. of drug after removal of foreign matter (g)	Wt. of foreign matter (g)	Foreign matter (%)
10.00	9.896	0.104	1.04
10.00	9.885	0.115	1.15
10.00	9.895	0.105	1.05
Mean	-	-	1.08

Table 2.40. Loss on Drying

Wt. of drug + China dish (Before drying) (gm) A	Wt. of drug + China dish (After drying) (gm) B	A-B (gm)	Loss on Drying (%) (w/w)
10.000 +105.651 = 115.651	141.153 141.551 141.551	0.851 0.841 0.861	8.51 8.41 8.61
Mean			8.51

Table 2.41. Flourescence analysis of *E. hirta*

Solvent used	*Day Light*	U V light	
		254nm	*366nm*
Powder as such	Dark green	Slight green	Blackish green
1N HCl	Greenish brown	Brownish green	Dark green
50% HCl	Light green	Green colour	Dark green
50% HNO_3	Green	Brownish green	Light green
50% H_2SO_4	Slightly green	Brownish green	Light green
1N NaOH	Light yellow	Green colour	Moderate green
Alcohol NaOH	Light green	Green colour	Dark green
Methanol	Light green	Dark green	Dark green
Benzene	Slightly Yellow	Slight buff	Green
$FeCl_3$	Brownish yellow	White	Green
1% KOH	Brownish black	Light buff	Dark green

Table 2.42. Powdered drug reaction with different reagent

Treatment	Observation
Conc. HCL	Dark green
Conc. HNO3	Light brown with whitish foam
Conc. H2SO4	Greenish yellow
Glacial Acetic acid	Light yellow
Iodine solution	Dark brown
NaOH in Methanol	Light green

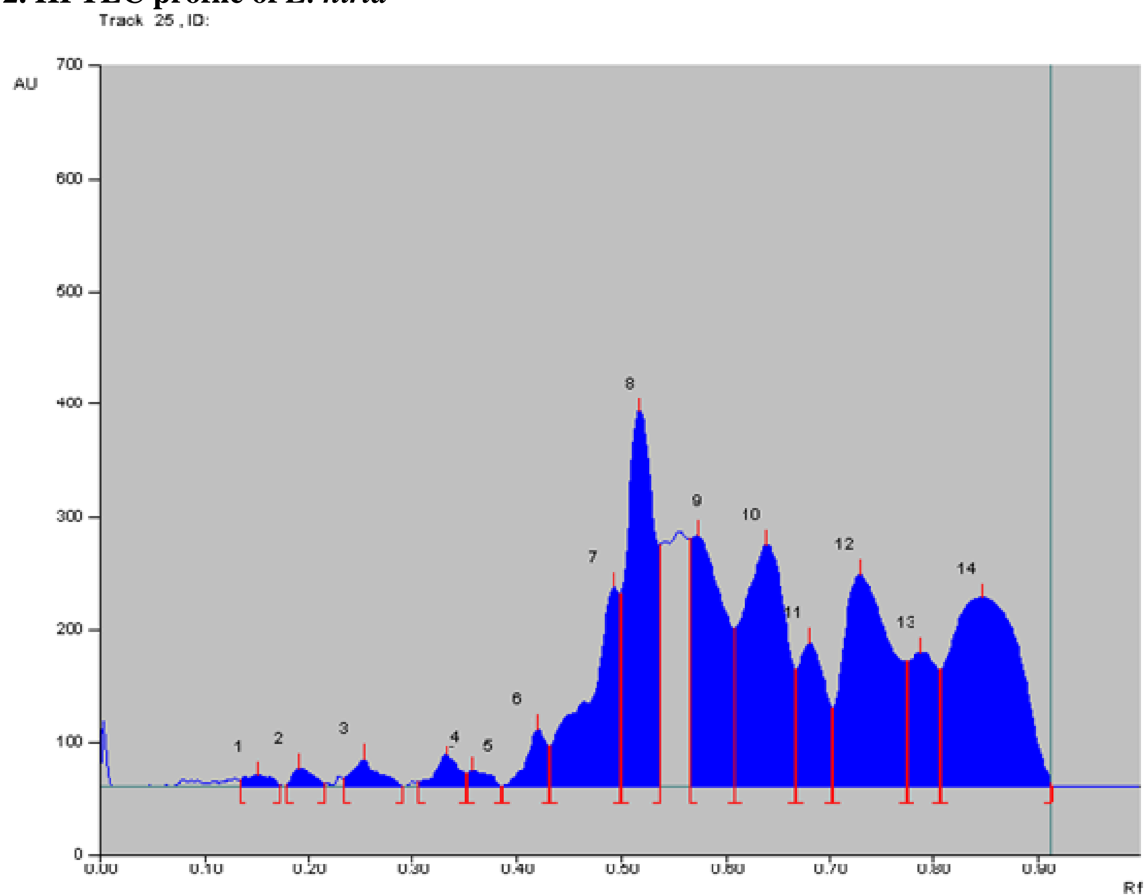
Table 2.43. Determination of pH of the drug

Sample	pH of 1 % solution	pH of 10 % solution
E. hirta	5.22	5.46
	5.21	5.50
	5.18	5.44
Mean	5.20	5.46

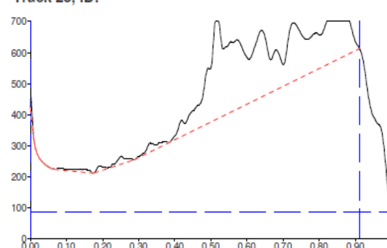
Table 2.44. Thin layer chromatography of extracts.

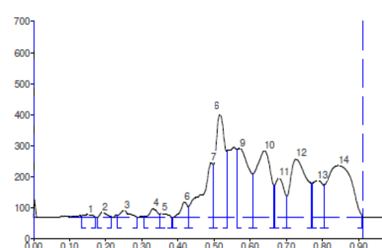
S.no.	extracts	Solvent system	Number of spots	R_f value
1	Methanolic extract	Toluene :Ethyl acetate: Formic acid (3:1:1)	4	0.28,0.45, 0.68,0.74

2. HPTLC profile of *E. hirta*



Track 25, ID:





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.13	5.6	0.15	10.5	0.62	0.17	1.5	248.0	0.45	unknown *
2	0.18	0.9	0.19	17.5	1.02	0.22	2.7	299.4	0.54	unknown *
3	0.23	7.1	0.25	25.1	1.47	0.29	0.1	568.9	1.03	unknown *
4	0.31	3.9	0.33	29.6	1.74	0.35	11.5	580.3	1.05	unknown *
5	0.35	11.9	0.36	14.8	0.87	0.38	0.4	262.0	0.48	unknown *
6	0.39	0.4	0.42	51.4	3.02	0.43	36.6	910.1	1.65	unknown *
7	0.43	36.7	0.49	177.3	10.41	0.50	171.0	5034.0	9.15	unknown *
8	0.50	171.1	0.52	333.6	19.58	0.54	215.1	7919.1	14.40	unknown *
9	0.56	220.0	0.57	223.7	13.13	0.61	141.0	6630.9	12.05	unknown *
10	0.61	141.3	0.64	215.5	12.65	0.67	104.6	8231.6	14.96	unknown *
11	0.67	104.8	0.68	127.9	7.51	0.70	69.4	2992.2	5.44	unknown *
12	0.70	69.6	0.73	189.0	11.09	0.77	111.8	7981.5	14.51	unknown *
13	0.77	111.9	0.79	119.9	7.04	0.81	105.6	3096.7	5.63	unknown *
14	0.81	106.0	0.85	167.8	9.85	0.91	3.1	10253.7	18.64	unknown *

Fig: 2.7. HPTLC Finger printing of methanol extract of *E. hirta*

2.4. Discussion

As a part of standardization, the macroscopical examination of *A. racemosus* and *Euphorbia hirta* was studied. It showed important characteristics. It is a technique of qualitative evaluation which is settled on the study of morphological and receptive interprets of drugs (Figure 2.1 and 2.5). The anatomy of plants (Figure 2.2 and Figure 2.3) and (Figure 2.6) were examined by taking transverse sections. The microscopical analysis of the transverse section was the recognizing features and can be utilized as anatomical marker. It revealed the histological features of both the drugs.

The extracts obtained by the plant materials with specific solvents are indicative of extractive value. The drug was extracted with different solvents in order of their increasing polarity. Higher extractive values were seen in *Asparagus racemosus* (Table 2.7, 2.8, 2.9 and 2.10) and *Euphorbia hirta* (Table 2.28, 2.29, 2.30 and 2.31) in hot extraction, indicating the effect of elevated temperature on extraction. The percent extractive in different solvents indicates the quantity and nature of constituents in the extract. The extractive values are also useful in determining the unique constituents soluble in particular solvent (Hussain et al, 2010).

The residue remaining after complete burning of plant material is the ash content or ash value, which simply represents inorganic salts. It was determined by three different methods, which measured total ash, acid-insoluble ash, and water-soluble ash, for *Asparagus racemous* 8.2, 2.946 and 5.65 respectively illustrated in the (Table 2.15, 2.16, 2.17) for *Euphorbia hirta* the values were 5.50, 2.20 and 4.03 illustrated in the (Table 2.36, 2.37 and 2.38). These ash values are important quantitative standards. Foreign matter analysis was performed in order to detect the contaminatiom of the plant material *Asparagus racemous* contained 1.10 (Table 2.18) while *Euphorbia hirta* contained 1.08 (Table 2.39) debris in the known amount of the plant material.

The fluorescence analysis of the powdered drug from root of *Asparagus racemosus* (Table 2.20) and *Euphorbia hirta* (Table 2.41) in various solvents was performed under normal and UV light. It is the phenomenon presented by various chemical constituents present in the plant material. Powdered drug reaction *Asparagus racemous* (Table 2.21) *Euphorbia hirta* (Table 2.42) with different reagents was also performed resulting in the colour change. Some components show fluorescence in the visible range, if chemical constituents themselves are not fluorescent, they may be converted into fluorescent derivatives by utilizing different reagents. In this way some crude drugs are assessed qualitatively and it is an important parameter of pharmacognostical evaluation. pH determination was performed to detect the nature of the particular drug. *Asparagus racemous* shows pH of 1% solution and 10% solution 4.71 and 5.56 respectively (Table 2.22) and *Euphorbia hirta* shows 5.20 and 5.46 (Table 2.43).

The percentage of active chemical constituents is mentioned on air-dried basis in raw drugs. Therefore, the loss on drying of plant materials should be determined, for *Asparagus racemous* 5.53 (Table 2.19) and *Euphorbia hirta* 8.51 (Table 2.40).

Thin layer chromatography (TLC) is a valuable technique for the separation of the plant constituents. The chromatographic profile includes the characteristic finger print for qualitative evaluation of plants. The values of *Asparagus racemous* are summarized in (Table 2.23) and *Euphorbia hirta* in (Table 2.44). A validated qualitative HPTLC method has been developed for both the drugs and it reveals the presence of different constituents.

2.5. Conclusions

Preliminary phytochemical screenings of the plants were studied along with physicochemical parameters using TLC and HPTLC profile. After the investigation it can be concluded that the standardization and preliminary phytochemical studies of *Asparagus racemosus* and *Euphorbia*

extacts yielded a set of standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant materials. These studies can also help the ayurvedic manufacturer for the quality, purity, safety and for the selection of the raw material for various formulations.