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Pharmacognostical Studies & Phytochemical Evaluation of the Stem Barks of *Embilica Officinalis* Gaertn.

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ABSTRACT: To investigate the pharmacognostic parameter, physico-chemical parameters, fluorescence analysis, preliminary phytochemical screening, and Thin Layer Chromatographic analysis of the stem barks of the plant *Embilica officinalis* Gaertn. The air-dried and powder bark of the plant *Embilica officinalis* Gaertn (Euphorbiaceae) were studied by morphology, microscopy, florescence analysis, preliminary phytochemical screening and thin layer analysis of powdered drug. Other physicochemical parameters were also performed as per WHO guidelines. The dried powder leaves were investigated by morphology. The results of physic-chemical parameters such as loss on drying, ash values and extractive values, fluorescence analysis, preliminary phyto-chemical screening and TLC are summarized here. The present information on the pharmacognostic evaluation of the plant drug *Embilica officinalis* Gaertn delivered the qualitative and quantitative parameters serve the important information to the identity and to determine the quality and purity of the plant material in the future. It also signify the important information of the closely related other species and varieties.

KEYWORDS: *Embilica officinalis* Gaertn; Physico-Chemical Evaluation; Phyto-Chemical Screening; Quality Control Test.

INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to serve mankind with new remedies. At present, there is a worldwide movement for assessing the plant resources which are of medicinal and economical value and importance. Researchers are focusing mainly on ethnobotanical & ethnomedicinal investigations to fulfill the increasing demand of herbal products. In the last few decades there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects [1]. WHO estimate that, about 80% of the population in the developing countries depends directly on plants for its medicine [2, 3]. In India 2000 medicinal preparations used are of plant origin. India has a rich heritage of traditional medicine and the traditional health care system have been flourishing for many centuries.

The North-East region of India (22^0-29^0 N; 89^0-97^0 E) comprises the Sikkim and the seven sister states namely Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura. This region of India has several hill ranges interspersed with valleys and is by large sparsely populated. Nearly 40% of the total geographical area of this region is covered by evergreen forest. A large no of people

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belonging to various groups of the northeastern region of India still practicing their own traditional heath care systems.

Emblica officinalis Gaertn (syn. *Phylanthus emblica*) is a well known Indian traditional medicinal plant which is popularly known as AMLA (AMLAKHI –in Assam), belonging to the family Euphorbiaceae. It is widely available roadside tree of this region. The different parts of the trees have multi-disciplinary utility to human. In traditional system of medicine the fruits and barks are used as a tonic, diuretic and a laxative. The fruits are useful in treating asthma, bronchitis, intermittent fever and cardiac disorder. The roots are said to be an emetic. The root barks are useful in treating ulcerative stomatitis. The leaves juice is used to treat fever, conjunctivitis, diarrhea and dysentery. The powder of seeds is useful to treat asthma, bronchitis and biliousness [4].

The bark of *E. officinalis* may can be easily adulterated with inferior materials. This adulteration can be prevented by means of various evaluation parameters like microscopic study. Microscopy is an important tool for authentification of crude drugs and study of powdered drugs [5]. Evaluation of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. The evaluation of a crude drug is necessary because of these main reasons i) biochemical variation in the drugs ii) detoriation due to treatment and storage, and iii) substitution and adulteration, a result of carelessness, ignorance or fraud. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituents present in the crude drug, in addition to its morphological and microscopic analysis. With the advent of separation techniques and instrumental analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative in nature [6, 7]. The present study on this plant was undertaken to determine the pharmacognostical parameters for evaluating the plant material. Various parameters like macro and microscopic properties, physico-chemical evaluations like ash value, extractive value, loss on drying (LOD) and phyto-chemical screening test, TLC profiling and fluorescence analysis of powdered crude drug were carried out and some salient qualitative as quantitative parameter were mentioned.

MATERIALS & METHODS

Plant material

The stem barks of *Emblica officinalis Gaerts.* (Family - Euphorbiaceae) were collected from campus garden of Dibrugarh university, Dibrugarh, Assam, India in the month of August. The plant was identified and authenticated taxonomically by Dr. B.K. Sinha at National Botanical survey of Shillong, India. A voucher specimen DU/PSc/HRB-01/2011 of the collected sample was deposited in the institutional herbarium for future reference.

Plant profile

Taxonomical classification [8]

- Kingdom : Plantae (Plants)
- Subkingdom :*Tracheobionta* (Vascular plants)
- Superdivision : *Spermatophyta* (Seed plants)
- Division : Angiospermae (Flowering plants)
- Class : Dicotyledonae (Dicotyledons)
- Subclass : Rosidae
- Order : Geraniales
- Family : Euphorbiaceae
- Genus : *Emblica*
- Species : *officinalis* Geartn.

Vernacular name [9]

- English : Indian Goose berry
- Sanskrit : Aamalaki
- Assamese :Amlokhi
- Bengali : Amloki
- Hindi : Amla

Reagent and Chemicals

All reagents and chemicals used for pharmacognostic evaluation and phyto-chemical screening were analytical grade obtained from SRL Chemical, Rankem, Otto, Himedia Pvt. Ltd. India.

Organoleptic evaluation

Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation.

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Physico-chemical evaluation

Physico-chemical parameters such as the percentage of loss on drying (LOD), total ash, acid insoluble ash, water soluble ash were determined as per guideline of the Indian Pharmacopoeia [10]. Water and alcohol soluble extractive were estimated by cold maceration according to the method prescribed by WHO [11]. All the parameters were taken in triplicate and the results which were obtained presented as mean $\pm$ standard error of mean (SEM).

Macroscopic evaluation

The bark was morphologically studied for its size, shape, surface, fracture and configuration. The macroscopy of crude drug includes its visual appearance to the naked eyes and its sensory characteristics [12, 13]. Simple microscope of magnification 10xs was used for the perception of special structural features such as:

- Size and shape of the drug,
- Colour and external marking
- Fracture and degree of uniformity of the particles.
- Surface appearance by reflected light, shining particles, fibres and crystals.

Microscopic characters

Sectioning

Observation of Transverse Section (TS) and Longitudinal Section (LS) of a plant part reveals the arrangement of cells in a tissue and structure and morphology of a particular cell from all angles. Thin Transverse Section (TS) and Longitudinal Section (LS) were cut using hand sectioning method and stained with saffranin. Then these were mounted in glycerine and observed under microscope.

Microscopic description of tissues are supplemented with micrographs wherever necessary, photographs of different magnification were taken with Nikon Labphoto2 Microscopic unit. For normal observation bright field was used for the study of crystals, cork, parenchymatous cell and fibres. Descriptive terms of the anatomical features are mentioned as given in the standard anatomy books **[**14].

Fluorescence Analysis

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations [15, 16]. The colors observed by application of different reagents in various radiations were recorded.

Phytochemical screening

Phytochemistry is the study of phytochemicals that is derived from plants. In other word it is the study of plant's secondary metabolites, their identification, isolation, estimation and standardization [17, 18, 19].

The dried and coarsely bark sample was extracted successively with petroleum ether (60-80^{0}C), chloroform, acetone, methanol in a soxhlet extractor & water by cold maceration. The concentrated extracts were evaporated to dryness and the extracts obtained with each solvent were weighed. Their percentage was calculated in terms of initial air dried plant material. The colors of extracts were observed. The successive extract, as mentioned above, were subjected to various qualitative phyto-chemical test for the identification of chemical constituents present in the plant material.

Thin Layer Chromatographic Analysis

Preparation of plates:

Glass plates of 5 x 20-cm^2 size were coated with silica gel G with the help of spreader to a layer thickness of 0.25 mm. After spreading, the plates were first air dried and then activated at 110 ^{o} C for 30 min [20, 21].

Application of sample:

Sample solutions of tests were spotted using a capillary tube. The spots were placed at equidistance from each other. The spot area was kept 2cm above the base of the plate so that the spotting area does not be immersed in mobile phase in the development chamber.

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Mobile phases: The following solvent systems (SS-1, SS-2 & SS-3) were selected to run the TLC plates-

SS-1: Petroleum ether (40-60ºC): Ethyl acetate: Glacial acetic acid (4: 1: 2)

SS-2: Toluene: Ethyl acetate: Formic acid (9: 0.5: 0.5)

SS-3: Petroleum ether (40-60ºC): Ethyl acetate: Glacial acetic acid (2.5: 1.5: 1)

Development of chromatogram:

The extracts were spotted on the plates with the help of fine bore capillaries and chromatogram were developed in chromatographic chamber using different solvent systems in a room temperature (31 º C and at an angle of 75^0). In all the cases the solvent system was allowed to run to a distance of 10 cm from the point of application of the extract in the plates. The time required for the development varied from 30-40 min. After completion of run the plates were removed from the chamber and allowed to dry in air. These plates were observed after development in Iodine-chamber for the presence of the spots and the R_f values of the spots were calculated and recorded. Retardation factor is the ratio of distance travelled by the solute to the distance travelled by the solvent.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

RESULTS & DISCUSSION

Macroscopic Evaluation The characters recorded are described below.

General appearance – Single or branched and entire or longitudinally sliced stems.

Colour- Light grey

Odour – Characteristics

Taste- Astringent

Shape- Rough surface.

Physico-Chemical Evaluations

The values of all determinations viz. Ash values, Extractive values and Loss on drying are summarized in Table 1. In this evaluations the amount of acid insolubale ash is lesser than water soluble ash, where as the amount of total ash was nearly double of their water soluble ash.

Microscopic Transverse section (T.S) and longitudinal section (L.S.) of stem barks *Emblica officinalis Gaertn.* are shown in fig. 1 and 2.

Sl. No.	Parameters	Average values of three replicates ± S.D of stem barks
1.	Total ash	10.334 ± 0.4714
2.	Acid insoluble ash	0.567± 0.0008
3.	Water soluble ash	6.507± 0.8448
4.	Water soluble extractive	11± 0.8164
5.	Alcohol soluble extractive	7± 0.8164
6.	Loss on drying	7± 0.8164

Table 1 Values of Physico-chemical parameters (Ash values, Extractive values and Loss on drying)

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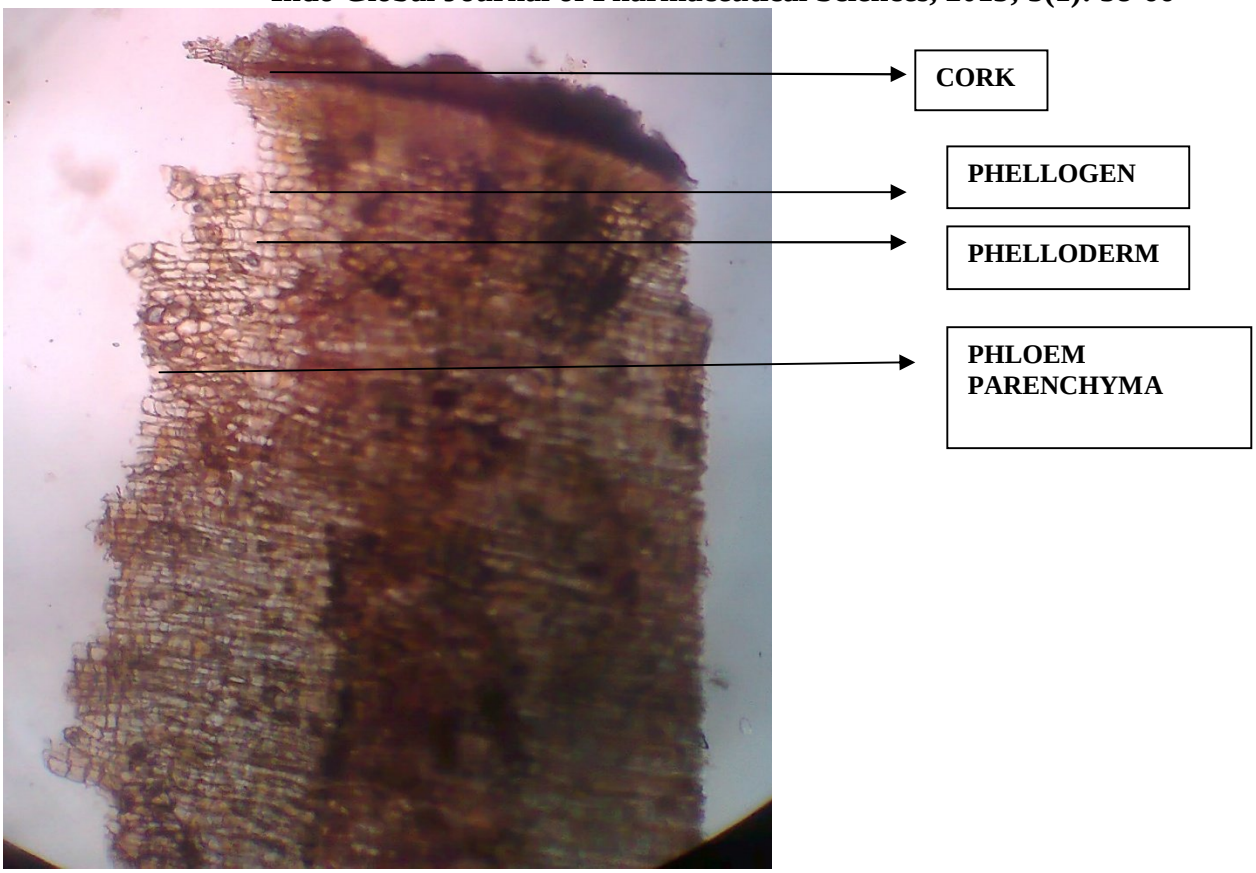

Fig.1. Transverse section (T.S) of stem barks *Emblica officinalis Gaertn.*

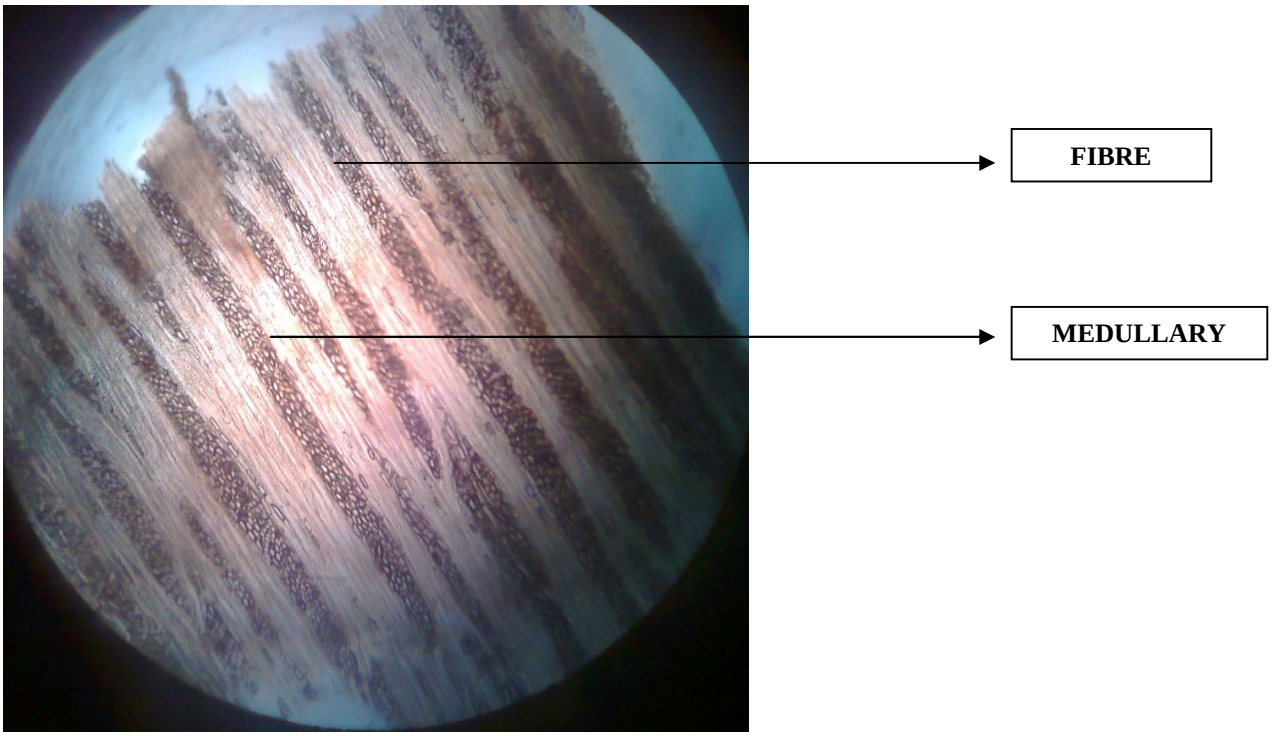

Fig. 2 Longitudinal section (L.S.) of stem barks *Emblica officinalis* Gaertn.

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Fluorescence Analysis

The results were summarized in Table no 2.

Phytochemical screening: Refer Table 3.

Thin Layer Chromatographic Analysis

The leaves extract showed three distinct spots with different intensities. The colors are yellow, yellowish green and violet in color respectively. The resultant R_f values were summarized in Table -4.

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder drug as such	Light brown	Brown	Yellowish brown
Powder + Methanol	Light brown	Yellowish brown	Brownish black
Powder + 1% glacial acetic acid	Brown	Dark brown	Blackish brown
Powder +10% NaOH	Yellowish brown	Dark yellowish brown	Bluish brown
Powder + dil. NH_3	Yellowish brown	Light brown	Brown
Powder + Conc. HNO_3	Brown	Blackish brown	Dark brown
Powder+ dil.NH_3+Conc.HNO_3	Yellowish brown	Light brown	Blackish brown
Powder +1M H_2SO_4	Brown	Dark brown	Yellowish black
Powder +1M HCl	Brownish yellow	Brown	Dark brown
Powder + 10% $FeCl_3$	Reddish brown	Light brown	Brownish yellow
Powder +Acetone+ Methanol	Light brown	Brown	Black
Powder +10% Iodine	Yellowish brown	Dark brown	Blackish brown

Table 2 Shows fluorescence analysis of powdered bark of E. officinalis Gaertn.

Sl. No.	Plant Constituents	EXTRACTS (STEM BARKS)				
		Petroleum ether (40°-60°c)	Chloroform	Acetone	Methanol	Water
1.	Alkaloids:	_	+	+	+	_
2.	Carbohydrates	_	_	+	+	+
3.	Glycosides	_	_	+	+	+
4.	Steroids:	+	+	+	+	_
5.	Fats and Oils:	_	_	+	+	_
6.	Tannins and Phenolic compounds:	_	_	+	+	+
7.	Proteins:	_	_	+	+	+
9.	Gums:	_	_	+	+	_
10.	Mucilages:	_	_	_	_	_
11.	Flavonoids:	_	_	+	+	+
12.	Lignin's:	_	_	_	_	_
13	Triterpenes:	_	_	_	_	_

Table 3 Shows phyto-chemical screenings of successive extracts of stem barks of Emblica officinalis Gaertn. Here, '+' indicates presence '-' indicates absence

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Sl. No.	Chromatography Solvent	Extracts	Number of Spots	R_f Values	Visualizing Agents
1.	SS-1:Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid (4 : 1 : 2)	Petroleum Ether	2	0.80 0.86	Iodine
		Chloroform	2	0.69 0.84	Iodine
		Methanol	3	0.83 0.65 0.76	Iodine
2.	SS-2:Toluene : Ethyl Acetate : Formic Acid (9 : 0.5 : 0.5)	Petroleum Ether	3	0.72 0.77 0.81	Iodine
		Chloroform	2	0.54 0.71	Iodine
		Methanol	2	0.64 0.67	Iodine
3.	SS-3:Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid (2.5 : 1.5 : 1)	Petroleum Ether	2	0.63 0.74	Iodine
		Acetone	1	0.73	Iodine
		Methanol	3	0.28 0.57 0.73	Iodine

Table 4. TLC profile of Emblica officinalis Gaertn. Stem barks extracts.

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [22]. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs [7]. The Organoleptic studies shows the important characteristics of the drugs, the outer structure of the barks, the surface of the barks, the typical tongue sensation and the odour may screen the preliminary phytochemical constituents.

Ashing involves an oxidation of the component of the product. A high ash value is indicative of contamination, substitution or adulteration. The Total ash usually contains carbonates, phosphates, silicates which includes both physiological and non-physiological. Acid-insoluble ash usually indicates the contamination with silicon material like earth and sand. Water-soluble ash was used for the estimation of the amount of inorganic elements. Extractive value represent the extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. By following the cold maceration method, the yield of water-soluble extractive was found greater than alcohol soluble extractives.

Loss on drying value is an important parameter of crude drug evaluation, which helps in its preservation. The objective of drying of fresh material is to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug and to reduce their weight and bulk.

Microscopic method is one of the cheapest and simplest methods to start with establishing the correct identification of the source material [24]. The transverse section of *E. 0fficinalis* bark exhibited different distinguished layers of cells consisting of cork cells, cortex, phelogen and pheloderm

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tissues. The Cork consists of elongated, thin walled parenchymatous cells. Cortex consists of 3-4 layers of collenchymatous cells. Phellogen consists of a row of tangentially elongated cells, Phelloderm consists of parenchymatous cells and Phloem parenchyma consists of rectangular cells and in the T.S of bark the groups of fibers are frequently found associated with medullary rays. Medullary rays are of 2-3 seriate and parenchyma consists of lignified cells.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [15, 23].

Five solvents were selected for the extraction of stem bark of *Emblica officinalis Gaertn.* i.e Petroleum ether, Chloroform, Acetone, Methanol, and Water and different phytochemical tests were performed. The petroleum ether and the chloroform extracts of stem bark shown negative response for the entire test, except sterols in the extract. But the chloroform extract which represents the presence of alkaloids. The acetone extract and the methanol extract showed most of the test positive. The presence of carbohydrates, proteins, glycosides and tannins are in the water extract but the other compounds such as alkaloids, steroids, fats etc. are absence in this solvent. The preliminary phytochemical studies exhibited the presence of the maximum number of phytoconstituents in the methanolic extract of the stem bark of *E. officinalis.*

TLC is produced with the aim of identifying the individual substances in a mixture and also for testing purity or for separation of mixtures. The R_f value indicates the position at which a substance is located in a chromatogram. It is appropriate to regard R_f value as a guide for identification. Three different mobile phases and iodine used as visualizing agents as for consideration of TLC determination.

Two spots were visualized in petroleum ether & chloroform extract & three spots were shown in methanol extract when eluted with solvent system-1. In the SS-2, three spots were observed in petroleum Ether & two spots in chloroform & methanol extract while in SS-3; two spots were noticed in petroleum ether, single spot in acetone & three spots in methanolic extract.

CONCLUSION

The present pharmacognostic data emphasize the knowledge of quality and identity of the plant *Emblica officinalis* Gaertn. The qualitative and quantitative parameters serve the important information of the plant E. *officinalis* Gaertn. These information will also be helpful to differentiate *Emblica officinalis* Gaertn from the closely related other species and varieties. In conclusion, the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug.

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