CHAPTER 2

Pharmacognostical standardization of *Elytranthe parasitica*

2.0.1. **KEYWORDS:** - Standardization, *Elytranthe parasitica*, HPTLC fingerprinting


2.1. **INTRODUCTION**

The most primitive evidence of human dependence on plants for healing and therapeutic benefits can be traced back to the Neanderthal period (Winslow & Kroll, 1998). Consequently, plant-derived herbs have been reckoned to interact favorably with the human body and so produce numerous health benefits. In various parts of the world, folklore therapy advocates the use of medicinal herbs and plants for the treatment of several ailments. In this regard, traditional medicinal plants are a treasure trove of innumerable biologically active phytochemicals (Namdeo, 2007). An approximate 80% of the non-industrialized countries bank on medicinal plants as the vital source of medicine for their primary healthcare needs (Fransworth & Soejarto, 1985). Astoundingly, swiftly industrializing countries such as the People’s Republic of China depends on Traditional Chinese Medicine (TCM) and thereby invests heavily on TCM and other traditional practices such as reiki, acupuncture, etc. (Houghton, 1995). Similarly, the science of Ayurveda with its origins in India has made gigantic leaps, and there is an overall positive trend towards holistic and integrative medicine worldwide (Patwardhan et al., 2004).

An estimated 80% of pharmaceuticals which include antimicrobial, cardiovascular, immunosuppressive and anticancer drugs are derived from plants (Gordaliza, 2009). Pharmaceutical medicines obtained from flowering plants, primarily from Fabaceae, Malvaceae, Asteraceae, Cruciferae, Solanaceae, Liliaceae, etc. account for almost 30% of the prescribed medications (Balandrin et al., 1985). An estimated 85% of traditional medicine involves the use of plant extracts (Farnsworth NR) which may be referred as “modern herbal medicine.” The
number of patients seeking herbal approaches for therapy has also grown exponentially, with an approximate 38.2 million in the United States relying on herbal supplements for healthcare, according to a survey in 2002 (Zhang et al., 2011). Various studies have shown the increased dependence on alternative medicine by a substantial number of patients on chemotherapy primarily for boosting their immunity and alleviating the side effects of conventional chemotherapeutics (Damery et al., 2011, Poonthananiwatkul et al., 2015).

Owing to their potential to cure diverse ailments, the demand for plant-derived substances has risen tremendously (Baris et al., 2006). The market value of herbal pharmaceuticals sold in the United States alone exceeded US$ 65 billion in 2003 (Gordaliza, 2009). The value of the global herbal market is projected to reach an estimated $5 trillion by 2050 according to a WHO forecast (Aneesh et al., 2009).

2.2. REVIEW OF LITERATURE
The development of herbal drugs involves numerous steps, beginning with acquiring a passport data on raw materials, accurately identifying the pharmacognostic features, evaluating chemical quality, assessing safety and efficacy via preclinical studies which ultimately gather sufficient data to perform randomized, controlled clinical trials. Plant materials are often obtained from natural sources, although many of the medicinal plants are also cultivated. The dynamic evolutionary process could modify and have a significant effect on the identity and structure of natural materials. Besides, several botanical species are on the verge of extinction. Even if they are available, their properties as documented in classic texts may have undergone considerable change due to time and environmental factors. Due to these reasons, the correct identification and supply of raw materials to avoid adulteration have indeed become a challenge during commercialization of herbal products. Therefore, addressing the need for standardization is crucial and requires broader consideration (Patwardhan et al., 2004).

Additionally, assessment of the plant identity and its chemical composition is highly essential to acquire the anticipated pharmacological effect (Houghton, 1995). Hence, plant parts, extract, fractions and isolated compounds proposed for therapy should meet certain strictly determined standards such as pharmacopoeial requirements included in the pharmacopoeia. Hence standardization of the plant material and herbal preparations would ultimately certify their therapeutic value. There are a wide number of methods to perform standardization plant material,
namely macroscopic (focusses on botanical identity and purity of the plant material); microscopic (focused on histochemical investigations, which provides the basis for identification of the material); biological (microbiological, biomolecular studies) and chemical methods. Chemical investigations of the plant material have a variety of goals, such as quantitative analysis of bioactive compounds, isolation of phytocomponents from the plant tissues for their further identification, or physicochemical characterization, and, finally, structural analysis of the isolated unknown compounds (Waksmundzka, Sherma & Kowalska 2008). Due to the lack of established regulatory standards and implementation protocols, there is a growing need for efficient purity control of the plant material (Chawla et al., 2013). In general, the regulations for a herbal product in Europe and United States are more rigorous than in India (Verma, 2013). The World Health Organization (WHO) has come up with series of technical guidelines which would help ensure the quality of medicinal products for the safety of the consumers (Kokate CK, 1994).
2.3. **OBJECTIVE**
The present study aimed to standardize the stem parts of *Elytranthe parasitica* (L.) Danser (Loranthaceae) as per the WHO guidelines.

2.4. **MATERIAL AND METHODS**
2.4.0.1 **Chemicals and reagents**
Ethanol was procured from Hayman Ltd, Essex, UK. Petroleum ether, diethyl ether and butanol were purchased from Finar Limited, Ahmedabad, India. Methanol was purchased from Merck Specialties Pvt Ltd, Mumbai, India. Sodium carbonate was purchased from Nice Chemicals Pvt Ltd, Kochi, Kerala, India. Aluminium chloride anhydrous, Folin & Ciocalteau’s phenol reagent (#F9252), Folin-Denis’ reagent (#47742), Gallic acid (#147915) and quercetin (#Q4951) were purchased from Sigma Aldrich, St Louis, USA. Pre-coated HPTLC and TLC silica gel aluminium plates 60F254 (20 cm × 20 cm with 0.2 mm thickness) were procured from Merck Life Sciences Ltd Pvt, Mumbai, India. All reagents and chemicals used were of analytical grade.

2.4.0.2 **Instrumentation**
UV Spectrophotometer (UV-1650PC, Shimadzu), CAMAG high performance TLC system (CAMAG Chemie-Erzeugnisse & Adsorptionstechnik AG, Muttenz, Switzerland) consisting of Linomat 5, Scanner 4, UV Cabinet 4, Twin trough chambers, WinCATS 3.00 software, Microplate ELISA reader (ELx800, BioTek Instruments Inc., Winooski, Vermont, USA).

2.4.1. **Collection and authentication of plant material**
Stem of *Elytranthe parasitica* (EP) growing on the sacred fig tree, *Ficus religiosa* was collected from Manipal, Karnataka, India. The plant was authenticated by Dr. Gopala Krishna Bhat, Taxonomist, Department of Botany, Poornaprajna College, Udupi, Karnataka, India. A voucher specimen (PP 565) has been deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal for future reference.

2.4.1.1 **Extraction of plant material and preliminary fractionation**
EP stem (10 kg) was shade-dried, coarsely powdered and extracted with methanol by Soxhlet extraction to obtain the methanol extract (EP.M). After completion of extraction, the extract was concentrated under reduced pressure and controlled temperature and stored in the desiccator until further use. EP.M (200 g) was fractionated sequentially with solvents petroleum ether, diethyl ether, chloroform, methanol, and water.
ether, ethyl acetate, butanol and water to obtain petroleum ether fraction (EP.PE), diethyl ether fraction (EP.DEE), ethyl acetate fraction (EP.EA), butanol fraction (EP.But) and aqueous fractions (EP.Aq) respectively. All fractions were concentrated on a rotary evaporator to complete dryness and their percentage yield was calculated. The fractions were stored at 4°C until further use.

2.4.2. Pharmacognostical standardization as per WHO guidelines

Coarsely powdered EP stem parts were subjected to macroscopic, microscopic and physicochemical standardization. EP extract/fractions (which would be taken up for bioactivity guided fractionation) was subjected to phytochemical standardization in accordance to WHO guidelines (Geneva Organisation Mondiale De La Sante, 1992).

2.4.2.1 Macroscopic Standardization

Various morphological features of EP stem parts such as its size, shape, colour, surface, texture, fracture and appearance of cut surface were studied.

2.4.2.2 Microscopic Standardization

Transverse Section - Free hand sections of EP stem were taken and warmed with chloral hydrate to remove the hydrophobic pigments such as chlorophyll. Uniform, clear and transparent sections were selected, stained with phloroglucinol/HCl (Hydrochloric acid) and viewed at low magnification (10 X) and high magnification (40 X).

Powder Microscopy - Powder of EP stem was examined for its microscopic characters. The powder was passed through sieve no. 60, warmed with chloral hydrate and viewed under microscope for calcium oxalate crystals and other characters. The clarified powder was later stained with phloroglucinol in the presence of HCl for lignified structures such as stone cells. The powder was viewed for starch grains after adding iodine to it.

2.4.2.3 Physicochemical Standardization

2.4.2.3.1 Determination of Ash value

Ash value indicates the inorganic salts present in the crude drugs or adhering to the crude drug. It also helps to detect inorganic substances added to the crude drug to adulterate it. Total ash refers to the residue obtained after incineration. Part of the total ash, which is insoluble in an acid (e.g.
HCl) is known as acid-insoluble ash; part of the total ash which readily dissolves in hot water is called water soluble ash. Percentage of total ash, acid insoluble ash and water-soluble ash was performed as per the WHO guidelines (Geneva Organisation Mondiale De La Sante, 1992).

Briefly,

A. **Total ash** - About 2 g of the powdered drug was accurately weighed in a tarred silica crucible. The powdered drug was spread as a fine layer at the bottom of the crucible and total weight of crucible was noted. The crucible was incinerated at a temperature not exceeding 450°C for 5-6 h until powdered material was free from carbon. The crucible was cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated in triplicate with reference to the air-dried drug.

B. **Acid insoluble ash** - The ash obtained in the determination of total ash was boiled with 25 mL 0.1 N dil. hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper by filtration and it was washed with hot water. The insoluble ash was transferred into a tarred silica crucible, ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

C. **Water soluble ash** - The ash obtained in the determination of total ash was boiled for 5 min with 25 mL of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited at a temperature not exceeding 450°C. The procedure was repeated until a constant weight was observed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

2.4.2.3.2 **Determination of Extractive value**

A. **Ethanol soluble extractive** - 5 g of powdered drug was taken to a stoppered 100 mL conical flask. 100 mL of 95% ethanol was added. The flask was corked and set aside for 24 h. For better extraction by maceration, the flask was shaken frequently. The contents were filtered rapidly taking precautions against loss of the solvent, following which the final volume was
noted. The solvent obtained was taken in a previously weighed china dish and heated. Final drying was performed in an oven at 100°C. Afterwards, the china dish was stored in the desiccator and final weight was taken. The percentage of ethanol soluble extractive value was calculated.

**B. Water soluble extractive** - 5 g of powdered drug was taken in a stoppered 100 mL conical flask, to which 100 mL distilled water was added. The flask was corked and set aside for 24 h with periodic maceration. The contents were filtered rapidly taking precautions against loss of the solvent, following which the final volume was noted. The solvents so obtained was taken in a previously weighed china dish and heated. Final drying was performed in an oven at 100°C. Afterwards, the china dish was stored in the desiccator and final weight was taken. The percentage of water soluble extractive value was calculated.

**C. Ether soluble extractive** – 5 g of powdered drug was taken to a stoppered 100 mL conical flask, to which 100 mL diethyl ether was added. The flask was corked and set aside for 24 h. The flask was shaken carefully frequently. The contents were filtered rapidly taking precautions against loss of the solvent, following which the final volume was noted. The solvents so obtained was taken in a previously weighed china dish and heated. Final drying was performed in an oven at 100°C. Afterwards, the china dish was stored in the desiccator and final weight was taken. The percentage of ether soluble extractive value was calculated.

**2.4.2.3.3 Determination of Loss on drying**

An excess of water in medicinal plant materials encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Hence limits for water content should be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water.

**Procedure:** About 5 g of powdered crude drug was weighed in a petri plate (that was previously dried and tarred). Powder was distributed evenly and placed in the oven at a regulated temperature of 100-105°C. The petri plate was removed from the oven and closed promptly with its lid. Thereafter, the plate was kept at room temperature and weighed. The experiment was repeated till two consecutive weighing did not differ by more than 5 mg, unless otherwise stated in the test procedure. The loss in weight on drying was then calculated.
2.4.2.3.4 Determination of foaming index

Numerous plants contain phytoconstituents known as saponins; when the aqueous solutions of these plants are shaken, they produce foam which persists for a long time. To assess the foaming potential, it is useful to measure the foaming index.

Procedure: 1 g of the coarse powder (passed through sieve size 1250) was weighed accurately and transferred into 500 mL conical flask. To this, 100 mL of boiling water was added. It was maintained at the same temperature for 30 min. The mixture was cooled and filtered into 100 mL volumetric flask. Thereafter, the volume was made up to 100 mL with distilled water. The above solution was placed into ten stoppered test tubes, in a series of successive portions of 1 mL, 2 mL, 3 mL, and so on up to 10 mL. Subsequently, the volume was adjusted to 10 mL with distilled water in each tube. The tubes were stoppered and shaken for 15 secs at two frequencies per second. The solutions in the test tube were kept for 15 min and the foam formed was noted. Height of the foam formed in each tube was measured. The results were assessed as follows:

- If the height of the foam in every tube was less than 1 cm, the foaming index was less than 100.
- If the height of foam of 1 cm was measured in any tube, the volume of the plant material decoction in this tube (a) was used to determine the index. If this tube was first or second in a series an intermediate dilution was prepared for more precise result.
- When the height of the foam was more than 1 cm in every tube the foaming index was over 1000. In this case, the determination must be made on a new series of dilutions of the decoction in order to obtain a result.

Foaming index was calculated according to the following formula:

\[ \text{Foaming Index} = \frac{1000}{a} \]

Where ‘a’, is the millilitre of decoction used to make the dilution in the test tube where maximum foam formation was observed.

2.4.2.3.5 Determination of swelling index

Procedure: 1 g of the powdered plant material, moistened with alcohol (1 mL) was taken in a ground glass stoppered cylinder (25 mL). 25 mL of water was subsequently added. The cylinder was closed and shaken vigorously every 10 minutes for 1 hour. It was allowed to stand for 3 h and the final volume was noted.
2.4.2.3.6 Determination of Fluorescence analysis

Fluorescence of the powdered crude drugs has been studied as a pharmacognostic character to distinguish between plants and their species.

Procedure: The powdered crude drug was observed under day light and UV light (254 nm and 366 nm). The test material was further treated with different reagents namely, 1 N Hydrochloric acid, 1 N sodium hydroxide (aqueous), ferric chloride, 1N nitric acid, ammonia, iodine, 1 N sodium hydroxide (alcoholic), picric acid and 1N Sulphuric acid, and then observed for any colour change in daylight and under 254 nm (Chase and Pratt, 1949). The change in colour was recorded, accordingly.

2.4.2.4 Phytochemical Standardization

2.2.2.4.1 Phytochemical screening

A small portion of EP extracts and fractions were used for the detection of various phytochemicals such as alkaloids, carbohydrates, saponins, tannins, flavonoids, proteins, sterols, triterpenoids, glycosides, fixed oils and fats according to standard protocol (Evans and Evans, 2009).

2.4.2.4.2 Quantitative estimations

2.4.2.4.2.1 Estimation of polyphenolic content

a. Total Phenol content

The total phenol content present in EP extracts/fractions was determined using the Folin-Ciocalteu method (Adesegun et al., 2009). Calibration curve was detected by mixing methanol solution of gallic acid (1 mL; 3.125–100 mg/mL) with 5 mL Folin-Ciocalteu reagent (diluted ten-fold) and sodium carbonate (4 mL, 0.7 M). The absorbance was measured at 765 nm and the calibration curve prepared accordingly. One mL of plant extract/fractions were also mixed with the reagents above and after 2 h the absorbance was measured to determine total plant phenolic contents. All determinations were carried out in triplicate. The total content of phenolic compounds in the extracts in gallic acid equivalents (GAE) was calculated by the following formula: \( T = \frac{C \times V}{M} \); where \( T \) = total content of phenolic compounds, milligram per gram plant extract, in GAE; \( C \) = the concentration of gallic acid established from the calibration curve,
milligram per millilitre; \( V \) = the volume of extract, millilitre; \( M \) = the weight of plant extract, gram.

b. **Total Flavonoid content**

The aluminium chloride colorimetric method was followed to determine the total flavonoid content in plant extract and fractions according to the method of Chang et al., 2002. Quercetin was used as the standard to make the calibration curve. One mg of quercetin was dissolved in methanol and then diluted to obtain concentrations in the range of 15.625 to 500 \( \mu g/mL \). To each diluted solution, methanol (1.5 mL), 10\% aluminium chloride (0.1 mL), 1M potassium acetate, distilled water (2.8 mL) was added. After incubation for 30 min, optical density of the diluted solution was measured at 415 nm with a Shimadzu UV-1650 PC spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, USA). Similarly, flavonoid content in EP extracts and fractions were determined. The total content of flavonoids in the extracts in quercetin equivalents (QE) was calculated by the following formula: \( T = \frac{C \cdot V}{M} \); where \( T \) = total content of flavonoids, milligram per gram plant extract, in QE; \( C \) = the concentration of quercetin established from the calibration curve, milligram per millilitre; \( V \) = the volume of extract, millilitre; \( M \) = the weight of plant extract, gram.

c. **Total tannin content**

Folin Denis method was employed to detect the presence of tannins in the investigational plant (Bubba et al., 2009). Accordingly, the total tannins were estimated spectrophotometrically using the Folin- Denis reagent comprising of phosphomolybdate and phosphotungstate. Calibration curve of standard gallic acid (at concentrations ranging from 12.5 - 1000 \( \mu g/mL \)) was prepared by mixing 9.3 mL of ultrapure water to 100 \( \mu L \) of gallic acid and 300 \( \mu L \) of Folin-Denis reagent. After a time gap of 3 minutes, 300 \( \mu L \) of aqueous solution of 0.7 M Na\(_2\)CO\(_3\) (saturated) was added. Reaction mixture was kept in dark for incubation for one hour and finally absorbance was measured at 760 nm. Likewise, the procedure was repeated with EP extract/fractions (at concentration of 1000 \( \mu g/mL \)). The total tannin content (in GAE) was computed as follows: \( T = \frac{(C \cdot V)}{M} \); Where in, \( T \)=total tannin content, milligram per gram plant extract/fraction, in GAE; \( C \)=the concentration of gallic acid (as derived from the standard calibration curve), milligram per milliliter; \( V \)=the volume of extract/fraction, milliliter; \( M \)=the weight of plant extract/fraction, gram.
2.4.2.4.2.2. Identification and quantification of bioactive makers by TLC (Thin Layer Chromatography) and HPTLC (High Performance Thin Layer Chromatography) fingerprinting

Optimization of mobile phase – TLC and HPTLC fingerprinting of EP initial extract (EP.M) and partitioned fractions was attempted in different solvent systems based on comprehensive literature review search. TLC fingerprinting of initial EP extract and partitioned fractions was performed alongside various reference compounds such as gallic acid, lupeol, β-sitosterol, quercetin, quercitrin, kaempferol, epicatechin and ellagic acid in order to identify their presence by Retention factor, Rf value. Following this, the TLC plates were dried and run in the following optimized solvent systems:

- Solvent system 1- Toluene: methanol (9: 1) for Lupeol (Tandon & Sharma, 2010)
- Solvent system 2- Toluene: ethyl acetate: Formic acid: methanol (3:3:0.8:0.2) for Gallic acid (Sawant et al., 2010).

Following identification of standard markers by TLC, the percentage content of markers in EP extract/fractions was quantified by HPTLC according to the following protocols:

2.4.2.4.2.2.1. Quantification of Gallic acid by HPTLC fingerprinting

Gallic acid was quantified in EP extract and fractions by HPTLC fingerprinting (Sawant et al., 2010). Briefly, test solutions of EP extract/fractions were prepared at a 2 mg/mL concentration in methanol. Gallic acid solution was prepared at a concentration of 100 μg/mL in methanol. HPTLC Silica gel 60 F254 plate (Merck Life Sciences Ltd Pvt, Mumbai, India) with a dimension of 20 cm x 10 cm served as the stationary phase. Mobile phase was prepared by saturating the 20 cm x 10 cm Twin trough chamber with 10 mL of Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) for twenty minutes. Using the Camag Linomat 5 sample applicator, outfitted with liquid nitrogen tank, 10 μL per sample/standard was sprayed in the form of bands onto the HPTLC plate at a distance of 1.5 mm from the plate base. Sample solutions were loaded onto the sample applicator with a 100 μL HPTLC syringe (Camag Linomat syringe 695.0014, Hamilton Bonaduz, Schweiz). The plate was air dried and developed in the Twin trough chamber up to a distance of 80 mm from the point of application. Quantitative analysis of gallic acid was done by scanning the plates at 280 nm using Camag TLC scanner 3 equipped with win-CATS-V.
1.2.3 software (Camag). The peak areas were recorded and percentage content of gallic acid in column fraction was calculated using the following formula:

\[
\text{% Content of reference standard} = \frac{\text{AUC of sample}}{\text{AUC of standard}} \times \frac{\text{Conc of standard}}{\text{Conc of sample}} \times \text{% Purity}
\]

Subsequently, UV absorption spectrum of bands corresponding to gallic acid was examined at the same wavelength.

2.4.2.4.2.2.2 Quantification of lupeol by HPTLC fingerprinting

Lupeol was quantified in EP extract and fractions by HPTLC fingerprinting (Tandon & Sharma, 2010). Briefly, test solutions of MP extract/fractions were prepared at a 2 mg/mL concentration in methanol. Lupeol solution was prepared at a concentration of 100 μg/mL in methanol. HPTLC Silica gel 60 F_{254} plate (Merck Life Sciences Ltd Pvt, Mumbai, India) with a dimension of 20 cm x 10 cm served as the stationary phase. Mobile phase was prepared by saturating the 20 cm x 10 cm Twin trough chamber with 10 mL of toluene: methanol (9:1) for twenty minutes. Using the Camag Linomat 5 sample applicator, outfitted with liquid nitrogen tank, 10 μL per sample/standard was sprayed in the form of bands onto the HPTLC plate at a distance of 1.5 mm from the plate base. Sample solutions were loaded onto the sample applicator with a 100 μL HPTLC syringe (Camag Linomat syringe 695.0014, Hamilton Bonaduz, Schweiz). The plate was air dried and developed in the Twin trough chamber up to a distance of 80 mm from the point of application. Using Camag TLC Scanner 3, the HPTLC plate was scanned densitometrically under a scanning speed of 20 mm/s at a single wavelength of 620 nm after derivatization with Anisaledehyde sulphuric acid (ANS) reagent.

2.4.3 Statistical Analysis

Results are expressed as Mean ± Standard Error of Mean (SD). All experiments were performed in triplicate. One-way ANOVA, followed by Tukey’s test was used to analyse the results.
CHAPTER 2-PHARMACOGNOSTICAL STANDARDIZATION

2.5. RESULT AND DISCUSSION

2.5.1. Collection, extraction and fractionation (yield) of plant material

EP stem (10 kg) was shade-dried, coarsely powdered and extracted with methanol by Soxhlet extraction to obtain the methanol extract (EP.M). After completion of extraction, the extract was concentrated under reduced pressure and controlled temperature and stored in the desiccator until further use. The % yield of EP.M was estimated to be 10.75% w/w. The percentage yield (%w/w) of partitioned fractions were estimated as follows: EP.PE (35.7%), EP.DEE (1.53%), EP.EA (10.06%), EP.But (10.75%), EP.Aq (40.5%).

2.5.2. Pharmacognostical Standardization

2.5.2.1. Macroscopical Standardization

Description of morphological feature of *Elytranthe parasitica*: Parasitic honeysuckle or *Elytranthe parasitica* is a large hemiparasitic shrub, which grows on host trees such as neem, peepal and mango tree (Figure 2.1, 2.2). Its leaves are coriaceous, glabrous, ovate-oblong or lanceolate, obtusely acuminate at apex, rounded and acute at base. The leaves are entire, oppositely arranged and slightly crenulate. Flowers are sessile, grow in 2-3 decussate pairs in short axillary peduncles. Peduncle carrying the spikes was up to 1 cm long. Sepal cup is cup-like. The flowers had a long, variegated, reddish flower tube, which was slightly curved. Petals are pink coloured. Style was long, stigma nearly spherical. Corolla was up to 5 cm long; tube pink, divided half-way down, lobes green, reflexed. The shrub flowers in the season between February to May (Bhat, 2003) The fruit is a greenish ovoid berry with persistent sepals.

Figure 2.1. *Elytranthe parasitica* growing on host plant

Figure 2.2. *Elytranthe parasitica* stem and flowers
The stem and wood of EP is yellowish-brown to greenish-brown in colour. Its stem is thickened at the nodes, characteristic of mistletoes. Stems have small twigs of aerial branches ranging from 2 cm to 2.5 cm in thickness with bulged nodes having two opposite leaves; the bark of the stem is thin, dark brown and specked with lighter brown uniformly distributed lenticels. The stem surface is slightly rough to touch and has an irregular and fibrous fracture. The stem has an astringent taste with no distinct odour (Figure 2.3).

![Figure 2.3. Morphology of EP stem and leaves](image)

### 2.5.2.2. Microscopical Standardization

A. Transverse section

Transverse section of the stem was circular in outline. The outermost layer consisted of cork with few layers of dark brown, irregular parenchymatous cells. The inner cork was made up of few layers of radially arranged in regular rows of lignified parenchymatous cells. Below the cork the region consisting of cortex was made up of many layers of tangentially elongated cells interspersed with few stone cells either single or in groups of two. Patches of pericyclic fibres appeared outside phloem throughout the cortex; phloem was seen in several thin patches around the well-developed xylem. Xylem occupied one-third of the transverse section and traversed regularly by 1 to 4 seriate radially elongated lignified medullary ray cells and consisted of well-developed vessels, xylem fibres, tracheids and xylem parenchyma. The pith occupied the central part of the stem and consisted of thin walled, rounded or polygonal lignified parenchymatous cells; small groups of sclereids were also seen in this region (Figure 2.4).
B. Powder Microscopy

Powder microscopy of EP stem powder revealed the presence of cork cells, lignified wood elements, lignified pith cells, lignified pericyclic fibres, starch grains, tannin and stone cells (Figure 2.5).

**Figure 2.4.** Microscopical Features of *Elytranthe parasitica* Transverse Section of EP stem. CK: Outer cork cells; IN: Inner cork cells; PF: Pericyclic fibres; PH: Phloem SC: Stone cells; MR: Medullary rays; XV: Xylem vessels; PT: Pith

**Figure 2.5.** Microscopical features of EP stem. Powder microscopic features of EP stem. (A) Cork cells (B) Lignified wood elements (C) Lignified pith cells (D) Pericyclic fibres (E) Lignified pericyclic fibres (F) Starch grains (G) Stone cells (H) Tannins


2.5.2.3. Physicochemical Standardization

2.5.2.3.1. Ash value

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Being a validity parameter, it assesses the degree of purity of a given drug. Table 2.1 depicts the ash value (total, acid insoluble and water soluble) of EP stem powder.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of ash values</th>
<th>Ash value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Ash</td>
<td>3.50 ± 0.216</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble Ash</td>
<td>0.48 ± 0.139</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble Ash</td>
<td>1.19 ± 0.157</td>
</tr>
</tbody>
</table>

Table 2.1: Ash value of EP stem

Results are represented as mean ± SD (n=3)

2.5.2.3.2. Extractive value

The amount of extractable matter in EP, extracted by different solvents namely, water, ethanol and ether were determined by cold maceration, the values of which are tabulated below.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of Extractive value</th>
<th>Extractive value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water soluble</td>
<td>8.62 ± 0.44</td>
</tr>
<tr>
<td>2</td>
<td>Alcohol soluble</td>
<td>5.82 ± 1.25</td>
</tr>
<tr>
<td>3</td>
<td>Ether soluble</td>
<td>3.02 ± 0.66</td>
</tr>
</tbody>
</table>

Table 2.2: Extractive value of EP

Results are represented as mean ± SD (n=3)

It was specifically noted that EP stem powder had higher content of water soluble matter as compared to alcohol or ether soluble content. This implies that EP stem parts have sufficiently high carbohydrate, tannin and amino acid content.

2.5.2.3.3. Loss on drying

Moisture is an unavoidable constituent in plants, which builds up on storage. Drying of the crude drug will help in its preservation and will stop enzymatic or hydrolytic reactions which might alter or degrade the nature of the phytoconstituents in the drugs. Moisture content was
determined by loss on drying which is the loss of mass on heating. It is expressed as percent w/w (Indian Pharmacopoeia, 1996). The moisture content of the dried powdered material was determined by loss on drying method and was found to 7.62% w/w. EP dried plant material was observed to be highly hygroscopic, and was therefore stored in the desiccator until further use.

2.5.2.3.4. Foaming index

The foaming index of EP was found to be 125.

<table>
<thead>
<tr>
<th>Volume of plant material (mL)</th>
<th>Volume of water (mL)</th>
<th>Height of foam (cm)</th>
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<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Results are represented as mean ± SD (n=3)

2.5.2.3.5. Swelling index

EP was found to have very low swelling property. The swelling index of EP stem was observed to be zero or nil.

2.5.2.3.6. Fluorescence analysis

In order to confirm the identity of EP stem parts, fluorescence analysis was performed. Results are shown in Table 2.4.
<table>
<thead>
<tr>
<th>S.no.</th>
<th>Treatment</th>
<th>Short UV light</th>
<th>Long UV light</th>
<th>Day light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder</td>
<td>Green</td>
<td>Light green</td>
<td>Dark green</td>
</tr>
<tr>
<td>2.</td>
<td>Powder + 1N HCl</td>
<td>White</td>
<td>Pale black</td>
<td>White</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + 1N H₂SO₄</td>
<td>Light lemon yellow</td>
<td>Light violet</td>
<td>No colour</td>
</tr>
<tr>
<td>4.</td>
<td>Powder + 1N HNO₃</td>
<td>Light green</td>
<td>Violet</td>
<td>Yellow</td>
</tr>
<tr>
<td>5.</td>
<td>Powder + 1N Aq. NaOH</td>
<td>Blackish green</td>
<td>Black</td>
<td>Orange brown</td>
</tr>
<tr>
<td>6.</td>
<td>Powder + 1N Alc NaOH</td>
<td>Light greenish yellow</td>
<td>Light brown</td>
<td>Light yellow</td>
</tr>
<tr>
<td>7.</td>
<td>Powder + Picric acid</td>
<td>Greenish yellow</td>
<td>Blackish green</td>
<td>Bright yellow</td>
</tr>
<tr>
<td>8.</td>
<td>Powder + 5% FeCl₃</td>
<td>Greenish yellow</td>
<td>Blackish green</td>
<td>Yellow</td>
</tr>
<tr>
<td>9.</td>
<td>Powder + Iodine</td>
<td>Brownish green</td>
<td>Reddish brown</td>
<td>Brown</td>
</tr>
</tbody>
</table>

**2.5.2.4. Phytochemical Standardization**

**2.5.2.4.1. Phytochemical screening**

Secondary metabolite profiling is valuable in the rapid identification of known compounds in plant extracts (Sasidharan et al., 2012). EP extract and fractions were screened for the presence of secondary metabolites. EP.M, EP.PE and EP.DEE gave a positive test for Liebermann-Burchard and Salkowski test revealing phytosterols. EP.DEE, EP.EA and EP.But gave an instant crimson red color in Shinoda’s test, implying presence of flavonoids in these fractions. EP.M, EP.DEE, EP.EA, EP.But and EP.Aq tested positive (greenish-black color) for Ferric chloride test for phenols and tannins. Saponins and carbohydrates were detected in EP.M extract and EP.Aq fraction. Table 2.5 summarises the phytochemical constitution of EP extract/fractions.

---

**Table 2.4: Fluorescence analysis of EP stem**

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Treatment</th>
<th>Short UV light</th>
<th>Long UV light</th>
<th>Day light</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Powder</td>
<td>Green</td>
<td>Light green</td>
<td>Dark green</td>
</tr>
<tr>
<td>2.</td>
<td>Powder + 1N HCl</td>
<td>White</td>
<td>Pale black</td>
<td>White</td>
</tr>
<tr>
<td>3.</td>
<td>Powder + 1N H₂SO₄</td>
<td>Light lemon yellow</td>
<td>Light violet</td>
<td>No colour</td>
</tr>
<tr>
<td>4.</td>
<td>Powder + 1N HNO₃</td>
<td>Light green</td>
<td>Violet</td>
<td>Yellow</td>
</tr>
<tr>
<td>5.</td>
<td>Powder + 1N Aq. NaOH</td>
<td>Blackish green</td>
<td>Black</td>
<td>Orange brown</td>
</tr>
<tr>
<td>6.</td>
<td>Powder + 1N Alc NaOH</td>
<td>Light greenish yellow</td>
<td>Light brown</td>
<td>Light yellow</td>
</tr>
<tr>
<td>7.</td>
<td>Powder + Picric acid</td>
<td>Greenish yellow</td>
<td>Blackish green</td>
<td>Bright yellow</td>
</tr>
<tr>
<td>8.</td>
<td>Powder + 5% FeCl₃</td>
<td>Greenish yellow</td>
<td>Blackish green</td>
<td>Yellow</td>
</tr>
<tr>
<td>9.</td>
<td>Powder + Iodine</td>
<td>Brownish green</td>
<td>Reddish brown</td>
<td>Brown</td>
</tr>
</tbody>
</table>
2.5.2.4.2. **Quantitative estimation**

2.5.2.4.2.1. **Estimation of polyphenolic content**

a. **Total phenol content**

Polyphenols are a diverse group of phytoconstituents comprising anthocyanins, isoflavonones, flavonoids, tannins and catechins. Innumerable health benefits have been attributed to this group of phytochemicals largely due to their key role in protecting the human body against multiple diseases (Scalbert et al., 2005). In the past, several epidemiological studies have investigated the chemopreventive effects of polyphenols against various malignancies (Kampa et al., 2005). In this regard, green tea polyphenols were found to exert powerful anticancer effects by regulating the immune function (Chen et al., 2014). Therefore, polyphenol content of EP extract/fraction was evaluated to explore any relation between their occurrence and the chemotherapeutic potential of the plant. Gallic acid was used as the reference standard for determining total phenol content.

<table>
<thead>
<tr>
<th>S. NO</th>
<th>PHYTOCHEMICAL TESTS</th>
<th>EP.M</th>
<th>EP.PE</th>
<th>EP.DEE</th>
<th>EP.EA</th>
<th>EP.BUT</th>
<th>EP.Aq</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phenolic compounds &amp; tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Sterols &amp; triterpenoids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7.</td>
<td>Proteins &amp; amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Fixed oils &amp; Fats</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Volatile oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Gums and mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- Absent; + Present in low concentration; ++ Present in high concentration

**Table 2.5:** Phytochemical screening of EP extract/fractions
phenolic content by Folin-Ciocalteau method and a calibration curve was plotted (R²=0.9981) accordingly (Figure 2.6). EP fractions, notably EP.EA and EP.DEE fractions were found to be highly enriched with phenolic compounds with an estimated phenolic content of 91.01 ± 2.68 and 72.48 ± 2.43 mg GAE/ g of plant fraction respectively. TPC of EP extract/fractions is listed in Table 2.6.

**Table 2.6: Total phenol content in EP extract/fractions**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts/fractions</th>
<th>Total phenol content (mg GAE/g of plant extract/fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>EP.M</td>
<td>66.97 ± 4.78(^b)</td>
</tr>
<tr>
<td>2.</td>
<td>EP.PE</td>
<td>36.85 ± 1.05(^d)</td>
</tr>
<tr>
<td>3.</td>
<td>EP.DEE</td>
<td>84.38 ± 1.55(^b) ,(^c)</td>
</tr>
<tr>
<td>4.</td>
<td>EP.EA</td>
<td>92.17 ± 4.48(^a)</td>
</tr>
<tr>
<td>5.</td>
<td>EP.BUT</td>
<td>77.43 ± 6.25(^b) ,(^c)</td>
</tr>
<tr>
<td>6.</td>
<td>EP.AQ</td>
<td>31.56 ± 1.47(^d)</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SEM (n=3). Results are analyzed by one-way ANOVA followed by Tukey’s test. Values in the same column followed by a different superscript (a-d) are significantly different (P < 0.05). GAE= Gallic Acid Equivalent

b. **Total flavonoid content**

Flavonoids, a sub group of polyphenolic compounds have been widely acknowledged to possess remarkable anticancer potential (Ren et al, 2003). For estimation of flavonoid content by
aluminium chloride colorimetric method, quercetin was employed as the reference standard and used in concentrations ranging from 15.625 to 500 μg/mL to plot a calibration curve (R²=0.999) as depicted in Figure 2.7. Among the EP fractions and extract, the highest flavonoid content was observed in the diethyl ether fraction, EP.DEE (14.41 ± 0.52 mg QE/g of plant fraction). EP.EA also had sufficiently high flavonoid content (12.93 ± 0.65 mg QE/g of plant fraction).

![Figure 2.7. Standard plot of quercetin](image)

**Table 2.7:** Total flavonoid content in EP extract/fractions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts/fractions</th>
<th>Total flavonoid content (mg QE/g of plant extract/fractions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>EP.M</td>
<td>10.87 ± 0.85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>EP.PE</td>
<td>12.97 ± 1.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.</td>
<td>EP.DEE</td>
<td>15.72 ± 2.85&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>EP.EA</td>
<td>22.48 ± 1.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.</td>
<td>EP.BUT</td>
<td>12.29 ± 5.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.</td>
<td>EP.AQ</td>
<td>2.29 ± 0.21</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SEM (n=3). Results are analyzed by one-way ANOVA followed by Tukey’s test. Values in the same column followed by a different superscript (a-d) are significantly different (P < 0.05). QE= Quercetin Equivalent.
c. Total tannin content

Tannins, another sub group of polyphenol, comprising numerous hydroxyl (–OH) groups as well as other suitable groups (such as carboxyl, –COOH) also exhibit exceptional anti-tumor activity (Chung et al., 1998). For instance, gallic acid and its analogues (eg: lauryl, octyl, dodecyl gallates) can trigger apoptosis in several tumor cell lines of melanoma, leukemia, lung and stomach cancer (Locatelli C et al., 2009). Moreover, tannins seem to be most common in several plants belonging to Loranthaceae (Gill & Hawksworth, 1961). A study by Ibrahim J et al detected more than 70% of Loranthacea plants to contain tannins (Ibrahim et al., 2014), with both phlobotannin and gallo-tannin being frequently detected in plants belonging to Loranthacea. For tannin content estimation by Folin-Denis’ method, gallic acid was used as the positive control. Calibration curve of gallic acid was plotted to estimate the tannin content in EP extract and fractions (R²=0.998). The level of tannins in EP extract and fractions, estimated by Folin – Denis method and expressed in Gallic acid Equivalent (GAE) were similar to the level of phenolics quantified by Folin-ciocalteau method. The highest content of tannin was found in fractions EP.EA fraction (22.80 ± 0.19 mg GAE/ g of plant fraction) and EP.DEE fraction (17.10 ± 0.13 mg GAE/ g of plant fraction). Sufficiently high tannin content was noted in the butanol fraction (13.57 ± 0.20 mg GAE/ g of plant fraction) as well. Table 2.8 lists the total tannin content in EP extract and fractions.

![Figure 2.8. Standard plot of gallic acid](image)
Identity based TLC and HPTLC fingerprinting

TLC fingerprinting

Thin layer chromatogram was performed wherein, EP extract/fractions were spotted alongside standard markers gallic acid and lupeol. Gallic acid eluted in the selected solvent system, with an Rf of 0.63; corresponding bands were detected in EP extract/fractions in short UV light of 254 nm (Figure 9A). Following derivatization with Anisaldehyde reagent, triterpene lupeol eluted as a distinct purple coloured band at an Rf of 0.76 in the mobile phase system, toluene: methanol (9:1), this was vividly observed in EP.DEE fraction (Figure 2.9B).

Table 2.8: Total tannin content in EP extract/fractions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts/fractions</th>
<th>Total tannin content (mg GAE/ g of plant extract/fractions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>EP.M</td>
<td>7.18 ± 0.12</td>
</tr>
<tr>
<td>2.</td>
<td>EP.PE</td>
<td>4.37 ± 0.15</td>
</tr>
<tr>
<td>3.</td>
<td>EP.DEE</td>
<td>17.10 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.</td>
<td>EP.EA</td>
<td>22.80 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5.</td>
<td>EP.BUT</td>
<td>13.57 ± 0.20</td>
</tr>
<tr>
<td>6.</td>
<td>EP.AQ</td>
<td>0.64 ± 0.09</td>
</tr>
</tbody>
</table>

Data is presented as mean ± SEM (n=3). Results are analyzed by one-way ANOVA followed by Tukey’s test. Values in the same column followed by a different superscript (a-d) are significantly different (P < 0.05). GAE= Gallic acid Equivalent.
2.5.2.4.2.2.2 HPTLC fingerprinting

2.5.2.4.2.2.1 Quantification of gallic acid

Gallic acid, a principal polyphenolic molecule that occurs naturally in several plants based foods has been credited with remarkable anti-tumor activity (Verma & Mishra, 2013). Numerous studies have proven gallic acid to exhibit potent cytotoxic activity against various cancer cell lines (Sun et al., 2016, Maurya et al., 2011, Schuck et al., 2013, Inoue et al., 1994, Tan S et al., 2015, Isuzugawa et al., 2001). Moreover, gallic acid also showed an appreciable selectivity index when tested in normal cell lines (Faried et al., 2007, Inoue et al, 1994). Gallic acid showed 3-fold higher sensitivity towards hepatic cancer cell lines HepG2 and SMMC-7721 when compared to normal hepatocyte cell line HL-7702 (Sun et al., 2016, Li T et al., 2010). The underlying Structure Activity Relationship (SAR) of gallic acid’s selective anticancer activity was due to its carboxyl group which differentiated between cancer and non-cancerous cells and so exclusively triggers apoptosis in cancer cells (Inoue et al., 1995). Several studies have also indicated gallic acid to possess potent pro-oxidant potential (Apak R et al., 2013). Hence, presence of gallic acid was appraised in the EP extract/fractions.

Figure 2.9: Thin layer chromatogram of EP extract/partitioned fractions along with standard marker (A) Detection of Gallic acid at short wavelength, 254 nm. Stationary phase: Silica gel 60 F254, Mobile phase: Toluene: ethyl acetate: Formic acid: methanol (3:3:0.8:0.2); Samples applied: (1) EP.M (2) EP.PE (3) EP.DEE (4) Gallic acid (5) EP.EA (6) EP.But (B) Detection of Lupeol in white light following derivatisation by spraying with Anisaldehyde reagent. Stationary phase: Silica gel 60 F254, Mobile phase: Toluene: methanol (9:1), Samples applied: (1) EP.M (2) EP.DEE (3) Lupeol (4) EP.EA (5) EP.But
HPTLC with gallic acid as reference standard afforded well resolved peaks in EP extract and fractions, with good separation of gallic acid from other phytoconstituents. Figure 2.10 depicts the HPTLC fingerprint profile of EP extract and fractions alongside marker gallic acid. Reference standard gallic acid eluted at Rf (Retention factor) of 0.63. Gallic acid was detected in EP extract and bioactive fractions, EP. DEE and EP.EA. EP.DEE, the diethyl fraction gave mainly eleven peaks, of which gallic acid was peak no. 7 (Rf value - 0.63, 2.173 ± 0.053 %). EP.EA gave eleven peaks, of which gallic acid was peak no. 10 (Rf value - 0.63, 0.528 ± 0.01 %). EP methanol extract, EP.M also contained gallic acid, however in comparatively less quantity (peak no 7, Rf value - 0.63, 0.293 ± 0.016 %). Gallic acid was not observed in other EP fractions EP PE, EP. But and EP. Aq. Table 2.9 lists the percentage content of gallic acid in EP extract/fractions. To confirm the presence of gallic acid in EP, the UV spectrum of gallic acid at 280 nm was compared with the corresponding peak (at Rf 0.63) in EP extract/fraction. The UV spectral characteristics matched, verifying the presence of gallic acid in EP.M, EP.DEE and EP.EA (Figure 2.11).

Table 2.9: HPTLC quantification of gallic acid in EP extract/fractions

<table>
<thead>
<tr>
<th>S.NO</th>
<th>SAMPLES</th>
<th>GALLIC ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rf value</td>
</tr>
<tr>
<td>1</td>
<td>EP.M</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>EP.PE</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>EP.DEE</td>
<td>0.63</td>
</tr>
<tr>
<td>4</td>
<td>EP.EA</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; ND - Not detected
Figure 2.10. HPTLC based identification of gallic acid in EP extract/fractions (A) EP.M, Methanol extract (B) EP.PE, Petroleum ether fraction (C) EP.DEE, Diethyl ether fraction (D) EP.EA, Ethyl acetate fraction (E) EP.But, Butanol fraction (F) EP.Aq, Water fraction fraction (G) Gallic acid (reference standard)
2.5.2.4.2.2.2.2. Quantification of lupeol

Lupeol is a pharmacologically bioactive pentacyclic triterpenoid present in olives, mango, acacia, figs and other dietary sources (Siddique and Saleem, 2011). Numerous studies have reported substantial anti-cancer activity of lupeol against several cancer cell lines (Tarapore et al., 2013). Studies indicate that lupeol, a tripterpenic chiefly exhibits chemopreventive efficacy by regulating crucial signaling pathways such as PI3K/Akt, MAPK and Wnt/β-catenin (Saleem, 2010). Lupeol content in the various fractions of EP was quantified using High-Performance Thin Layer Chromatography. Lupeol afforded a well-resolved peak in the solvent system toluene: methanol (9:1) at retention factor (Rf) of 0.76. A peak corresponding to lupeol was chiefly spotted at Rf value of 0.76 in EP.M extract and non-polar fractions, EP.PE and EP.DEE. The remaining fractions EP.EA, EP.But and EP.Aq did not contain the peak corresponding to lupeol perhaps due to the polar nature of the fractions. Table 2.10 lists the percentage content of

![Figure 2.11. Overlay of UV absorption spectra of reference gallic acid and corresponding band in EP extract/fractions (A) Gallic acid and EP.M extract (B) Gallic acid and EP.DEE (C) Gallic acid and EP.EA](image-url)
lupeol in EP extract/fractions. Figure 2.12 shows the HPTLC densitogram of EP extract and fractions along with reference standard lupeol.

![Figure 2.12](image.png)

**Figure 2.12.** HPTLC based identification of lupeol in EP extract/fractions (A) EP.M, Methanol extract (B) EP.PE, Petroleum ether fraction (C) EP.DEE, Diethyl ether fraction (D) Lupeol (reference standard). The arrows specify the lupeol peaks in the tested samples.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>SAMPLES</th>
<th>RUPEOL</th>
<th>% Lupeol content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EP.M</td>
<td>0.76</td>
<td>1.32 ± 0.23</td>
</tr>
<tr>
<td>2</td>
<td>EP.PE</td>
<td>0.76</td>
<td>5.02 ± 1.06</td>
</tr>
<tr>
<td>3</td>
<td>EP.DEE</td>
<td>0.76</td>
<td>0.29 ± 0.47</td>
</tr>
<tr>
<td>4</td>
<td>EP.EA</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; ND - Not detected

---

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2.6. CONCLUSION

Efforts to monitor quality and ensure the safety/efficacy of herbal products are gradually evolving worldwide. Global definitions of herbal products are being developed with international cooperation and a new perspective of standardization, validation, safety and efficacy of herbal medicines is evolving, which is a good sign. Inhouse monograph for the standardization of the stem parts of the candidate plant, Elytranthe parasitica (L.) Danser was developed in accordance to WHO guidelines in terms of their morphology, microscopy and powder characteristics. Phytochemical characterization studies in EP revealed presence of polyphenols in the plants with especially higher flavonoid, tannins and phenolic content in the active fractions EP.DEE and EP.EA. Bioactive standard markers, lupeol and gallic acid were identified and quantified in EP extract/fractions.

2.7. REFERENCE


**CHAPTER 2-PHARMACOGNOSTICAL STANDARDIZATION**


Tandon, N., Sharma, M., (2010). *Quality standards of Indian medicinal plants*, Indian Council of Medical Research (ICMR), New Delhi, India.


