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Pharmacognostic evaluation, GC-MS analysis and anti-oxidant activity of leaves of *Mimusops Elengi*

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ABSTRACT

The plant Mimusops elengi commonly known as 'Spanish cherry' is found mostly in South Asia. The stem, barks, leaves and fruits of the plant are used in the preparation of various Ayurvedic medications to treat some ailments. The pharmacognostic studies were carried out in terms of microscopy, macroscopy and fluorescence analysis of the dried powdered leaves as well as for the fresh leaf. The present study involves the preparation of ethanol, ethyl acetate, petroleum ether and aqueous extracts of the M. elengi leaves by soxhlet extraction method. The crude extracts were further subjected to preliminary phytochemical testing which reveals that petroleum ether extract contains fats, fatty acids and sterols. Aqueous and ethanolic extracts enriched with phenolics, flavonoids, sterols and carbohydrates. Moderate amount of phenolics and carbohydrates were present in ethyl acetate extract. GC-MS analysis on crude extracts of petroleum ether and ethyl acetate confirms the presence of squalene, Z,Z-6,28-Heptatriactontadien-2-one, Methyl 3-bromo-1-adamantaneacetate, 2,6,10,14,18,22-tetracosahexane, 2,6,19,15,19,23-hexamethyl ester, Chloroacetic acid, tetradecyl ester. The leaves of M. elengi contain a high percentage of an isoprenoid compound called Squalene. Squalene is used to control cholesterol related problems. Crude extracts of M. elengi was evaluated for their antioxidant properties by performing Hydroxyl Radical Scavenging Assay. Quercetin was used as the standard. Aqueous and ethanolic extracts shows potent antioxidant activity compared to ethyl acetate and petroleum ether extracts. The antioxidant properties of the leaf extracts aids in the treatment of diabetes, coronary heart disease, cancer and also retards the age related eye problems like cataract.

Keywords: *Mimusops elengi*, phytochemical screening, pharmacognostic profile, proximate analysis

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INTRODUCTION

Medicinal plants are used world-wide in the treatment of many diseases. They predate the development of many modern medications as well. One such medicinal plant is *Mimusops elengi* (belongs to the family Sapotaceae) which is commonly found in the southern and the eastern countries of Asia. Almost all parts of the plant have some medicinal properties to offer [1]. The plant is commonly called as 'Spanish Cherry' or 'Bullet Wood' but in India it is very popularly known as 'Maulsari' or 'Bakul' [2].

Bullet-Wood is a large and a beautiful tree whose medicinal properties were first discovered by the tribes living near the tropical forests of the Asian countries. The plant is immensely popular for its timber. The leaves are a glossy green and the flowers are cream colored with a pleasant fragrance. In many house-holds this is used as an ornamental plant. The tree may reach up to a height of 9-18m with a thick and sturdy bark. The bark appears to be

brownish black or greyish black in color with a circumference of 1m. The bark is scaly with several striations which covers its outer surface [3].

The leaf is enriched with flavonoids, phenolics, sterols and carbohydrates [4]. The plant possesses an diuretic and astringent properties [4]. The various parts of the plant are also used to strengthen the teeth and deal with various gum related problems. The bark confers wound washing properties and a solution containing bark extract is used to heal sore eyes. Plant extracts can be used to treat gastric problems as well. Extract of flowers are used against heart diseases and act as antidiuretic in polyuria and antitoxin [5]. The snuff made from the dried and powdered flowers used in a disease called Ahwa, which is seen in Bengal, in which strong fever, headache and pain in the neck, shoulders and other parts of the body occurs [6].

Ripened fruits facilitates in burning urination. The fruit obtained from the plant is crushed and made into a paste and applied to aid during child birth. Powder of dried flowers is made into a snuff to treat cephalalgia [7]. Based on the literature survey, there are no reports on the pharmacognostical profile and preliminary phytochemical screening of the constituents of the leaf of *Mimusops elengi.* Hence we focused to study the proximate analysis and other physicochemical parameters by using standard monographs protocol. The objective of the present study is to standardize the fresh leaf and the dried leaves for its macroscopy, microscopical characters as well as percentage of different crude extract and its preliminary phytochemical screening. The crude extracts were further given for GC-MS analysis and their antioxidant properties were studied as well.

MATERIALS AND METHODS

A. Chemicals and instruments

Ethanol, methanol, petroleum ether, ethyl acetate, sulphuric acid and all the other various chemicals used were of analytical grade and were purchased from sigma Aldrich laboratories, Bangalore. Among the instruments we used the Soxhlet apparatus for extraction purposes, sonicator (Branson, Model-3800), U.V (Hitachi, U-2900/2910), colorimeter and the compound microscope (Pro-way,XSZ-PW108).

Plant material:

The sample was collected from the botanical garden of Vellore Institute of Technology, Vellore, Tamil Nadu and the sample was submitted for authentification. The authentication number was PARC/2013/2160.

B. Methods

Microscopic and macroscopic studies:

The leaves of *Mimusops elengi* were collected and first were subjected to Microscopic and macroscopic analysis. The stomatal index and the palisade ratio were studied by peeling out the upper and the lower epidemis after boiling the leaves, staining with safranin and then mounting it on glycerin. The rest of the protocol was followed according to the methods given by Kokate C.K [8]. The colour of the leaves were seen to be dark green with a faint mint-like odour and has a tinge of bitter taste.

Sample preparation:

The leaves from our sample plant were collected and dried for one week. Then the leaves were grinded and crushed in an automatic mixture and reduced to a powder form.

This powdered form of the leaves was further subjected to various kinds of pharmacognostical evaluation.

Pharmacognostical Evaluation:

Determination of loss on drying

About 1gm of the powdered sample was taken in a porcelain dish and it was kept in the oven at 100°C for one hour. Then it was cooled by keeping the container in a desiccator. The loss in weight of material was recorded as per (WHO/QCMMPM guidelines, 1992).

Determination of total ash content

About 1gm of the powdered sample was taken in a tarred crucible and incinerated by gradually increasing the temperature to 500°C. The residue was cooled and weighed. The process was repeated for constant value. The percentage of total ash was calculated [9][10].

Determination of water soluble ash content
The total ash obtained was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and heated for 15 minutes at a temperature not exceeding 450^0C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated [9][10].

Determination of acid insoluble ash content
The ash obtained as directed under total ash was boiled with 25ml of 2N hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with acid, dried and weighed. The percentage of acid insoluble ash was calculated [9][10].

Preparation of the extract:
The leaves obtained of the plant sample was dried, powdered and weighed. About 25 gms of dried powdered leaves was packed in the soxhlet extractor and extracted with petroleum ether (60°-70°C). All the fatty substances like the fats, waxes and sterols got extracted from the powdered sample. For the preparation of aqueous extract the defatted material was extracted with hot water (70°-80°C) for 8-10 hours. The aliquots of extracts were collected and passed through Whatmann No.1 filter paper. Then the solvents were completely evaporated into dryness at 60°C by vacuum distillation. The crude extract was run on a prepared silica plate by an appropriate solvent system to identify the number of spots. The residues were designated as petroleum ether (PE), ethyl acetate (EA), ethanol (EtOH) extracts, aqueous (Aqs) respectively (Lakshmi et al., 2006) [10].

Fluorescence Analysis:
The fluorescence properties of the powdered sample was carried out by using various reagents and under long UV (365nm) and short UV (254nm) (Kokoshi et all., 1958) [13].

Preliminary Phytochemical screening:
Crude extracts of *M. elengi* was dissolved in distilled water (ethanol and aqueous) whereas petroleum ether and ethyl acetate extracts were dissolved in respective solvents itself and subjected to the following phytochemical tests. The study was carried out by using standard procedures described by Kokate (1986), Harborne (1992) and Evans (1996) [8][11][12].

Test for Phenolics and Tannins
Ferric Chloride Test: To 2ml of extract solution, few drops of neutral $FeCl_3$ solution were added. Formation of dark green colour indicates the presence of phenolics.

Lead Acetate test: To 2ml of extract solution few drops of 10% of lead acetate were added. White precipitate appeared which confirms the presence of phenolic compounds.

Test for Flavonoids
Alkaline reagent test: To 2ml of extract solution few drops of 10% NH_4OH was added. Formation of yellow fluorescence indicates the presence of flavonoids.

Shinoda test (Magnesium Hydrochloride reduction test): Extract (10mg) was dissolved in 5ml of alcohol. To this solution, magnesium ribbon and concentrated hydrochloric acid were added. The mixture is heated in a water bath. Magenta colour appeared which proves the presence of flavonoids.

Test for Carbohydrates
Molische's test: 2 drops of alcoholic solution of 1-naphthol was added to 2 ml of extract solution. Conc.H_2S0_4 (1ml) was added slowly along the sides of the test tube and allowed it to stand for a few seconds. Formation of violet ring indicates the presence of carbohydrates.

Fehling's Test: Few drops of Fehling solution A and Fehling solution B was added to 2 ml of extract solution and boiled the mixture for 5 minutes. Appearance of a red precipitate indicates the presence of carbohydrates.

Benedict's test: Few drops of Benedict's reagent was added to 2 ml of extract solution. The mixture was boiled for 2 minutes. A brownish red color precipitate appeared which confirms the presence of carbohydrates.

Test for Sterols
Salkowski test: About 2 ml of extract solution was mixed with 2ml of $CHCL_3$ and 3 ml of conc.H_2S0_4. A reddish brown coloration of the interphase indicates the presence of sterol.

Libermann Burchards test: Extract (5mg) was dissolved in a minimum of chloroform; to this 2ml acetic anhydride and two drops of concentrated sulphuric acid was added. Array of colours indicates the presence of phytosterols.

Detection of proteins and amino acids
Biuret test: To the extract of *M. elengi* copper sulphate solution (2%) was mixed. To the above solution 1 ml of ethanol and excess of potassium hydroxide pellets were added. Formation of pink color indicates the presence of proteins.

Test for saponin
Foam test: The extract solution was shaken in a graduated cylinder for 10 minutes. Layer of foam appeared which indicates the presence of saponins

Detection of Alkaloids
Mayer's test: Alkaloids will give a cream colored precipitate with Mayer's reagent (Potassium mercuric iodide solution). No characteristic change observed, which confirms the absence of alkaloids in the *M. elengi* extracts.

Detection of Fats and Oils
Saponification test: Alcoholic KOH solution (0.5 N) was mixed with constant stirring to the extracts and a drop of phenolphthalein was added. Formation of soap indicates the presence of fixed oils and fats.

GC-MS Analysis:
The crude extracts of *Mimusops elengi* were given for GC-MS analysis. And the results were further reported.

Hydroxyl Radical Scavenging Assay:
The hydroxyl radical scavenging activity was determined according to the method of Beara et al [14]. 2 mL of extract solution (10, 20, 30, 40 and 50 mg), 1.0 mL ascorbic acid, 1.0 ml of Butylated hydroxyl toluene, 5.0 mL of phosphate buffer (0.2 M, pH 6.6), 1.0 mL of ferrous sulfate (7.5 mmol/L) and 1.0 mL of H_2O_2 (0.1 %) were mixed and diluted to 25 mL with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm. The scavenging percentage (P%) was calculated as P% = ((A – A1)/(A2 – A1))×100, where A, A1 and A2 are the absorbance value of the system with all solution including H_2O_2 and the extract solution and the system without extract solution, respectively.

RESULTS AND DISCUSSION

A. Pharmacognostical Evaluation of *M. elengi*

Proximate Analysis:
Standardization of crude drugs can be done by quantitative determination of pharmacognostical parameters. It helps in the determination of various chemical components of the mixture. In **Table 2.1** we see that the moisture content of the leaves of *M .elengi* is extremely low as the value is 6.9%. This is helpful since it will arrest bacterial and fungal growth, as the general requirement for moisture content in crude drug is not more than 14% as per the African Pharmacopoeia (African Pharmacopoeia, 1986).

Total ash values and acid insoluble ash values are equally important parameters in evaluating the crude drugs. Ash values help to set the purity standards of the sample. It also tells about inorganic substituents which are present such as metallic salts or silica. The total ash value obtained from the leaves of *M. elengi* was 29.69%. The values of water soluble ash and acid insoluble ash are extremely high i.e. 94.28% and 94.97% respectively. These findings can be used as a quality parameter to evaluate *Mimusops elengi* leaves for any adulteration. From this investigation we can state that the total ash values obtained from the sample were extremely high.

Determination of Extractive Index:
The extractive index values helps in determining the various chemical constituents present in the sample, which are soluble in a particular solvent. This separates the various compounds present the crude extract and also helps in their

identification. All parts of a plant are usually rich in various kinds of substituents and because of which plants used in the formulations of medicines to treat all kinds of diseases. Plants have non-toxic effects used in treatments.

When the various components of the plant extract is not determined by any other way then extractive index helps in their evaluation. These values indicate the nature of the constituents present in the leaf extract after subjecting to Soxhlet Extraction by using petroleum ether, ethyl acetate, ethanol and water was tabulated in **Table 2.1**

Table 2.1: Physiochemical standard values of *M.elengi*

Sl. No	Physico-Chemical Constant	Percentage (%)
1.	**Loss on drying**	6.9
2.	**Ash values**	
A.	Total Ash	29.69
B.	Water soluble Ash	94.28
C.	Acid insoluble Ash	94.97
3.	**Extractive values**	
A.	Water	11.45
B.	Alcohol	37.14
C.	Petroleum ether	2.12
D.	Ethyl acetate	11.07

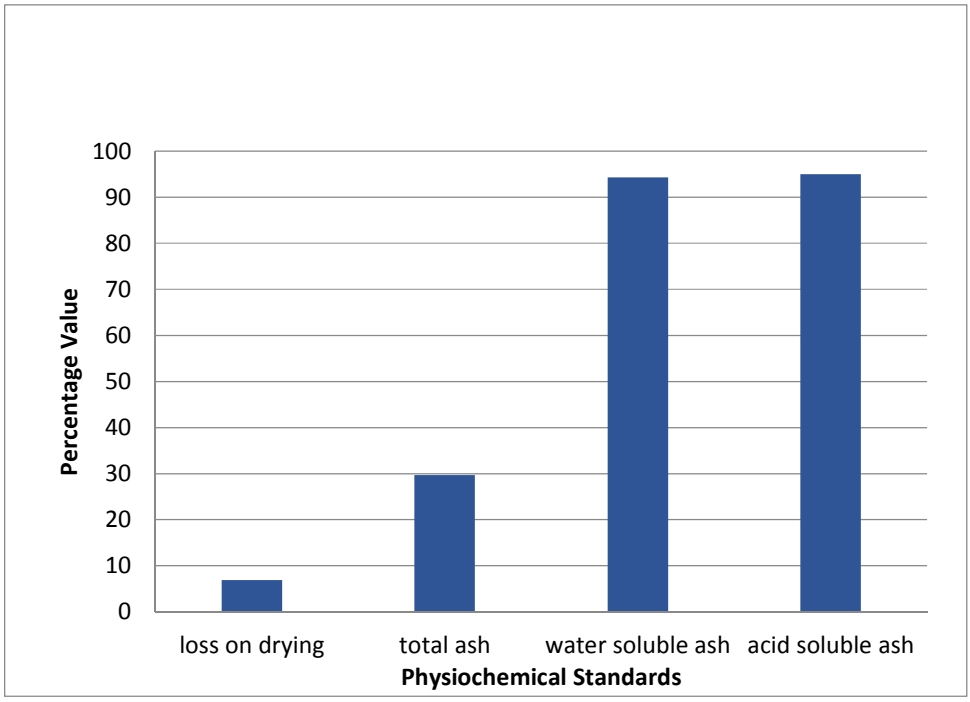


Figure 2.1: Percentage values of Physiochemical Standards of *M.elengi*

Table 2.2: Nature and yield of crude extracts of *M. elengi*

Crude Extracts	Color	Duration (hr)	Yield (%)
Petroleum Ether	Dark Green	26	2.12
Ethyl Acetate	Olive Green	35	11.07
Ethanol	Green	37	37.14
Aqueous	Greenish-brown	2	11.45

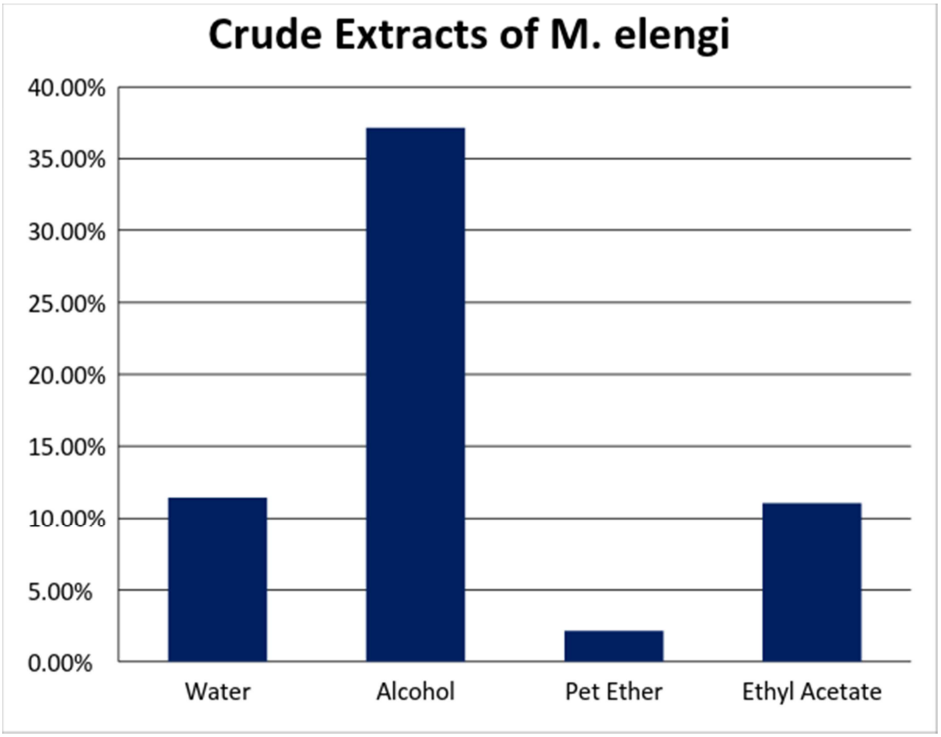


Figure 2.2: Percentage yield of crude extracts of *M. elengi*

Fluorecence Analysis of leaf extracts of *Mimusops elengi:*

Fluorescence is the ability of any compound or substance to emit light due to absorbed radiation. It is also an important parameter which helps in the evaluation of the crude sample. This property is exhibited by any sample due to the presence of various constituents in it. Thus, by this method we can determine the presence of these constituents. The compound when treated with concentrated acids and viewed under long and short U.V doesn't show any colour. However, it exhibits a strong red colour when acetic acid is added to it and it's viewed under short U.V light. The intensity of this colour decreases and it displays a reddish-brown colour when iodine is added and it's viewed under short U.V light. The various colour changes are shown in **Table 2.3**. These differences in colour indicate the presence of various functional groups/phytoconstituents present in the Mimusops species.

Preliminary phytochemical screening of *M .elengi:*

Preliminary phytochemical screening determines the various constituents/functional groups/classes of compounds which are present within the sample. It identifies the chemical compounds present. This is done by performing the respective tests for each group with the crude extract. We obtained extracts from petroleum ether, ethyl acetate, ethanol and water. Due to the solubility of various compounds in their respective solvents, the compounds can be determined with the help of phytochemical tests. This is one of the basic methods of evaluating the crude extract of the plant.

According the various tests which were performed we saw that the extracts of ethanol and water were enriched with phenolics, flavonoids, sterols and carbohydrates. Moderate amount of phenolics and carbohydrates were present in ethyl acetate extract. Minimal presence of carbohydrates and sterols were observed in the aqueous extracts. Proteins, saponins and alkaloids were absent in all the extracts.

The determination of these compounds plays a major role in health care since medicines can be formulated from theses extracts depending on the constituents it is rich in. These findings are useful is conferring additional information to the already present knowledge with regarding to the standardization and identification of *M .elengi* and distinguishes it from substitutes an adulterants.

Table 2.3: Fluorescence Analysis of powdered fruiting bodies of *M. elengi*

Sl. No	Reagents	Observation Under U.V light	
		Long U.V (365nm)	Short U.V (254nm)
1.	Powder as such	Olive Green	Olive Green
2.	Powder + Conc. HCl	Light Green	Green
3.	Powder + Conc. HNO_3	Light Green	Green
4.	Powder + Conc. H_2SO_4	Light Green	Green
5.	Powder + NaOH	Green	Green
6.	Powder + Water	Green	Green
7.	Powder + Acetic Acid	Dark Green	Bright Red
8	Powder + Iodine	Dark Green	Reddish-brown

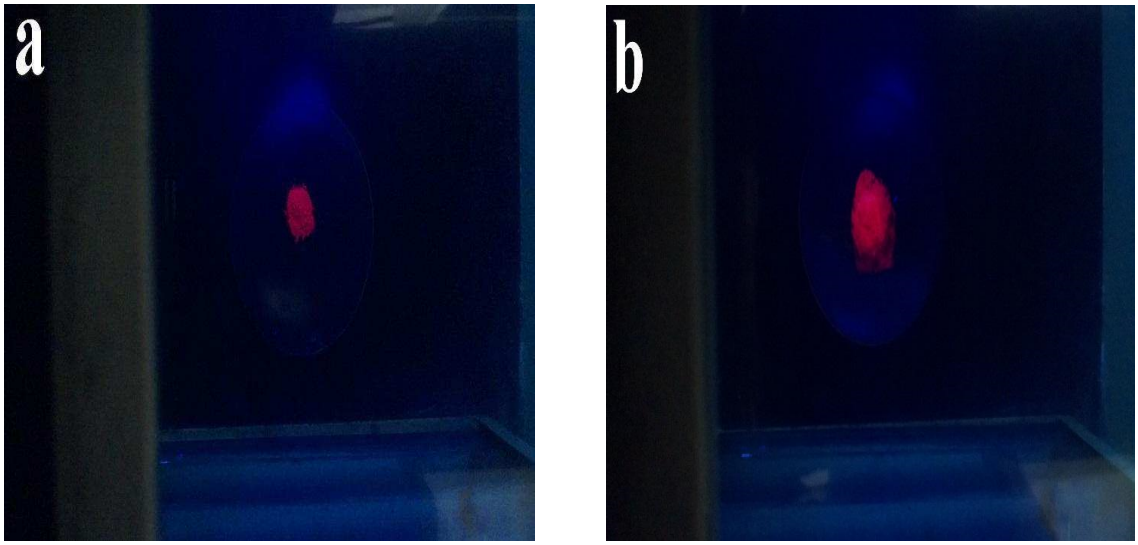


Figure 2.3: Fluorescence analysis of leaf extracts (a) Powder + Acetic acid (b) Powder + iodine

Table 2.4: Tests for Preliminary identification of phytochemicals present in crude extracts of *M .elengi*

Sl. No	Class of compounds	Tests	PE	AE	EtOH	Aqs
1.	Phenolics and Tannins	Ferric chloride	-	+	+++	++++
		Lead acetate	-	-	++	+++
2.	Flavanoids	Alkaline reagent	--	-	-	+
		Shinoda (Mg – HCl)	-	-	-	+
3.	Carbohydrates	Fehling's	-	+	+	++
		Molisch's	-	-	-	-
		Benedict's	-	-	-	-
4.	Sterols	Salkowski	-	-	+	+
		Libermann-Burchard	-	-	-	-
5.	Proteins	Biuret test	-	-	-	-
6.	Saponin	Foam test	-	-	-	-
7.	Alkaloids	Mayer's test	-	-	-	-
8	Fats & fixed oils	Saponification	-	-	-	-

(-)-Absent; (+)-Weak; (++)-Moderate; (+++) Strong;

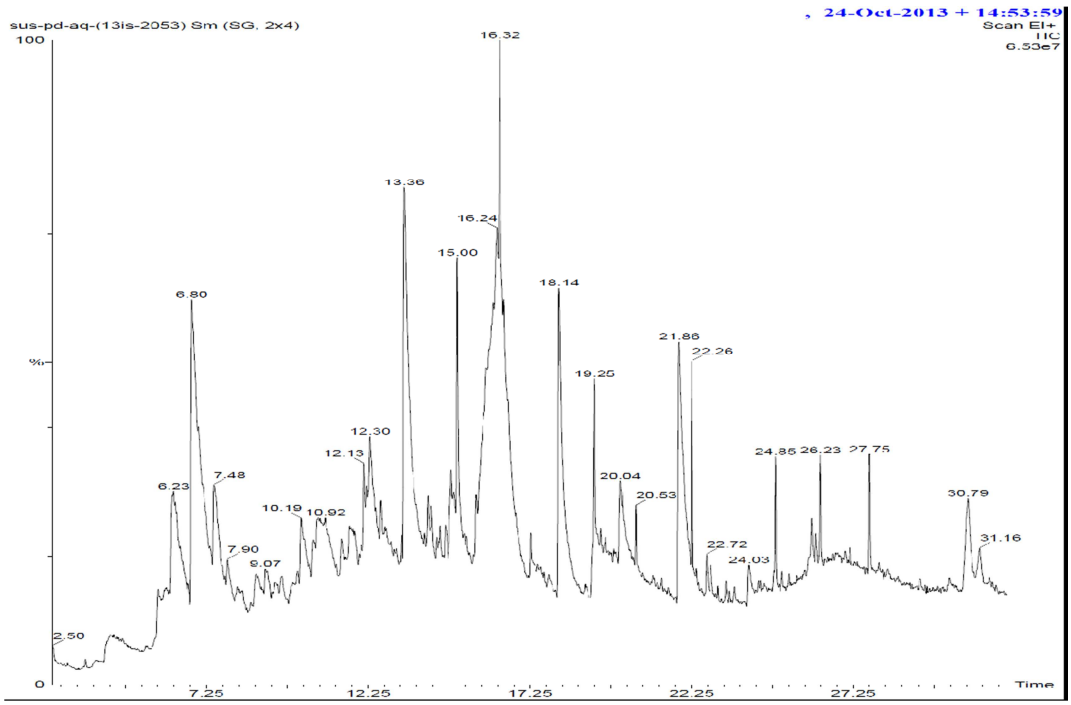
Determination of stomatal number and stomatal index:

Stoma are the two kidney shaped guard cells which are present in dicot leaves. They are surrounded by epidermal cells. They help in the exchange of gasses in leaves. The stomatal number and index was calculated and used in the standardization of the leaf.

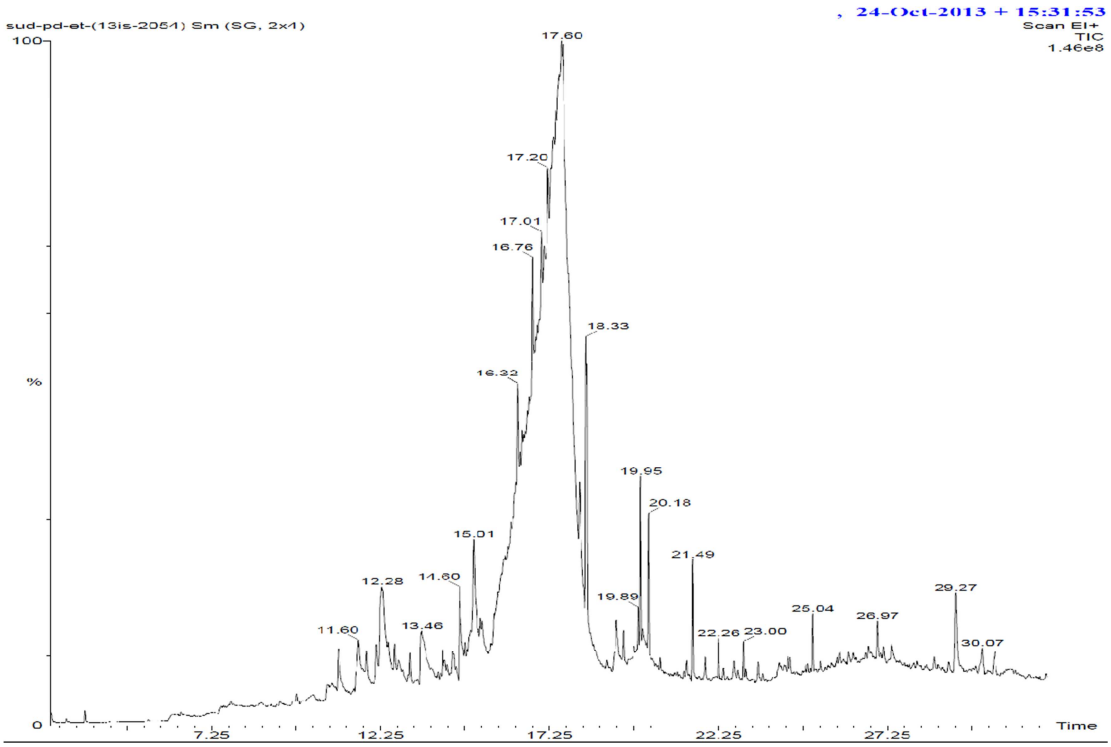
Figure 2.3: Chromatograms of all the crude extracts

(a). Chromatogram of PetroleumEther extract

(b). Chromatogram of Ethyl Acetate extract



(c). Chromatogram of Aqueous extract



(d). Chromatogram of Ethanol extract

GC-MS Analysis:
GC-MS results of all the four crude extracts of *Mimusops elengi* showed that several compounds are present in the ethanol and the aqueous extracts. Whereas very few compounds of medicinal value are present in the petroleum ether and ethyl acetate extracts. GC-MS analysis on crude extracts of petroleum ether and ethyl acetate confirms the presence of squalene, Z,Z-6,28- Heptatriactontadien-2-one, Methyl 3-bromo-1-adamantaneacetate, 2,6,10,14,18,22-tetracosahexane, 2,6,19,15,19,23-hexamethyl ester , Chloroacetic acid, tetradecyl ester. Out of this Squalene is the most important compound of biological significance. Chromatograms of all the four extracts can be viewed in Fig. 2.3 (a),(b),(c),(d)

Hydroxyl Radical Scavenging Assay:
Quercetin was used as a standard in Hydroxyl radical scavenging assay. And it was seen that the anti-oxidant scavenging activity decreased progressively with the decrease in the amount of sample taken. The anti-oxidant values of the crude extract was ethyl acetate was slightly more than the values of the other extracts. This can be seen in **Fig. 2.4**.

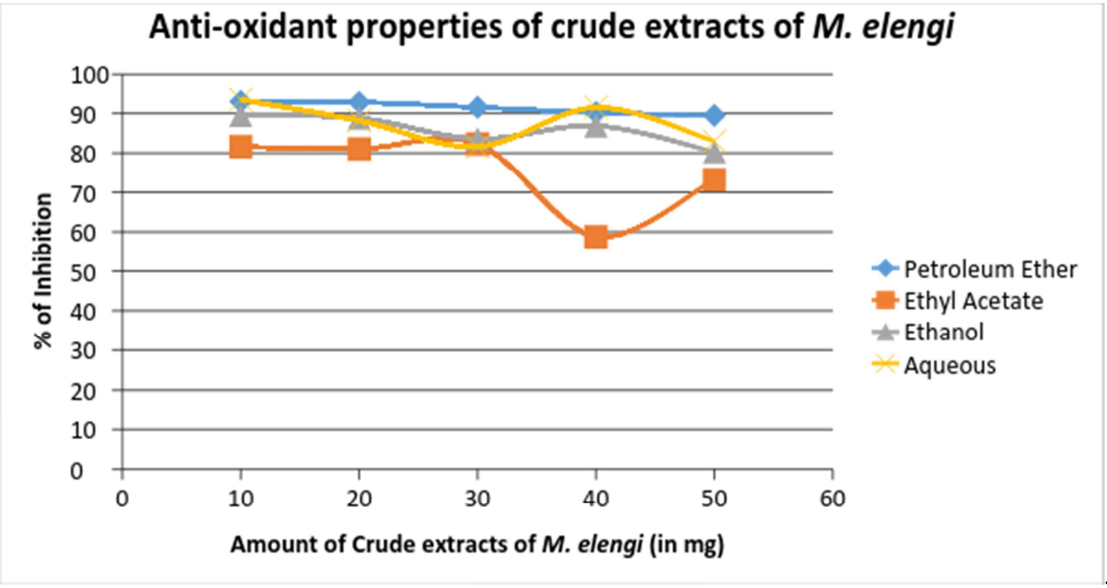


Figure 2.4: Anti-oxidant properties of crude extracts of *Mimusops elengi*

CONCLUSION

From the above results and discussions we can conclude that evaluating the physicochemical parameters will help in the standardization of this plant *M. elengi*. This pharmacognostical profile of the leaf of the plant will help in quality control and also prevent it from adulteration by other sample or constituents.

Further the GC-MS analysis helped to identify the potential bioactive compounds present in the leaf of *Mimusops elengi*.

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