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PHYTOCHEMICAL EVALUATION OF *MIMUSOPS ELENGI LINN* BARK

Anuradha.S.N*[1], Ting Ying Ying[2], Toong Jyh Kae[2], Arunkumar.S[3]
*[1]*Lecturer, Faculty of pharmacy,Aimst University, Malaysia.*
[2]*Student, Faculty of pharmacy,Aimst University, Malaysia.*
[3]*Scientist, IPDO,Dr.reddys lab, Hyderabad,India.*

ABSTRACT
World Health Organization (WHO) encourages, recommends and promotes traditional or herbal remedies in national health care programmes as these drugs are easily available at low cost, safe and people have faith in them. One of the medicinal plants is *mimusops elengi*. *Linn* which is also known as Bakul and Spanish cherry has a very large number of medicinal properties. Thus, it has a lot of therapeutic effects not only during ancient time, but also in the modern research. This study aims to perform the standardization of *mimusops elengi* bark by studying its pharmacognostical, physicochemical and phytochemical characteristics. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. This article provides useful and constructive information with regards to the identification, characterization and standardization of *mimusops elengi.*

Keywords: *Mimusops elengi linn*, Herbal, Phytochemicals

INTRODUCTION

Natural product is defined as a chemical substance produced by a living organism. It is a term used commonly in reference to chemical substances found in nature that have distinctive pharmacological effect. Plants have been one of the most important sources of medicine for thousands of years. [1]

Mimusops elengi is also known as Bakul and Spanish cherry is considered as a sacred plant among Hindus. *Mimusops elengi* has a very large number of medicinal properties thus it is known to make an important contribution to the field of science during ancient time and also to the modern research.

Mimusops elengi is classified under: Kingdom: Plantae, Order: Ericales, Family: Sapotaceae, Genus: Mimusops, Species: *Mimusops elengi Linn.* [1]

The bark of *Mimusops elengi* is found to be used as a cardio tonic, tonic, and anthelmintic. It is sometimes used as an astringent which help to cures biliousness and also the diseases of gums and teeth. The pentacyclic triterpene from bark have shown its moderate inhibitory activity against B-glucuronidase enzyme which is complies with ulcers in the gastric. [1, 2]

The barks of *Mimusops elengi* are basically greyish black in colour and it is channelled in shape which is normally at a length of range 15-25cm long and its width is about 10-15cm. While for the external part of the stem bark, it is basically rough surface on the external side. This is due to the vertical lenticels, crack and also the longitudinal fissures of the external surface of the *Mimusops elengi*.[3] These are all the characteristics for the fresh bark that are being cultivated not long ago. For the bark that has been dried under the normal room temperature, the bark is found to be brownish black in colour, and it has a curved, fibrous, thin and also longitudinally striated factures along with it [2, 3, 4]

There are major chemical constituents that are able to be found in the bark of the *Mimusops elengi*. Betulinic acid, ursolic acid, spinasterol and taraxerone are the constituents found in the stem bark of *Mimusops elengi*. [1, 5] Besides that, lupeol, B-amyrin and also the B-D-glucoside of B-sitosterol are also the chemical constituents found inside the stem bark.[1, 6] Many researchers found number of chemical compounds[6-13] with proved pharmacological activites[29,32,36,40]. The world health organization assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards. [14, 35, 37, 39] Few of the researchers around the world, had done phytochemical studies in this plantto prove the quality, chemical constituents and therapeutic activity.[17-24,30] Recent studies shows that the fraction of extract from bark of mimusops elengi has anticancer activity.[28]

Standardisation of herbal drugs used to confirm the drug`s identity, quality and purity[25] confirmation and amount of phyto- constituents were done by

Address for correspondence:
Anuradha.S.N,
Lecturer, Faculty of pharmacy,
AIMST University,Bedong
Semeling, Kedah, Malaysia- 08100

using RP-HPLC and TLC.[37,41,42] The scope of this study is to do standardisation of mimusops elengi bark using phytochemical analysis in Malaysia.

MATERIALS AND METHODS

Sample Collection:

The *Mimusops elengi* barks were collected twice in a year at Bedong village of Sungai Petani district, Kedah state in Malaysia. The samples were authenticated by Aimst University, Malaysia where the herbarium was deposited with a voucher specimen sample (AIMST/FOP/07). The barks collected were cleaned with water to remove the dust particles on the surface and then dried under natural sunlight for one week. The fresh bark was used for the study of macromorphological and microscopical characters; whereas the bark powders were subjected for preliminary phytochemical investigations like powder microscopy, physico-chemical properties and fluorescence analysis. [17-24, 32, 37, 40-42]

Preparation of Extract:

Bark powders are macerated with 3 different solvents systems, (distilled water, 50% methanol and 50% ethanol) After filtration, the remaining marc is soaked and filtered again. The second filtrates are added into first filtrates and evaporated until the volume decreased to 200mL. Concentrated aqueous, hydromethanolic and hydroethanolic extracts, were subjected to qualitative chemical tests.

Macroscopic Evaluation:

Size, shape, colour, odour, taste, length, thickness, surface characteristics, texture, and fracture were examined.

Microscopic Evaluation:

The bark sample specimen has been observed through a light microscope with a magnification of 4x, 10x and 40x.

Powder Characteristics:

A little quantity of bark powder was taken onto a microscopic slide, mounted in glycerol and examined under microscope.

Physico-Chemical Properties:

Total ash content, acid insoluble ash, water soluble ash, sulphated ash, water and alcohol soluble extractives have been determined according to the standard procedures.

Chemical Tests:

Chemical tests of saponin, flavonoid, phenolic compound, protein, tannins, triterpene, carbohydrates, steroid, alkaloid, and glycosides have been done.

Fluorescence Analysis:

Bark sample was placed in a test tube and different solvents are added, then viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations.

HPLC of Ursolic Acid:

RP-HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 10% ammonium acetate: methanol (pH 4.5) (30:70, v/v), and detection was made at 215 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation. [8,27,33,34]

Standard Solution Preparation:

10 µg of ursolic working standard was accurately weighed and dissolved in methanol and made up to the volume with the same solvent to produce a 1µg/ml of drug.

Sample Solution Preparation:

10g of bark powder was macerated with 30ml of 3 different solvents, which are distilled water, methanol and distilled water, ethanol and distilled water. After filtration, these extracts were evaporated until target volume of 20mL then used for HPLC.

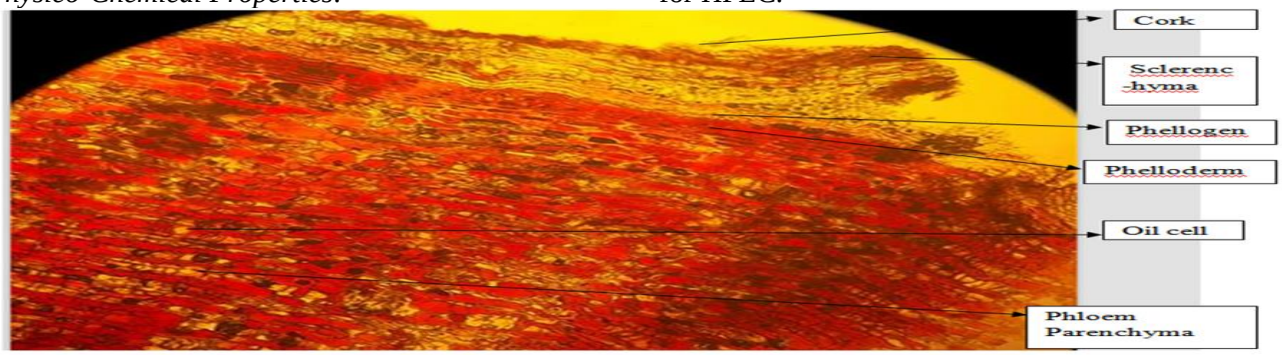

Figure- 1: Transverse section of Mimusops elengi bark shown under microscope.

RESULT AND DISCUSSION

Macroscopic evaluation:

Table- 1: Macromorphology descriptions of *mimusops elengi linn* bark

S.No	Characters	Observation
1	Colour	Dark brownish black
2	Odour	Characteristics
3	Taste	Astringent
4	Length	5cm (average)
5	Thickness	4.6mm (average)
6	Shape	Curved
7	Texture	Rough
8	Fracture (Inner surface)	Longitudinal Fissured
9	(Outer surface)	Longitudinal Striated and Fibrous

Powder characteristics:

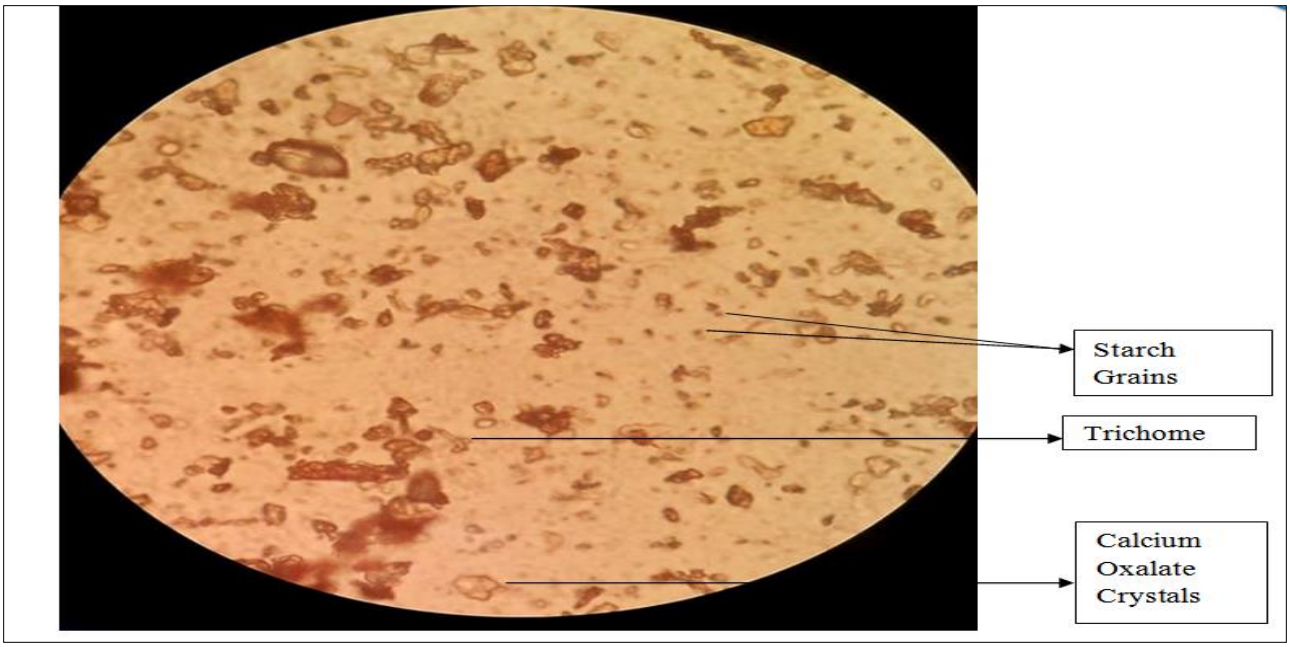

Figure- 2: Mimusops elengi bark powder shown under microscope.

The characteristic microscopy of bark powder showed the presence of calcium oxalate crystals, starch grains, oil cells and trichome.

The microscopic examination of transverse section of the bark shows the presence of cork cells, sclerenchyma, phellogen, phelloderm, phloem parenchyma, pericyclic fibers, medullary rays, and oil cell. The outermost layer consists of cork which is composed of rectangular compactly arranged cell 5-6 layered. Sclerenchyma cell layer has been observed with compactly arranged lignified cells. This is followed by 2-3 layered Phellogen cells. Cortex is composed of 6-8 layered Phelloderm cells. Phloem parenchyma show irregular cells alternating with lignified group of fibers and oil cells, along with numerous number of medullary rays.

PHYSICO-CHEMICAL PROPERTIES

Table- 2: Physicochemical properties of *mimusops elengi linn* bark

Parameters		sample
Ash Values (% w/w)	Total ash	6.5
	Acid Insoluble Ash	0.26
	Water soluble ash	1.835
	Sulphated ash	5.95
Extractive values (% w/w)	Water soluble extractive	4.2
	Acid soluble extractive	8.6

Ash value and extractive value are parameters used for the characterization of botanical drug, and are the preliminary steps of the quality control of herbal drugs. Ash value of medicinal plants reflects the carbonate, phosphate, oxides, silicate, and silica. Present investigations, considerable amount of total ash were noticed in bark. Sulphated ash was higher than the total ash, acid insoluble ash, and water soluble ash. The result shows that alcohol soluble extractive values are higher than water soluble extractive values, which indicates that the sample is more soluble in alcohol than water.

CHEMICAL TEST

Table-3: Chemical tests of aqueous, hydromethanolic and hydroethanolic extracts of *mimusops elengi.* bark.

Chemical Compound	Test	Observation			Results
		Aqueous extract	**Methanolic extract**	**Ethanolic extract**	
Saponin	Foam test	Pass. Foam persist for 10min	Pass. Foam persist for 10min	Pass. Foam persist for 10min	Presence of Saponins
Flavonoid	Lead Acetate test	Brownish precipitate	Small yellowish brown precipitate	Greyish precipitate	Presence of flavonoid
	Sulphuric acid test	Orange to crimson colour	Orange to crimson colour	Reddish orange colour formed	Presence of Flavonones
Phenolic compound	Ferric chloride test	Green colour formed	Green colour formed	Green colour formed	Presence of condensed tannin
	Lead acetate test	Brownish precipitate	Yellowish brown precipitate formed	Bulky red precipitate	Presence of phenol compound
Protein	Millon's Reagent	Light brown precipitate	Reddish brown precipitate	Light brown precipitate	Presence of protein
Tannins	Lead acetate test	Brownish precipitate	Yellowish brown precipitate formed	Bulky grey precipitate	Presence of tannins
Triterpene	Salkowski test	Bottom become	Bottom become yellow	Yellowish orange on	Presence of triterpene

		yellow		lower layer	
Carbohydrates	Benedict's test	Brick red precipitate	Reddish brown precipitate	Brick red precipitate	Presence of carbohydrate
	Barfoed's test	Reddish brown precipitate	Brick red precipitate	Brick red precipitate	Presence of carbohydrate
Steroid	Salkowski test	Yellowish orange on lower layer	Bottom become yellow	Yellowish orange on lower layer	Steroid is present
Alkaloid	Hager's test	Failed. No precipitate formed	Failed. No precipitate formed	Failed. No precipitate formed	No Alkaloids present.
Glycosides	Legal test	Light brown colour	Reddish colour	Bulky brown precipitate	Presence of glycosides
	Keller-killiani test	Brownish red precipitate form	Brown precipitate formed	Lower bulky white layer Upper reddish brown layer	Presence of glycosides

Chemical test stated in the Table 3 have been done for different types of extract. It show that bark of *Mimusops elengi* consists of most of the constituents that being test such as Saponin, Flavonoid, Phenolic compound, Protein, Tannins, Triterpene, Carbohydrates, Steroid, Glycosides in all types of the extract except for alkaloids. It shows negative results in all of the extract being tested with Hager's test. No precipitate form. Thus, alkaloids is absent in the bark of *Mimusops elengi.*

FLUORESCENCE ANALYSIS

Table- 4: Fluorescence analysis

S.N	Solvent treatment	Visible Light	Short UV (254nm)	Long UV (366nm)
1	Drug +Methanol	Yellowish Brown	Dark Brown	Milky
2	Drug + D.Water	Light pink	Dark Brown	Light Green
3	Drug + 10% sodium hydroxide	Reddish Brown	Mud Brown	Brownish Black
4	Drug + Ferric Chloride	Greenish Brown	Dark Brown	Brownish Black
5	Drug + Picric acid	Yellow	Light Brown	Yellowish Green
6	Drug + Chloroform	Colourless	Light Yellow	Light pink
7	Drug + Ammonia	Reddish Brown	Dark Red	Brownish Black
8	Drug + H_2SO_4	Yellowish Orange	Yellowish Brown	Dark Brown
9	Drug + HNO_3	Reddish black	Black	Bluish Black

Fluorescence is the essential parameter which acts as the 1st line standardization of crude drug. Ultraviolet (UV) light would produces fluorescence in many substance that normally does not even fluorescence under the daylight. This is because light which are very rich in short wavelength tends to be more active and higher tendency in producing fluorescence in the substances. The result of fluorescence is shown in the Table 4.

Among employed tests, bark powder of *Mimusops elengi* produced noticeable colour with several solvents like methanol, distilled water, chloroform, and ammonia under long UV, and thus can be an important character to ascertain genuineness of the powdered drug.

HPLC Analysis:

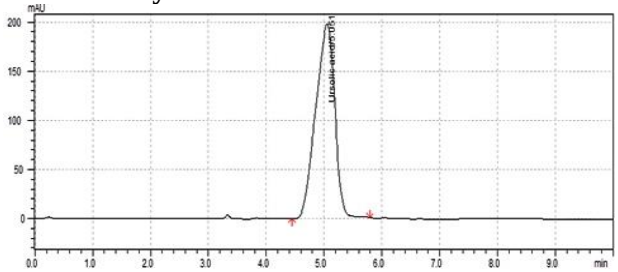

Figure-3: Standard Chromatogram

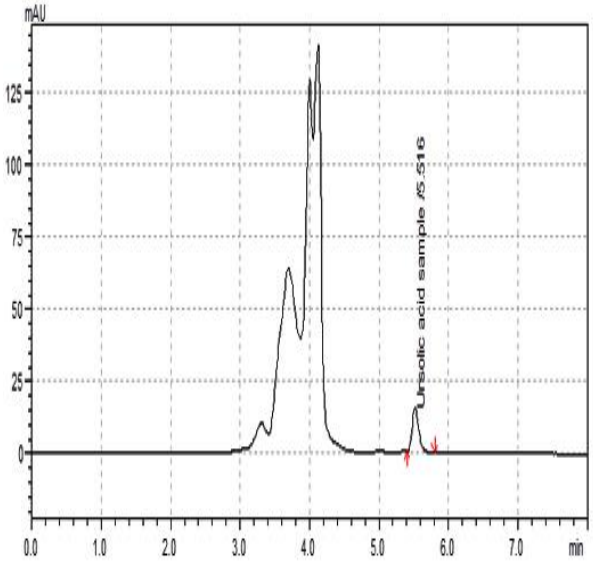

Figure- 4: Aqueous Extract Chromatogram

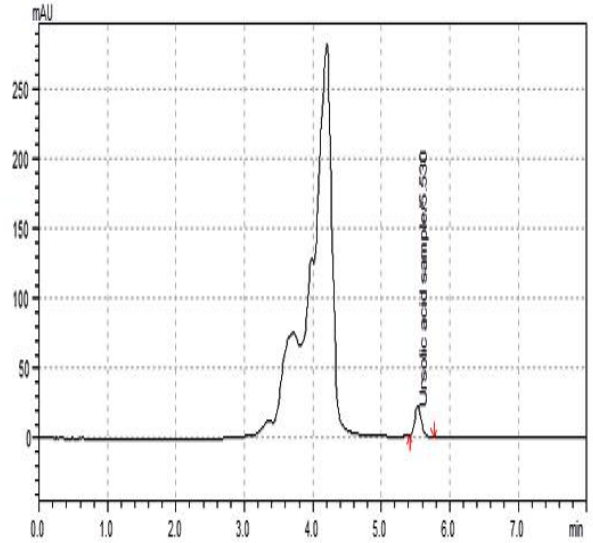

Figure- 5: Hydromethanolic Extract Chromatogram

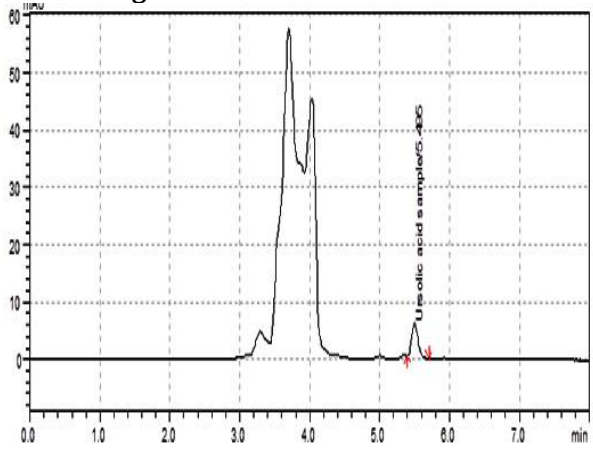

Figure- 6: Hydroethanolic Extract Chromatogram

RP-HPLC analysis was carried out in aqueous, hydromethanolic, hydroethanolic extracts. The chromatogram confirms the presence of one of constituents of *Mimusops elengi* bark (ursolic acid).

CONCLUSION

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects. Pharmacognostic evaluations like macromorphology and micromorphology characteristics, ash analysis, extractive value, fluorescence analysis, chemical tests and HPLC are useful as quality control parameters. This helps in determining the important constituents present in the extracts besides establishing the actual identity of the source materials.

REFERENCES

[1] PV Kadam, KN Yadav, RS Deoda, RS Shivatare, and MJ Patil. *Mimusops elengi*: a review on ethnobotany, phytochemical and pharmacological profile. *Journal of Pharmacognosy and Phytochemistry*.1 (3):64-74 (2012).

[2] S Rajkumara, A Pandiselvi, and G Sandhiya. Isolation of Chemical Constituents from *Mimusops elengi* bark and Evaluation of Anti-inflammatory activity. *Int J Phytopharm Res.* 3(1): 9-15 (2012).

[3] RS Shivatare, RS Deoda, PV Kadam, HU Bhusnar, NS Narappanawar, and MJ Patil.Pharmacognostic Standards for Mimusop selengi Linn-A Review. Journal of Pharmacognosy and Phytochemistry.2(3): 12-18 (2013).

[4] B Gopallrishnan, and SN Shimpi, Seeds of mimusops elengi Linn. Phrmacognosy and phytochemical studies. *International journal of pharmacognosy and phytochemical research.* 3(1):13-17 (2010).

[5] K Raghunathan, R Mitra. Pharmacognosy of Indigenous Drugs. Vol. I, Government of India, Central Council for Research in Ayurveda and Siddha. 158-183(2000).

[6] G Mishra, and CR Mitra,. Constituents of bark of *Mimusops elengi linn*. *Phytochemistry*, 6:1909 (1967).

[7] N Jahan, A Malik, G Mustafa, Z Ahmad, S Ahmad, E Anis, S.Malik, S Shujaat, N Afza,

and Atta-ur-Rahman. Triterpenes from *Mimusops elengi*. *Natural product letters*, 15(3):177-185(2001).

[8] Dighe, Vidya and Mestry, Dhanashri, Separation and determination of triterpene acids by using High Performance Thin Layer Chromatography from stem bark of mimusops elengi Linn. *International journal of pharmacy pharmaceutical Sciences* 6(1):313-317(2014).

[9] GA Akowuah, and I Zhari, Determination of betulinic acid from Orthosiphonstamineus leaf extract in rat plasma. *Die pharmazie*-An international journal of Pharmaceutical Sciences, 63(11), pp.788-790 (2008).

[10] Q Liao, W Yang, Y Jia, X Chen, Q Gao and K BI. LC-MS determination and pharmacokinetic studies of ursolic acid in rat plasma after administration of the traditional chinese medicine prepararion Lu-Ying extract. *yakugakuZasshi*, 125(6):509-515 (2005).

[11] M Roqaiya, W Begum, SF Majeedi, and A Saiyed. A review on traditional use and phytochemical properties of mimusops elengi Linn. *International Journal of Herbal Medicine*. 2(6 part A):20-23(2015).

[12] GV Rao, C Sharlene, and T Mukhopadhyay. Secondary metabolites from flowers of mimusops elengi linn. *Der pharmacia letter*. 4(6):1817-20 (2012).

[13] F Gaedcke, and B Steinhoff. Herbal medicinal products: scientific and regulatory basis for development, quality assurance and marketing authorisation. *CRC press*. (2003).

[14] R Roy chaudhury, Herbal medicine for human health. New Delhi: *WHO reginal office for South-East Asia* (1992).

[15] KR Kirtikar, and BD Basu. Indian Medicinal Plants Vol-3(1918).

[16] NS Madhav, K Upadhyaya and A Bisht. Phytochemical screening and standardisation of polyherbal formulation for dyslipidemia. *International journal of pharmacy and oharmaceutical sciences*. 3(3):235-238(2011).

[17] M Yunus, D Yunus, and M Iqbal. Systemic bark morphology of some tropical trees. *Botanical journal of the Linnean Society*.103(4):367-377 (1990).

[18] VD Rangari. Pharmacognosy and phytochemistry. New Delhi: *Career publications*. 1st ed :103-115(2006).

[19] WC Evans. Trease and evans pharmacognosy. *Edingburgh: Saunders Elsevier*. 541-570(2009).

[20] OF Kunle, HO Egharevba, and PO Ahmadu. Standardization of herbal medicines- A review. *International ournal of biodiversity and conservation*. 4(3):101-112 (2012).

[21] AK Yusuf. Studies on some physicochemical properties of the plant gum exudates of acacia Senegal (dakwara), Acacia sieberiana (farar kaya) and acacia nilotica (bagaruwa). *Journal of research in National development*. 9(2):10-17(2013).

[22] J Liu. Pharmacology of oleanolic acid and ursolic acid. *Journal of ethnopharmacology*, 49(2):57-68(1995).

[23] MK Gupta, PK sharma, SH Ansari, and Rekha Lagarkha, Pharmacognostical evaluation of grewiaasiatica fruits. *Int J Plants Sci*. 1(2):249-251(2006).

[24] VD Rangari. Pharmacognosy and phytochemistry. 1st ed. New Delhi: *Career Publications*.103-115(2006).

[25] A Pandey and S Tripathi. Concept of standardisation, extraction and pre phytochemical screening stratergies for herbal drug. *Journal of Pharmacognosy and phytochemistry*. 2(5):(2014).

[26] D Menpara, D Desai and S Chanda. Pharmacognostic, phytochemical, physicochemical and fluorescence analysis of Terminalia bellerica leaf and stem. *World journal of pharmaceutical Sciences.* 2(4):390-396(2014).

[27] AK Maji, S Pandit, P Banerji, and D Banerjee. A validated RP-HPLC method for simultaneous determination of botulin, lupeol and stigmasterol in AsteracanthaLongifoliaNees. *International journal of pharmacy and pharmaceutical sciences.* 6(5):(2014).

[28] Vonna aulianshah, uripHarahap, and Rosidah. Cytotoxic activity and phase G2-M Cell cycle inhibition active fraction of mimusops elengi stem bark on T47D cells. *International journal of PharmTech research.* 6(2):868-873(2014).

[29] D Mestry, and VV Dighe. Pharmacognostic evaluation and preliminary phyto-chemical screening of the dried powder of stem bark of mimusops elengi linn and leaf of Jasminumsambac Ait. *Journal of*

pharmacognosy and phytochemistry. 2(4): 107-112 (2013).

[30] F Amir, KK Wong, I Eldeen, MZ Asmawi and H Osman. Evaluation of biological activities of extracts and chemical constituents of mimusops elengi. *Tropical journal of Pharmaceutical Research*. 12(4):591-596 (2013).

[31] SF Zohra, B Meriem, S Saamira and MS Alsayadi-Muneer. Phytochemical screening and identification of some compounds from mallow. *J Nat prod Plant Resour*. 2(4):512-6 (2012).

[32] PV Kadam, RS Deoda, RS Shivatare, KN Yadav, and MJ Patil. Pharmacognostic, phytochemical and physiochemical studies of mimusops elengi linn stem bark(Sapotaceae). *Scholars Research Library*. 4(2):607-613 (2012).

[33] EMS Oliveira, et al. Isolation and quantitative HPLC-PDA analysis of lupeol in phytopharmaceutical intermediate products from vernonanthuraferruginea(Less), *H. Rob.Quimica Nova*. 35(5):1041-1045(2012).

[34] SV Taralkar, and SChattopadhyay. A HPLC method for determination of ursolic acid and betulinic acid from their methanolic extracts of Vitex Negundo Linn. *Journal of Analytical & Bioanalytical Techniques*. 3:3 (2012).

[35] N Choudhary, and BS Sekhon. An overview of advances in the standardisation of herbal drugs. *Journal of pharmaceutical education and research*. 2(2):55 (2011).

[36] V Lalitha, B Kiran, & KA Raveesha. Invitro evaluation of mimusops elengi plant for antibacterial activity and phytochemical analysis. *Pharmacophore*. 2(1):78-85(2011).

[37] B Gami, and MH Parabia. Pharmacognostic evaluation of bark and seeds of mimusops elengi L. *Int J Pharm PharmSci*. 2(Suppl 4):110-3(2010).

[39] P Vihar. Standardization of centipede minima, mimusops elengi, Orchismascula and Paeoniaemodii. *Indian Journal of Traditional knowledge*. 8(3):338-345(2009).

[40] MB Gallo, and MJ Sarachine. Biological activities of lupeol. *Int J Biomed Pharm Scvi*, 3(1): 46-66 (2009).

[41] S Satish, MP Raghavendra, DC Mohana, and KA Raveesha. Antifungal activity of a known medicinal plant mimusops elengi L. against grain moulds. *J. Agri. Technol.* 4(1):151-165 (2008).

[42] S Panchawat and K Rathor. Standardization and evaluation of herbal drug formulation. *Indian Journal of Natural Products*. 19:11-15 (2003).

[43] DV Awing. Standardization of herbal medicinal prducts. In XXVI International Horticultural Congress: *The Futuree for Medcinal and Aromatic Plants* .629:111-114 (2002).