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Analysis on Standardization of Medicinal Plant with Hepatoprotective Activity

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ABSTRACT: Bark of Mimusops elengiL. is an evergreen tree, belonging to family Sapotaceae, cultivated in gardens as an ornamental tree. It is traditionally used as cardiotonic, anthelmintic, in the treatment of leprosy and various ailments. The present study includes phytochemical and pharmacological evaluation of bark. Here the powdered bark was subjected to successive soxhlet extraction using different solvents of increasing polarity. The successive methanol and aqueous extracts were subjected for In-vivohepatoprotective evaluation. The methanolic extract was found more effective. So it was further subjected for preliminary phytochemical and chromatographic examination. Attempt was made to isolate the compounds using isocratic elution technique. The compounds isolated were characterized by UV, FT-IR, 1H-NMR, 13C- NMR, and Mass spectroscopy

I INTRODUCTION

Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Plants produce a variety of chemical substances that act upon the humanbody.Many plants bio synthesise chemical substances that are useful for the maintenance of health in humans and other animals. Many are secondary metabolites, of which at least 12,000 have been isolated-a number estimated to be less than 10% of the total. In many cases these substances (particularly the alkaloids) serve as plant defense mechanisms against insects and herbivores. Many of the herbs and species used by humans as season as food yields useful medicinal compounds.Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacologicalactivity.Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs; while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of rural population depend on indigenous system of medicine.

Ayurvedic, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit Au (life) and Veda (knowledge). As the name implies it is not only the science of treatment of the ill but covers the whole gamut of happy human life involving the physical, metaphysical and the spiritual aspects.Ayurveda recognizes that besides a balance of body elements one has to have an enlightened state of consciousness, sense organs and mind if one has to be perfectly healthy. Ayurveda by and large is an

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experience with nature and unlike in Western medicine, many of the concepts elude scientific explanation. Ayurveda is gaining prominence as the natural system of health care all over the world. Till today this system of medicine is being practiced in countries like Nepal, Bhutan, Sri Lanka, Bangladeshand Pakistan. While the traditional system of medicine in the other countries like Tibet, Mongolia and Thailand appear to be derived from Ayurveda.Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capsaicin, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Liver:

The liver is the largest glandular organ with a weight of about 1.5 kg. It is a reddish brown organ with four lobes of unequal size and shape. The liver is on the right side of the abdominal cavity just below the diaphragm and is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested food from the small intestine. These blood vessels subdivided into capillaries which then lead to a lobule. Each lobule is made up of thousands of hepatic cells which are the basic metaboliccells.

FUNCTIONS OF LIVER

The basic functions of liver can be divided into:

Its metabolic functions concerned with the majority of the metabolic system of thebody.

Its vascular functions, for storage and filtration ofblood.

Its secretory and excretory functions that is responsible for forming the bile that flows through the bile ducts into the gastrointestinaltract.

Metabolic functions of the liver:

Carbohydrate metabolism:

Synthesis of plasma proteins and most of the blood clotting factors like fibrinogen andprothrombin.

CLASSIFICATION OF LIVER FUNCTION TESTS:

Test dependent on hepatic secretion andexcretion:

Bilepigment

Clearance of foreign substances from theserum.

Test dependent upon specific biochemicalfunctions:

Protein metabolismtests

Carbohydrate metabolismtests

Lipid metabolismtests

Barbiturate metabolism tests (Increased sleepingtime)

Test dependent upon measurement of serum enzymeactivity:

SGPT

SGOT

SALP

Otherenzyme

Medicinal Plants With Hepatoprotective Properties

PHYTOCONSTITUENTS : [24]

Roots, Leaves and heart-wood –hentriacontane,β-carotene and lupeol.

Flowers- sterols.

Bark- Tannin and saponins. Major constituents: pentacyclic triterpenoids,
β-amyrin, bassic acid, betulic acid, lupeol, taraxerone, taraxerol and ursolic acid.

Others: steroids, glycoside ofβ-sitosterol,α-spinasterol, fatty acid ester of
α-spinasterol and flavanoid quercitol.

Fruits and seeds- quercitol, ursolic acid, glucose, quercetin, dihydroquercetin and
β-sitosterol glucoside.

Kernel- sapogenin and saponin.

TRADITIONAL USES: [19]

The bark is cardiotonic, stomachic, cures biliousness and diseases of the gums andteeth.

The flowers are expectorant, cures diseases of the nose andheadache.

The fruit and the seeds are aphrodisiac, diuretic and astringent to thebowels.

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Figure No: 01 PhotographsofMimusopselengiLinn. [25]

Fig.01 Tree

Fig.02 Leaves

Fig. 03 Bark

Fig.04 Flowe

Fig. 05 Fruit

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II.PHARMACOGNOSTICINVESTIGATIONS

The systematic pharmacognostic investigations of the plant material and its documentation will help the scientific community for further investigations. So effortsare made to contribute in thisdirection.
Collection and Authentication of the wholeplant.

Morphologicalcharacters.
Microscopiccharacters.

Transverse section of theBark
Powder microscopy

Proximatevalues.
Extractivevalues

Alcohol soluble extractivevalue.
Water soluble extractivevalue.

Petroleum ether soluble extractivevalue.
Moisture contentdetermination

Ashvalues
Totalash

Acid insolubleash
Water solubleash

Transverse section of the Bark

The microtome section of the bark was taken observed under low and high power magnification of microscope and results are as on page no.62 (Figure No: 03).

Powder microscopy

The dried bark ofMimusops elengiL. were coarsely powdered and boiled with chloral hydrate for 5-10 minutes and then stained with phloroglucinol and HCl in 1:1 ratio, observed under high power (40x), for different diagnostic characters suchas, cork cells, crystal fibres, medullary rays with attached fibres and cortex cells with starch grains. Results are as on page no.63 (Figure No: 04).

Proximate Values [41-43]

The following proximate values were determined for the bark powderedMimusops elengiL. Results are as on the page no.64 (Table No: 05).

Extractive values

Extractive values help to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with various solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.
Alcohol soluble extractive value.

Figure No: 01 UV spectra of isolated COMP-A

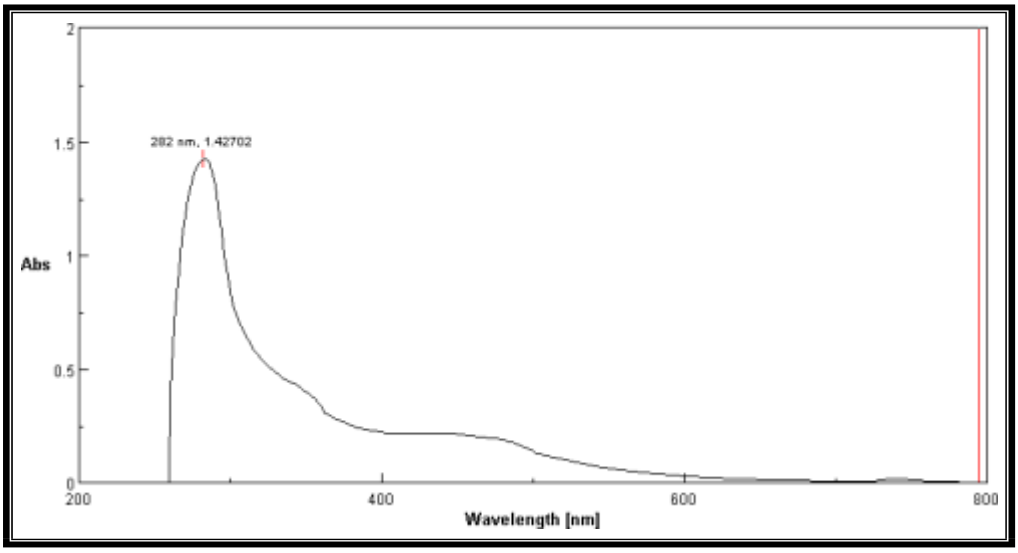

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Table No: 01. Proximate values of Mimusops elengiL bark.

Sl.No.	Parameter	Determined Value % w/w
A	Extractive values	
1	Alcohol soluble extractive value	22.00
2	Water soluble extractive value	28.00
3	Pet ether soluble extractive value	1.00
B	Moisture content	12.75
C	Ash Values	
1	Total ash	6.5
2	Water soluble ash	1.00
3	Acid insoluble ash	1.5
4	Sulphated ash	4.5

III. DISCUSSION

Present research includes Pharmacognostic, Phytochemical and Hepatoprotective investigations of the bark of **Mimusops elengiL.**

Pharmacognostic Contributions:

The bark was found to be black to reddish brown in color, odourless, single quilled with rough surface. The Transverse section and powder microscopy of the bark revealed the presence of cork, cortex, medullary rays, phloem fibre, fibrous sclereids, crystal fibres, medullary rays with attached fibres and cortex cells containing starch grains.

Proximate values:

Proximate values for the bark ofMimusops elengiL. are as follows: Alcohol soluble extractive value (22.00%), Water soluble extractive value (28.00%), Pet ether soluble extractive value (1.00%), Loss on drying (12.75%), Total ash (6.5%), Acid insoluble ash (1.5%), Water soluble ash (1.00%) and Sulphated ash (4.5%). These values are criterion to put the guidelines of identity and purity of crude drug.

Phytochemical Investigations:

Preliminary phytochemical investigations of chloroform, methanol and aqueous extract and ethyl acetate fraction of methanol extract revealed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids and phenolic compounds. Further, chromatography based separation of ethyl acetate soluble fraction of methanol extract was carried out. The ethyl acetate fraction was found to contain three spots in TLC then an attempt was made to isolate these compounds by column chromatography with isocraticelution.

The isolated compound was further characterized by physicochemical tests, chromatography and spectral analysis such as, UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopy.

The isolated COMP-A:revealed following analytical data.

UV spectra:one peak withλ_{max} at 282 nm.

IR spectra:wave numbers at, 3369.77 for OH Stretching, 2924.07 for C-H Stretching (Aromaticity), 2855.13 for C-H Stretching, 2363.93

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for OH Stretching.
^{1}H-NMR spectra: Values at 6.7-7.2 for Ar-H (5H), 4.5-4.62 for OH (3H) of phenolic, 3.75 for OCH_3 (6H).
^{13}C-NMR: Values at 175.55 (C=O, carbon), 111.09 – 162.88 (C=C, Aromatic), 47.40 – 79.10 (C–O, Carbon), 103.81 (C=C, Unsaturated carbon).
MS spectra: Base peak at 302 and molecular ion peak at 381.

Acid Butanol Assay:

Acid butanol assay was carried out for the identification of condensed tannins present inMimusops elengiL. bark. The formation of pink colour withλmax at 530nm indicates the formation of cyanidin. Thus it gives clear indication of presence of catechin or epicatechin extenders inM. elengibark.
Pharmacological Screening:

Acute toxicity study: Acute toxicity studies were performed according to OECD guidelines of Method no: 423 and dose was fixed as 200 mg/kg b.w. of successive methanol and aqueous extracts.
Hepatoprotective activity:
Positive control group (Liv-52): liver section showed foci of necrosis.Binucleate hepatocytes seen with regenerativeactivity.
Successive methanol extract treated group: sections from liver show minimal hepatocytes necrosis. Good number of binucleate regenerating hepatocytesare alsoseen.
Successive aqueous extract treated group: liver section showed dense foci of necrosis. Hepatocytes showing fatty change and few regenerating hepatocytesare alsoseen.

From the above studies it is evident that successive methanolic and aqueous extracts of bark ofMimusops elengiL. plays a promising role in the treatment of Liver disease and worth for further investigations for isolation of more bioactive phytoconstituents for the above treatment.

IV. CONCLUSION

The overall pharmacognostic, phytochemical investigations and Hepatoprotective research on bark of**Mimusops elengiL.**exhibited results to conclude as follows:Morphological evidences to identify and authenticate the drug are as follows.

Microscopy of bark ofMimusops elengiL. exhibited prominent histological features like cork, cortex, medullary rays, phloem fibre, fibrous sclereids, cork cells, crystal fibres, medullary rays with attached fibres and cortex cells containing starchgrains.

Preliminary phytochemical investigations of chloroform, methanol, aqueous extracts and ethyl acetate soluble fraction of methanolic extract have revealed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids and phenolic compounds.

The successive methanol and aqueous extracts exhibited more promising hepatoprotective activity at the dose of 200mg/kg body weight which is supported by Histopathology. Collectively these natural flavonoids and tannins ofM. elengi barks are promising in this research. However further investigations are needed to give some more evidences to support thisresearch.

The isolated COMP-A was characterized by UV, FT-IR, H ^{1}-NMR**,** ^{13}C-NMR and Mass spectral data co-relates with member of natural flavor noids

The product of acid butanol reaction yields cyanidin. This confirms the proanthocyanidin present inMimusops elengiL. bark contains catechin or epicatechinunits.

The results of the investigations justify the folklore use of bark ofM. elengiin the treatment of liver diseases and the plant is worth for further isolation of more bioactive molecules.

V. SUMMARY

The present study was carried out to investigateMimusops elengiL. bark for pharmacognostic, phytochemical andin vivohepatoprotective screening.

Collection and Extraction:

Mimusops elengiL. bark was collected from local areas of Ashoka Van (Gokarn), Karnataka and authenticated by renowned botanist. Authenticated plant material was subjected to morphological and microscopical analysis. Shade dried and powdered bark was subjected to successive soxhlet extraction with organic solvents of increasing polarity like chloroform, methanol and water. All the extracts were evaluated by

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qualitative chemical examination for the presence of important to constituents.

Isolation:

One phytoconstituent (COMP-A) was isolated from ethyl acetate soluble fraction of methanolic extract by column chromatography and an attempt was made to characterize the isolated compound by TLC, UV, FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic studies. Characterization revealed that COMP-A may be a flavonoid.

Acid Butanol Assay:

Acid butanol assay shown the presence of cyanidin inMimusops elengiL. bark which was found to contain catechin or epicatechin units.

Evaluation forin vivoHepatoprotective activity:

To confirm the Hepatoprotective activity of the drug the biochemical studies was carried out on Albino rats and significant reduction of various enzymes like SGOT, SGPT, ALP and bilirubin levels in blood serum of extract treated groups was observed in the following range: successive methanolic extract group > successive aqueous extract group. The above results were supported further by Histopathological studies, which revealed a significant regeneration of the hepatocytes with normal portal vein. The groups treated with above extracts were compared with CCl_4 group.

In this study an attempt was made to provide scientific background to the traditional claim. Since the results showed a significant Hepatoprotective activity in rats, the traditional use of**Mimusops elengiL**bark for the Hepatoprotective activity may be justified.

REFERENCES

[1]. http://www.holisticoline.com/Herbal-Med/hol_herb-intro.htm/11.10am/28-12-2009.

[2]. http://www.holisticoline.com/Herbal-Med/hol_herb-forms.htm#standardized/3.30pm/7-1-2010.http://stepticwiki.org/index.php/Herbal_medicine/2.45pm/16-1-2010.

[3]. Harsh Mohan. Textbook of pathology, 5th ed. New Delhi: Jaypee brothers. Medical publishers (p) Ltd; 2000.5. www.pharmainfo.net /11.50am/18-1-2010.

[4]. Dr.C.C. Chatterjee, Human Physiology Vol-I. Medical Allied Agency;2004.

[5]. Cotran RS, Kumar V, Collins T. Pathologic Basis of Disease. 6th ed. NewDelhi.

[6]. Harcourt India Pvt. Ltd, 27 M, Block market, Greater Kailash II; 2001.

[7]. Shah M, Patel P, Phadke M, Menon S, Mary Francis, Sane R.T. Evaluation of the effect of aqueous extract from powders of root, stem, leaves and whole plant ofPhyllanthus debilisagainst CCl_4 induced rat liver dysfunction. Indian Drugs 2002;39(6):333-7.

[8]. Vogel HG, Drug Discovery and Evaluation Pharmacological Assays. 2nd ed. Springer- Verlag, Heidel berg, (NY); 2002.Subrata De, Ravishankar B, Bhavsar G.C. Plants with Hepatoprotective Activity.Indian Drugs 1993;30(8):355-63.Baheti J.R, Goyal R.K, Shah G.B. Hepatoprotective activity ofHemidesmusindicusR. Br. in rats. Ind. J. Exp. Bio. 2006;(44):399-402.

[9]. Akare S.C, Sahare A.Y, Shende M.A, Bondre A.V, Wanjari A.D. Hepatoprotective activity ofAcacia ferrugineaDC. leaves against carbon tetrachloride induced liver damage in rats. Int. J. Pharm. Tech. Res. 2009;1(3):962-5.

[10]. Vetrivel Rajan A, Shanmugavalli N, Greety Sunitha C, Umashankar V. Hepatoprotective effects ofCassia toraon CCl_4 induced liver damage in albino rats. Ind. J. Sci. Tech. 2009; 2(3):41-4.

[11]. Usha K, Mary Kasturi G, Hemalatha P. Hepatoprotective effect ofHygrophila spinosa andCassia occidentalison carbon tetrachloride induced liver damage in experimental rats. Ind. J. Clin. Biochem. 2007;22(2):132-5.

[12]. Kuldeep Ganju, Brij Bhushan Tiwari, Goswami R.B, Pathak A.K. Hepatoprotective activity of tuberous roots ofGloriosa superba. Ind. J. Nat. Prod. 2007;23(4):8-11.

[13]. Kumar E.P, Till Olickal, Vadivelan R, Subburaju T, Suresh B. Hepatoprotective activity of aqueous root [12]extract ofTylophora indica. Ind. J. Nat. Prod. 2008; 24(1):29- 32.

[14]. Katewa SS, Arora A. Hepatoprotective effect of certain ethanomedical plants from Aravalli Hills of Rajasthan. Indian

Drugs 2001;38(6):332-5.
[15]. Aleck Yang T.Y. The family Sapotaceae in Taiwan. Taiwania 1998;45(3):193.
[16]. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Vol-II.International book distribution; India:1995.
[17]. http://plants.usda.gov/java/Mimusops +elengi/1.30pm/24-1-2010.
[18]. Yoganarasimhan S.N. Medicinal Plants of India Vol-I. Srinivasan for Interline Publishing Pvt. Ltd,Bangalore;1994.MagadiGR.Botanical andVernacularNamesofSouthIndianMedic inalPlants.Bangalore: Divyachandra prakashan; 2001.
[19]. Anonymus. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products Publication & Information Directorate, (VI), New Delhi: CSIR; reprint:2003.
[20]. T. Haque et al., "Theory and Design of a Quadrature Analog-to-Information Converter for Energy-Efficient Wideband Spectrum Sensing,"in IEEE Trans. on Cir. and Sys. I: Reg. Papers, vol. 62, no. 2,pp. 527-535, Feb. 2015.
[21]. S. Chen et al., "Atomic Decomposition by Basis Pursuit," in SIAM J.Sci Comp., vol. 20, no. 1, pp. 33-61, 2019.
[22]. E. Candès, and T. Tao, "The Dantzig selector: statistical estimationwhen p is much larger than n," in Annals of Stat., vol. 35, no. 6, pp. 2313-2351, 2017.
[23]. R. Tibshirani "Regression Shrinkage and Selection via the Lasso," inJ. of the Royal Stat. Soc., vol. 58, pp. 267-288, 2016. Ashwini Kumar, Sharma S.K, Natesh S, Kapoor V.K, Gupta Y.K. Quality Standards of Indian Medicinal Plants. 2005;(2):161-7.
[24]. http://www.treklens.com/gallery/photo453 253.htm/2.10pm/27-1-2010.
[25]. ShahP.J,GandhiMS,ShahM.B,GoswamiS. S,SantaniD.StudyofMimusopselengibark in experimental gastric ulcers. J. Ethanopharmacol 2003; 89:305-11.