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Research Article

Qualitative Pharmacognostical and Phytochemical Evaluation on *Moringa oleifera* Lam. Leaf

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Abstract

The plant *Moringa oleifera* Lam. is a small tree otherwise called as horseradish tree or drumstick tree which is one of the pan-tropical species. It is a most useful plant with highly nutritional value and medicinal properties. This current study is planning to evaluate the pharmacognostical and phytochemical properties of *Moringa oleifera* Lam leaves. Here the pharmacognostical parameter like Macroscopic, Microscopic nature like cellular structure of the leaf and leaf constant characters were evaluated. Preliminary phytochemical analysis was done by using standard procedure. Preliminary phytochemical screening of the extracts of the acetone, ethyl acetate, petroleum ether, chloroform, methanol and aqueous solvents give the positive report for the presence of alkaloid, carbohydrates, proteins tannins and phytosterol. The column chromatography is used to isolate the phytoconstituents in the methanol extract. A white crystalline compound is isolated in the ratio of petroleum ether: chloroform (60:40, 40:60, 20:80) and it was taken for the structural elucidation and subjected to UV-VIS,IR, NMR and Mass spectral studies. It shows the fraction contains pentadecan-1-ol. It may be a precursor or intermediate for the biogenesis for some other compound. Antimicrobial activity of methanol and aqueous extracts of the leaf was screened against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Aspergillus niger Candida albicans*. Both extracts shows antibacterial and fungal activity towards these organisms but it was more in alcohol extract. We finally conclude that this study provides Pharmacognostical, Physiochemical and Phytochemical details of the *Moringa oleifera* leaves which are useful for the researchers in laying down standardization and pharmacopoeia parameters.

Keywords: *Moringa oleifera;* Phytochemical; Antimicrobial; Standardization; Pharmacognostical

Abbreviations

UV: Ultra Violet; IR: Infrared; NMR: Nuclear Magnetic Resistance

Introduction

The plant *Moringa oleifera* Lam. is a monogeneric family, the *Moringaceae*. Itis called as "Miracle Tree" and the most widely cultivated species allover India and in tropical and subtropical regions [1]. It's the native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. All parts of the Moringa tree are edible and have long been consumed by humans [2]. Research on Moringa mainly pivoted around its leaves, stem bark, root bark, flowers, fruits and seeds because of their immense nutraceutical potential.

The leaves are rich in vitamin A and C, are considered useful in scurvy and respiratory ailments [3]. They are rich in protein, minerals, beta-carotene, thiamin, riboflavin, and other vitamins, particularly vitamins A and C. The Moringa seeds are relatively high yield of 36% Ben oil [4]. The oil contains: 65.7% Oleic acids; 9.3% Palmitic acid; 7.4% Stearic acid; and 8.6% Behenic acid. The seeds are good for hypertension, gout, asthma, cancer, and anti-aging. It is also having anti-arthritic activity of anti– inflammatory activity [5,6]. Oil extracted from Moringa flowers can also be helpful for arthritic pains and rheumatic and gouty joints [7-9]. Moringa flower is a rich reservoir of bioactive phytochemicals and crude flower extracts showed promising antibacterial, antifungal, anti-larval, antioxidant, anti-inflammatory and anticancer properties [10-12]. Its leaves are also used for hiccups, asthma, gout, backache, rheumatism, kidney stone and skin wounds and sores. The aqueous extract of root of this plant is having anti-inflammatory action and hepatoprotective action [13]. The leaf extract of Moringa is having therapeutic activity towards hyperlipidemia, hypocholesterolemic effect [14,15]. The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy leads to the discovery of newer and recent drugs for treating more diseases and for trading. The benefits towards the treatment and prevention of diseases towards various ailments of this plant are very interesting and motivated to precede this present work. The pharmacognostical, phytochemical, antimicrobial studies of this work has been used for documentation purpose.

Materials and Methods

Plant materials collection and authentication

The leaf of *Moringa oleifera* Lam is available locally and was collected in and around Coimbatore. The botanical identity has been authenticated by the Director, Botanical survey of India, Coimbatore, No: BSI/SRC/5/23/2012-13/Tech/496. The voucher specimen has been submitted and preserved in herbarium for future reference.

Processing of plant material

The plant material (leaf) was collected and shade dried at room temperature and was subjected to size reduction to get course powder of desired particle size. This powdered material was subjected to successive extraction. One kilogram powdered drug was extracted with methanol and water separately by cold maceration method for 7 days. Then the extracts were filtered and solvent were evaporated under reduced pressure in a rotary evaporator to get the dry extract. The yield of the dry extracts were calculated and stored in desiccators and used for further experiments.

Microscopical Evaluation of the plant Leaf

The required sample leaf was cut and removed from the plant and fixed in FAA (Farmalin-5 ml +Acetic acid -5 ml+ 70% Ethyl alcohol-90 ml). The standard procedure was followed as per the procedure given by Sass, 1940 [16].

Photographs of different magnifications were taken with NIKON Labphoto2 microscopic Unit. For normal observations bright field was used. For the study of Crystals, Starch grains and lignified cells, Polarized light was employed. Under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard Anatomy books [17,18].

Preliminary phytochemical identification

The plant material was extracted with different solvents separately prepared and subjected to preliminary phytochemical identification test to identify its chemical constituents by using the standard procedures [19,20].

Fluorescence analysis

The drug powder was treated with different chemical reagents, acids and alkalies. Then this solutions were make a spot on a TLC plate. The developed colours were observed under UV - Fluorescent light at 254 and 366 nm [21].

Column chromatography

Column chromatography was used to separate the compounds. For this purpose 10 gm of methanol extract of the plant was taken and placed in a column (column size 90 cm X 2.5 cm), Packed with Silica Gel (100 - 200 mesh) are the adsorbent used to complete separation of the component of the sample and various organic solvents in various proportions were used.

Silica gel was made into homogenous suspension by shaking with petroleum ether (first eluent). The bottom of the column was plugged with little cotton to prevent pass put and then the silica gel suspension was poured into the column, set aside for 10 minutes and used. Methanol extract of the sample was subjected to column chromatography over silica gel. The column was eluted with

solvents of increasing polarity. They were Petroleum ether, Chloroform, Ethyl acetate, Acetone, Ethanol. The column was run with organic solvents in various proportions to isolate compounds from the plant extract [22].

Spectral study

The isolated compound was examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis the sample was scanned in the wavelength ranging from 260 - 900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400 - 4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. NMR 30and MASS spectral studies also performed and recorded. Each and every analysis was repeated twice for the spectrum confirmation [23-25].

Evaluation of antimicrobial activity

The antimicrobial activity of the test sample (leaf) extracts were carried out by standard disc diffusion method (Kirby Bauer method). The bacterial strains used were *Staphylococcus aureus* (+ve) (NCIM 2079), *Bacillus subtilis* (+ve) (NCIM2063), *Pseudomonas aeruginosa* (-ve) (NCIM 2036), *Klebsiella aerogenes* (-ve) (NCIM 2098) and fungi *Candida albicans* (NCIM 3102) and *Aspergillus flavus* (NCIM105) were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on Nutrient agar and Sabourad dextrose agar medium for bacteria and fungi respectively. From the culture obtained, using sterilized Pasteur loop, one loop full each of the microorganisms were transferred into the test tubes containing sterile nutrient broth for screening studies. The pH of the above media were maintained at 7.2, it is then sterilized by autoclaving at 121°C at 15lbs pressure for 15 minutes, in which the nutrient broth was used for sub culturing and MHA (Mueller Hinton Media) media was used for screening studies [26,27].

Nutrient broth with standard modification was prepared and sterilized by autoclaving at 120°C (151b/in^2) about 30 ml of nutrient agar medium was transferred aseptically into every sterilized petriplates to get thickness of 5 to 6 nm. The plate were allowed to solidify and upturned to prevent the condensate declining on the agar surface. The plates were dried at 37°C sooner than organisms were inoculated in the plates prepared prior, by dipping sterilize swab in the previously standardized inoculums and spread the organism by shacking the swab all over the surface of the medium. The plates were left at room temperature. Reference standard disc (6 mm diameter) was used as positive antibacterial and antifungal (Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi) as control. Each extracts of leaf was reconstituted with solvents and tested at the concentration of 200 µg/ml, 400 µg/ml. The paper discs were impregnated appropriately labeled and evenly spaced sides over the inoculated plates. On incubation the bacteria grow on area of the plate excluding those approximately the inhibitory compound of the plant, which they are sensitive. In the duration of overnight the phytocompound present in the plant extract prevents the development of visible growth which indicates the extract is having antibacterial action. The inhibition was measuring the diameter of the inhibition zone after prior incubation and the experiment was executed two fold and the average determination was recorded. The effect produced by the sample was compared with the effect produced by the positive control (Reference Standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi). The antimicrobial action was evaluated by measuring the width of inhibition zone [28].

Results and Discussion

Phramacognostical studies

The leaves are bipinnate or more commonly tripinnate, up to 45 cm long, and are alternate and spirally arranged on the twigs. Pinnae and pinnules are opposite; leaflets are 1.2 to 2.0 cm long and 0.6 to 1.0 cm wide, the lateral leaflets elliptic, the terminal ones obovate; petioles of lateral leaflets are 1.5 to 2.5 mm long, those of terminal ones 3 to 6 mm long. The leaflets are finely hairy, green and almost hairless on the upper surface, paler and hairless beneath, with red-tinged midveins, with entire (not toothed) margins, and are rounded or blunt-pointed at the apex and short-pointed at the base. The twigs are finely hairy and green, becoming brown [29].

In transverse view the leaflet has thin lamina and fairly prominent spindle shaped midrib. The midrib is more convex on the abaxial side than the adaxial side. The midrib is 380 µm thick and 500 µm wide (Figure 1). The epidermal layer of the adaxial and abaxial sides are prominent thick walled. The adaxial epidermal cells are rectangular to squarish in outline. The abaxial epidermal cells are small circular with small tuber cut out growth on the surface. The ground tissue of the midrib includes thick walled, compa-

ct parenchyma cells. The vascular strand is tangentially stretched thick and collateral (Figure 3). It includes a few diffuse thick walled Xylem elements and small groups of phloem elements situated on the abaxial side of the vascular strand [30].

Microscopic observation revealed that the leaf provided with the anisocytic type of stomata. Stomata are present only in the lower surface (33.00 ± 1.00) of the leaf whereas, no stomata were present on the upper surface. The stomata measure about 28.75 ± 1.25µm in length and 22.00 ± 1.25µm in breadth. Transverse section of the leaf revealed that the epidermal cells consist of straight anticlinal walls (Figure 1). Beneath every upper epidermal cell there are about 3.26 ± 0.10 palisade parenchyma cells are present; they are very green and tightly packed. Unicellular trichomes are present on upper surface [31-33]. The leaf constant like stomatal number, stomatal index, vein islet number and termination, and palisade ratio of the *Moringa olifera* Lam. leaf was studied and recorded the data on given table 1.

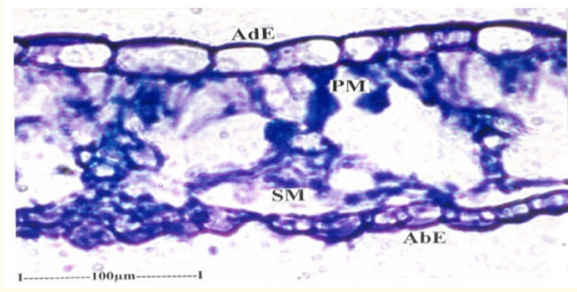


Figure 1: T.S of *Moringa oleifera* showing leaf lamina.

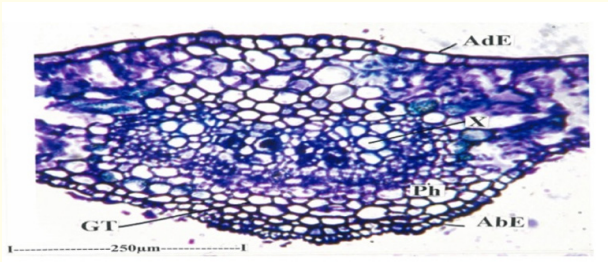


Figure 2: T.S. of *Moringa oleifera* Lam. leaf showing vascular bundles.

ADS- Adoxial side, ADE - Adoxial Epidermis, AbE- Abaxial Epidermis, GT- Glandular Trichome, MR- MidRib, LA- Lamina.

Parameters	Range	Mean ± SE
Palisade ratio	2.06 - 3.65	3.26 ± 0.10
Stomata number Upper surface	0	0
Stomata number lower surface	31.30 - 34.69	33.00 ± 1.00
Stomata index upper surface	0	0
Stomata index lower surface	13.95 - 16.56	15.25 ± 0.20
Vein islet number	26.47 - 33.52	30.00 ± 0.50
Vein termination number	32.64 - 43.75	38.20 ± 3.00
Epidermal cells Upper surface	152.87 - 180.32	166.60 ± 4.50
Epidermal cells lower surface	167.92 - 200.67	184.30 ± 2.50
Stomata length	27.43 - 30.06	28.75 ± 1.25
Stomata Breadth	20.02 - 23.97	22.00 ± 1.25

Table 1: Leaf constant of *Moringa oleifera* Lam.

Phytochemical studies

Preliminary phytochemical screening

The leaf extracts were separately prepared with different solvent system like acetone, ethyl acetate, petroleum ether, chloroform, methanol and water also subjected to chemical tests for the identification of its chemical constituents. The presence of different Phytoconstituents were identified and tabulated in table 2.

Fluorescent analysis

The leaf powder was treated with different reagents to find out the fluorescent capacity of colour development of drugs at 254 and 366 nm with different reagents and it was tabulated in table 3.

Spectral studies

A white crystalline compound was isolated from methanol extract of *Moringa olifera* in the fractions Petroleum ether : Chloroform in the ratio of (60:40), (40:60), and (20:80) ratio when applied in column chromatography and eluted with different solvents.

S.no	Chemical Test	Acetone	Ethyl acetate	Petroleum ether	Chloroform	Methanol	Water
(1)	Alkaloids	+	+	+	+	+	+
(2)	Carbohydrates	+	+	+	+	+	+
(3)	Proteins	+	+	+	+	+	+
(4)	Free Amino acids	-	-	-	-	-	+
(4)	Tannins and Phenolic substance	+	+	+	+	+	
(5)	Phytosterols	+	+	+	+	+	-
(6)	Flavanoids	+	+	+	+	+	+
(7)	Saponins	+	+	+	+	+	+

Table 2: Preliminary phytochemical screening.

S. No	Treatment	Fluorescence at	
		254 nm	366 nm
(1)	Powder as such	-----	----
(2)	Powder + Dilute Nitric acid	Light yellow	Yellow
(3)	Powder+ 10% Sodium hydroxide	Light orange	Orange
(4)	Powder + 1N hydrochloric acid	Yellow	Yellow
(5)	Powder + 50%Nitric acid	Orange	Orange
(6)	Powder + Acetic acid	Light brown	Yellowish Orange
(7)	Powder + Picric acid	D yellow	Yellowish Orange
(8)	Powder + 50% Ferric chloride	Orange	Orange
(9)	Powder + N/50 Iodine Solution	Dark brown	Brownish Red
(10)	Powder + 50% Sulphuric acid	Light blue	Blue
(11)	Powder + Ethanol	Light orange	Orange

Table 3: Fluorescent characters of *Moringa olifera* lam.

UV spectrum [34,35]

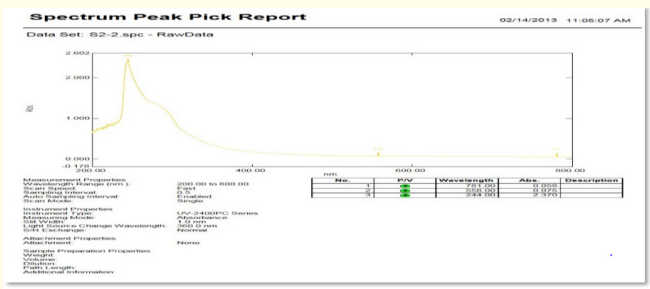


Figure 3: UV spectrum of isolated compound isolated *Moringa olifera* lam.

Infrared spectrum

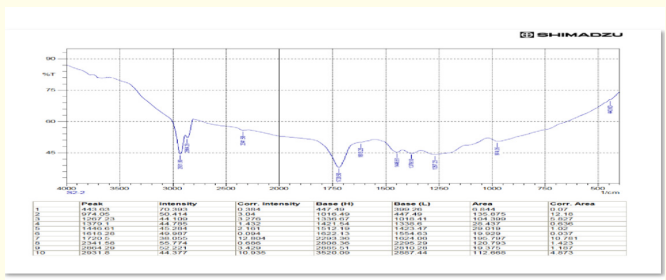


Figure 4: IR spectrum of compound from *Moringa oleifera lam.*

NMR spectrum

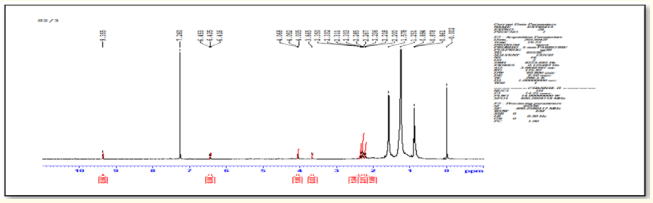


Figure 5: NMR spectrum of compound of from *Moringa oleifera* Lam.

Mass spectroscopy

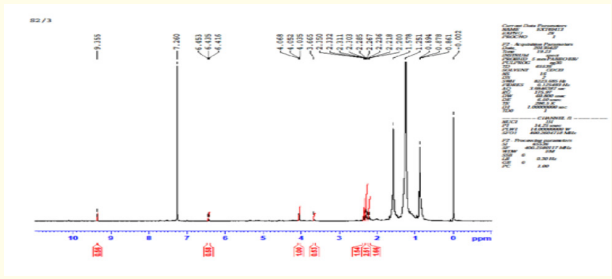
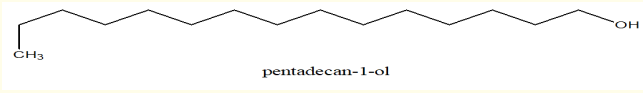


Figure 6: Mass spectrum of compound isolated from *Moringa olifera* lam.

Spectral description of the compound [36,37]

A compound (11.5 mg) was isolated from the methanolic extract of in the fractions of Petroleum ether: Chloroform (60:40), (40:60) and (20:80). It was White crystalline compound as per the spectral study report it has the singlet at δ 0.87 is owing to methyl group of protons. The broad singlet at δ 1.25 is owing to long chain methylene proton. The triplet at δ 4.05 is owing to -CH_2-OH group. The singlet at δ 2.30 is owing to β -CH_2 methylene group to –OH group. IR absorption band at 3317 cm^{-1} is owing to –OH group. Molecular formula CH_3-(CH_2)13-CH_2-OH. It may be a penta decanol [38-43].

Proposed structure of the compound



Figure

Antimicrobial studies

Methanol and water extract of the plant leaf was tested for their antibacterial and antifungal activity. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi). The inhibition of microbial growth was measuring the diameter of the inhibition zone after prior incubation and the experiment was done twice and the average determination was recorded and included [44]. The obtained results are tabulated in table 4.

The aqueous and methanol extract prepared from the selected plant was tested for their antibacterial and antifungal activity. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi). The inhibition of microbial growth was measuring the diameter of the inhibition zone after prior incubation and the experiment was done twice and the average determination was recorded and included Standard- Ciprofloxacin 5µg /disc for bacteria; Nystatin 100 units/disc for fungi [44,45]. The antibacterial and antifungal activity clearly shows that it is a dose dependent. The methanol extract shows better antibacterial activity than the aqueous extract. The extracts shows the activity towards the bacterial organisms in the given order like *Staphylococcus aureus>Pseudomonas aeruginosa > Bacillus subtilis > Klebsiella aerogenes*. In the dose 200 mg itself shows good activity related to that of the standard drug. The antibacterial activity is better in the dose of 400mg. The extract shows significant effect towards the fungal organisms like *Aspergillus niger > Candida albicans* in this order and also the activity increases with increasing the quantity. As per the literature different part of this selected plant is having more pharmacological activities anti-inflammatory, analgesic and antipyretic activities [46]. The leaf extracts with methanol and water are having antibacterial and antiviral activity. This study also reveals that the leaf of the selected plant is having antimicrobial activity.

S. No	Name of the Microorganisms	Zone of inhibition nm				Zone of inhibition nm			
		Water extract		Solvent control	Std	Alcohol Extract		Solvent control	Std
		200 µl	400 µl			200 µL	400µL		
1	*Staphylococcus aureus*	16	20	-	24	18	22	-	24
2	*Bacillus subtilis*	14	16	-	23	13	19	-	22
3.	*Klebsiella aerogenes*	08	12	-	22	12	18	-	23
4.	*Pseudomonas aeruginosa*	16	20	-	24	14	20		26
5.	*Aspergillus niger*	16	17	-	23	18	24	-	24
6.	*Candida albicans*	16	17	-	25	18	22	-	26

Table 4: The antimicrobial activity of the extracts.

Standard- Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 units/disc for fungi.

Conclusion

In this study the pharmacognostical parameters like morphological, microscopical and leaf constant were studied and documented. The Pharmacognostical study may be further used for documentation purpose. The preliminary phytochemical study confirms the presence of alkaloids, terpenoids, tannins, flavonoids and phytosterols in the different solvent extract. The methanol extract was subjected for the column chromatography, for the isolation of the active compound and one compound was isolated and its structural elucidation was done by UV, IR, NMR and MASS Spectral studies. The proposed structure of the isolated compound may be penta decanol. It may be the intermediate for the production of secondary metabolite or may be the precursor of the secondary metabolite molecule. The methanol and aqueous extracts shows antimicrobial activity but the methanol extract shows more significant activity than the aqueous extract.

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