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Studies on screening of phytochemicals with special reference to *Moringa oleifera*

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ABSTRACT:

The *Moringa oleifera* Lam. (Moringaceae) plant has excellent therapeutic and dietary value. It is indigenous to India but has since spread greatly throughout tropical and subtropical areas of the globe. Each component of this plant has unique biotechnological and pharmacological potential and is a good source of different nutrients. A comprehensive range of biological activities, including antioxidant, antihypertensive, tissue-protecting (for the kidneys, lungs, and heart), antiulcer, immunomodulatory, and analgesic effects, are present in *M. oleifera* leaf extract. Numerous secondary metabolites, including phenolics, polyphenols, flavonoids, and alkaloids, are present and are responsible for these effects. The aim of present study was toevaluate the characterization and screening of phytochemicals with special reference to *Moringa oleifera*.

Keywords: Characterization, Screening, Phytochemicals, *Moringa oleifera*

INTRODUCTION:

A highly valued plant, *Moringa oleifera*, is used in food, medicine, and industry. The plant is native to the Indian subcontinent and is primarily grown in tropical and subtropical climates worldwide. Since every part of the Moringa plant can be utilized for food or medicine, it is regarded as one of the most valuable trees in the world [1]. It contains nutrients with high nutritional value, including amino acids, minerals, vitamins, carbs, and organic acids. It is a good

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source of a variety of bioactive compounds. Some utilize it as cattle fodder, while others consume its flowers, leaves, and fresh pods as vegetables [2]. The most nutrient-dense food is made from Moringa leaves because they are high in antioxidants and other nutrients that people in underdeveloped nations frequently lack [3]. MO leaves have been used to cure a range of illnesses, including diabetes, typhoid, hypertension, and malaria. The pharmacological characteristics of MO leaves are due to their abundance of potent phytoconstituents. These pharmacological qualities have been validated by several in vitro and in vivo studies [4, 5].

MATERIALS AND METHODS:

Plant collection:

Different parts i.e. leaves, root, stem bark and root bark of Moringa oleifera were collected in the month of August and September from West Bengal, India. All the samples were properly labelled and collected in ice box from the fields. The botanical identity of plant was identified taxonomically and authenticated by the botanist.

Dryingandgrindingofsamples:

Different parts of Moringa were thoroughly washed with tap water and then with autoclaveddistilled water for about 2-3 minutes. Samples were dried under shade for 10-15 days. Driedparts of Moringa were grinded to make fine powder. Powder was sieved and stored in airsealed polythene bags at room temperature. The powder was used to make plant extractswhichisfurtherusedforqualitativeandquantitativeanalysisofphyto compounds.

Soxhletextractionofplantmaterial:

About 30 gms of plant powder was extracted using a Soxhlet apparatus with solvents (ethanol and petroleum ether) for 10-15 hrs or until the extracts was cleared at 5˚C less temperature than respective boiling point of the solvent. Dark gummy residue was obtained after evaporation of filtrate on water bath which wastransferredtoautoclavedfalcon tubesandwasstoredat-20˚C.Extractswerefurthersuspendedin DMSO(10%)givingthe concentration 50mg/mLforphytochemical andantimicrobialassay.

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PhytochemicalScreening:

Qualitativescreeningof phytochemicals:

The Phytochemical screening of ethanolic and petroleum ether extracts were carried out todetermine the presence of active secondary metabolites.The phytochemical research basedon ethnopharmacological information is generally considered an effective approach in thediscoveryofnewanti-infectiveagentsfrommedicinalplants.

The plant extracts were screened for the presence of reducing sugars, alkaloids, saponins,tannins,flavonoids,terpenoids,steroids andcardiacglycosidesaccording toestablishedprocedures.

TestsforAlkaloids:

- **Mayer'sTest:**Tothe1mLplantextract,fewdropsofMayer'sreagentwereadded.Creamco louredprecipitateindicated thepresenceofalkaloids.
- **Dragendroff'sTest:**Tothe1mLplantextract,2mLofDragendroff'sreagentwasadded. Appearanceoforangered precipitateconfirmedthepresenceofalkaloids.
- **Wagner'sTest:** To the 1mL plant extract, 1-2mL of Wagner'sreagent was added.Reddishbrownprecipitatesindicatedthepresenceofalkaloids.

TestsforSaponins:

- The1mLextractwasdilutedwith20mLofdistilledwaterandthen shakenfor15minutes.1cmlayeroffoamindicatedthepresenceofsaponins.

TestsforFlavonoids:

- **ShinodaTest:** Tothe2mLextract,fewmagnesiumturningswereaddedandconcentrated HCl was added dropwise to the test solution. Pink scarlet c hanges tobluecolourwhichconfirmedthepresenceofflavonoids.

TestsforTannins:

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- To the 1mL extract, 2-3 drops of $FeCl_3$ was added. The solution was allowed tostandforfewminutes.Theformationofgreenishblackcolourconfirmedthepresenceofta nnins.

TestsforTerpenoids:

- **Salkowaski Test:** 2 mL of plant extract was mixed with 2 mL of chloroform and 3mL of concentrated H_2SO_4 was carefully added toform alayer. A reddish browninterphasewasformedtoshowpositiveresultsforthepresenceofterpenoids.

TestsforPhenolics:

- **Ferric Chloride Test:** 3-4 drops of 5% $FeCl_3$ solution was added to 1 mL of plantextract and mixed thoroughly.The formation of bluish black colour indicated thepresenceofphenoliccompounds.

TestsforGlycosides:

- **Keller-Kilani Test:** 5mL of plant extract was treatedwith 2mL of glacial aceticacid containing 1drop of $FeCl_3$ solution. It was then underplayedwith 1mL ofconcentratedH_2SO_4.Abrown ringof theinterphaseindicatedthepresenceofcarbohydrates.

- **Fehling's Test:** To 1mL of plant extract, 1mL of distilled water was added. Then, 5-8 drops of Fehling solution was added at 65˚C. Brick red precipitates indicated thepresenceofreducingsugars.

TestforSteriods:

- 2mL of the acetic anhydride was added to 0.5 gm aqueous extract of sample with 2mLof H_2SO_4. The colour change from violet to blue or green indicated the presence ofsteroids.

Quantitativescreeningofphytochemicals:

After qualitative phytochemical estimation, quantitative phytochemical estimation was doneforthetotalphenolicandflavonoidscontent.

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Totalflavonoidsdetermination

The total flavonoids content was measured by a colorimetric assay. Each sample (0.5mL)was mixed with 2mL of distilled water and subsequently with 0.15 mL of a $NaNO_2$ solution(15%). After 6 minutes, 0.15 mL of $AlCl_3$ solution (10%) was added and allowed to stand for6 minutes, then 2 mL of NaOH solution (4%) was added to the mixture. Immediately, waterwas added to bring the final volume to 5 mL and the mixture was thoroughly mixed andallowed to stand for another 15 minutes. Rutin was taken as a standard for the calibrationcurve. Total flavonoid content of the different extracts was expressed as mg rutin equivalents(RU)pergramofsample(mg/g).

Totalphenolicsdetermination:

TotalphenoliccountofeachplantextractwasdeterminedwiththeFolin-Ciocalteumethod.2.5mLfresh Folin'sreagentwasmixedwith0.5mLofdifferentconcentrationsofextractsandimmediately2mLof Na_2CO_3(7.5%)wasaddedtoeachtubeandleftfor90minutesat30^{0}C.Theabsorbancewasmeasuredat765nm.Resultswereexpressedasgallicacidequivalents(mggallicacid/gdried extract).

MoringaleafextractusingTLC:

TLCAnalysis:

The plates used for TLC was prepared with silica gel G(2:1) and then plates were dried in oven at 105-110˚C for activation. After activationand drying ofplatesspottingofplateswasdone.Capillary was dipped in to sample solution and then smallamount of sample was drawn into the capillary. The end of the capillary was touchedto spot the TLC plate.The spot made by the capillary should not be more than 2mmindiameter.The development tank consists of three pieces; 500mlbeaker,glasscover,solventandplatesspottedwithsamples.The mobile phase was prepared and put into the TLC chamber.The TLC chamber was then allowed to saturate for 45 minutes. TLC plates wereplacedintotheglasschamberandallowedthemobilephasetomovethroughadsorbent phase up to a sufficient distance. The plates were activated for 1 hr at

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110˚Candallowedtocoolatroomtemperatureandhumidity.Thedevelopedchromatogramswereexaminedunder UVandvisiblelight.

Chromatogramanalysis:

The metabolites will be identified by taking RF (resolution front) value of spots and colordevelopedontheplates.Thesampleswillbeexaminedin UV/visiblelight.

$$\text{Resolutionfront(RF)} = \frac{\text{Distance travelled bythesample}}{\text{Distancetravelledbythesolvent}}$$

Isolationandcharacterization:

Isolationandpurificationofactivecompound:

TLC plates were developed and required fraction was scrapped and collected in eppendorfftube. Mixture was dissolved in HPLCgrademethanol and by shaking. The sample wascentrifuged at 5000 rpm for 10 minutes. Supernatant was dried under reduced pressure.FiltratewasdissolvedinHPLCgrademethanolforfurtheranalysis.

Antimicrobialdeterminationofactivecompound:

The isolated and purified active compound i.e., was tested for its antimicrobial activity againstgram-positive andgram-negativebacteriai.e.,*Staphylococcusaureus*and*Escherichiacoli.*

Agarwelldiffusionmethodforantibacterialassay:

Nutrient broth (5 mL) was inoculated with selected strains of bacteria and then incubated at37˚C overnight. Nutrient agar plates were prepared and checked for sterility. Wells wereprepared on the surface of agar with the help of sterile micro-tips. 100 µl of active compound(10 mg/mL) was added into the wells of the plates. Antibiotic ampicillin (10 mg/mL) andDMSO (10%) were taken as positive and negative control, respectively. The plates were thenincubated in upright position at 37˚C for 24 hrs. The plates were examined for the zone ofinhibitionwithzonemeasuringscale(Himedia).

RESULTS AND DISCUSSION:

Different partsof *Moringaoleifera*(MO)*viz.*leaves,stem bark,root

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barkandrootswerecollected as discussed in previous chapter.Different parts of Moringa plant were brought to lab in air tight bag, dried and stored in a safeplaceforfurtheruse.Storedsampleswerefurtherusedforqualitative,quantitativeandantimicrobial analysis. Different clinical strains of human pathogenic bacteria *viz. Escherichiacoli*, *Staphylococcus aureus*, Methicillin-resistant *S. aureus* (MRSA) and *Salmonella typhi* werecollected.

Qualitativeestimationofphytoconstituentsinethanolicandpetroleumetherextractsof *M.oleifera*:

The Phytochemical screening was carriedout to determinethe presence of active secondarymetabolites. Phytochemical screening of ethanolic and petroleum ether extracts were carried outfor the detection of phytoconstituents. The extracts were subjected to phytochemical screeningassays for alkaloids, flavonoids, phenolics, tannins, saponins,terpenoids and glycosides. Thescreening of phytoconstituents revealed the presence of alkaloids, phenolics, tannins, terpenoidsand saponins in ethanolic leaf and stem bark extracts of *M. oleifera*.Secondarymetabolites like alkaloids, phenolics and saponins were also found in root bark and root extractsof*M. oleifera*(Table1).

Table1:Qualitativephytochemicalanalysisofethanolicextractsofdifferentpartsof*M.oleifera*

S.No.	Phytoconsituents	Phytochemical Tests	Moringa Leaves	Moringa stembark	Moringa rootbark	Moringa roots
1.	Alkaloids	DragendroffTest	+	++	+	+
2.	Phenolics	Ferricchloride Test	++	++	+	+
3.	Tannins	GelatinTest	+	-	-	-
4.	Flavanoids	LeadacetateTest	++	+	-	-
5.	Carbohydrates	Barford'sTest	-	-	-	-
6.	Saponins	FoamTest	++	+	+	+
7.	Terpenoids	Salkowski'sTest	+	+	-	-
8.	Glycosides	Borntrager'sTest	-	-	-	-

("++"sign indicates the presence of good quantity of metabolites; "+"sign indicates the presenceofpoorquantityofmetaboliteswhereas"-

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"signshowedtheabsenceofphytocompounds)

The petroleum ether extracts (leaf and stem bark) of *M. oleifera*showed the presence of verylittleamountofalkaloids,phenolsandflavonoidsbutterpenoids,saponins,tanninsandglycosides werefound absent.Also, the phytochemical screening revealedthepresence of very little amount of alkaloids and phenols in root and root bark extracts of *M.oleifera*.

Table 2: Qualitative phytochemical analysis of petroleum ether extracts of different parts of *M.oleifera*

S. No.	Phytoconsituents	Phytochemical Tests	Moringa Leaves	Moringa stembark	Moringa rootbark	Moringa roots
1.	Alkaloids	Dragendorff'sTest	+	+	+	+
2.	Phenolics	Ferric chlorideTest	+	+	+	+
3.	Tannins	GelatinTest	-	-	-	-
4.	Flavonoids	LeadacetateTest	+	+	-	-
5.	Carbohydrates	Barford'sTest	-	-	-	-
6.	Saponins	FoamTest	-	-	-	-
7.	Terpenoids	Salkowski'sTest	-	-	-	-
8.	Glycosides	Borntrager'sTest	-	-	-	-

("++"sign indicates the presence of good quantity of metabolites; **"+"**sign indicates the presenceofpoorquantityofmetaboliteswhereas**"-"**signshowedtheabsenceofphytocompounds)

Ithasbeen reportedearlier that themethanol andethanol extractsof leaf of MOcontainsphenols, flavonoids, tannin, Saponin, alkaloids, glycosides and triterpenoids. Chloroform extractcontains less amount of flavonoids, tannin, triterpenoids and alkaloids. Pet-ether extract of leaf of*Moringa* contains phenols, flavonoids, tannin, alkaloids and glycosides.

Quantitative estimation of total phenolics and flavonoids in ethanolic and petroleumetherextractsof*M. oleifera:*

Qualitative phytochemical screening showed the presence of various secondary metabolites suchas phenolics and flavonoids in ethanolic and petroleum ether extracts of *M. oleifera*.

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Therefore,all the samples were further analyzed for the quantification of total phenolics and flavonoids byspectrophotometricanalysis.

Quantificationoftotalflavonoidcontent:

Totalflavonoid content wasmeasured by using aluminum chloridemethod and values were expressed in terms of RE. Significant variation in flavonoidcontent was observedin ethanolic andpetroleum ether extracts of *M.oleifera*. In ethanolicextracts, Moringa leaves showed more flavonoid content (mean=962.5±0.92 mg/g RE), followedby root bark (mean=386.74±2.3 mg/g RE), root (mean=385.41±3.01 mg/g RE) and stem bark(mean=337.31±0.7mg/g RE).In caseof petroleum etherextracts,Moringaleavesshowedhighestflavonoid content (mean=483.9±1.1mg/g RE),followedby root(mean=161.74±2.9mg/g RE), root bark (mean=119.31±1.2 mg/g RE) and stem bark (mean=114.01±1.9 mg/g RE).There is significant variation of flavonoid content in Moringa lea ves when compared with other partsof*M.oleifera*.However,Moringaleavesshowedasignificantlyhigheramountofflavonoidcontent.

Quantificationoftotalphenoliccontent:

Total phenolics were quantified by using FCmethod and thevalues were expressed as GAE. Significant variation in phenolic content was observed in theethanolic and petroleum extract of *M. oleifera*. The results indicated that the variation of totalphenolics existed among ethanolic and petroleum ether extracts. In ethanolic extracts, Moringaleavesshowedmoretotalphenoliccontent(mean=203.02±0.55mg/gGAE),followedbyroot (mean=80.23±0.1mg/gGAE),stembark(mean=71.68±0.18mg/gGAE)androotbark(mean=59.15±0.1 mg/g GAE). In case of petroleum ether extracts, samples from Kangra showedhighesttotalphenoliccontent(mean=165.14±0.7mg/gGAE),followedbystembark(mean=72.76±1.3mg/gGAE),rootbark(mean=60.04±0.25mg/gGAE)androot(mean=52.03±0.12mg/gGAE). However,ethanolicextractofMoringaleavesshowedasignificantly higheramountofphenoliccontentascompared to otherparts.

SecondarymetaboliteprofilingofleavesofMoringa

It was concluded from the present study that the Moringa leaves contains a variety of secondarymetabolitesincomparison tootherparts.Ethanolicextractof

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Moringaleaveswasfurtheranalyzedfortheidentification andcharacterizationofactivemolecules.

TLCanalysisofleafethanolicextractof*M.oleifera*:

Ethanolic leaf extract (1mg/ml) was dissolved and subjected for TLC analysis. TLC study wascarried out on silicagel aluminum plate. The solvent system usedfor TLC was chloroform:acetic acid: methanol: water (4:5:1:2). TLC characterization of ethanolic extract showedfourspots with Rf values ranging from 0.11- 0.51 in visible light. Itisevidentthatethanolicextractof*M. oleifera* showed 4 spots i.e. L-0, L-1, L-2 and L-3 having Rf values 0.11, 0.25, 0.30 and 0.51,respectively in the chromatogram observed in visible light. Compound having Rf values of 0.51wasmostprominentyellowcolorspot.

Antibacterialactivityofdifferentaccessionsof*M.oleifera*leafethanolicextract:

Differentaccessionsofleafextractshowedasignificantinhibitionofgrowthagainst*E.coli*and *S.aureus*.ItisevidentthatL-3accessionshowedthemaximumantibacterialactivitywiththezoneofinhibitionmeasuring9mmindiameteragainst*E.coli*and *S.aureus*.Theleastactivitywiththezoneofinhibitionmeasuring7mmwasfoundinL-1andL-0accessionagainst*E.coli*and *S.aureus*,respectively.

Table3:Antibacterialactivityofdifferentleafaccessionsagainst*E.coli*and*S.aureus*

BacterialStrains	L-0	L-1	L-2	L-3	Positive Control	Negative Control
E.coli	8mm	7mm	8mm	9mm	12mm	---
S.aureus	7mm	8mm	8mm	9mm	18mm	---

All the leaf accessions viz. L-0, L-1, L-2 and L-3 are active against gram negative and grampositive bacteria. Out of these, L-3 leaf accession showed maximum antibacterial activity againstboth*E.coli*and*S. aureus.*

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CONCLUSION:

A member of the family Moringaceae, Moringa oleifera (MO) is a plant that thrives in tropical and subtropical climates and is commonly cultivated in India. Traditional medicine makes use of M. oleifera leaves, roots, seeds, and flowers, while human nourishment is made from the immature pods, leaves, and seeds of the plant. Different Moringa tree sections have been utilized successfully against a variety of illnesses around the world. One of India's most valued medicinal trees, M. oleifera, has been utilized to treat a wide range of illnesses. In the current study, Moringa leaves were examined for the content of several nutrients as well as their phytochemical screening and antibacterial capability.

Moringa oleifera (MO) many components have shown impressive efficacy against all pathogenic strains. Samples were found to include secondary metabolites like alkaloids, flavonoids, phenolics, saponins, and tannins after phytochemical analysis. Ethanolic extracts have shown substantial antibacterial efficacy against all chosen pathogens in comparison to petroleum ether extracts. A phenolic antioxidant with an R_f value of 0.51 was extracted from *M. oleifera* leaves.*M.oleifera* is a fantastic plant to use for enhancing community health and may offer remarkable capabilities to create pharmaceutical goods. It is a tree with several uses that may either be processed into another form for use in different locations or consumed as food. The leaves of the Moringa oleifera plant or other plant parts have a wide range of antibacterial activity against specific diseases. Various minerals, antioxidants, and other bioactive phytoconstituents are abundant in Moringa leaves.In the experiment, the current results showed satisfactory results.

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