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PHYTOCHEMICAL SCREENING OF PHYLA NODIFLORA: A REVIEW

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Abstract: The aim of present study is to identify different chemical constituents. To study different activities of plant. To fabricate different types of formulation. Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Phytoconstituentsindividually or in the combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the importantphytochemicals with diverse biological activities. The pharmacological activity of a plantcan be predicted by the identification of the phytochemicals. Currently, phytochemicals are determined by various modern techniques, but the conventional qualitative tests are still popular for the preliminaryphytochemical screening of plants.

Index Terms: Phylanodiflora, Herbal plant, Phytochemical screening, Chemical Constituents

I. Introduction

The genus Lippia belongs to the family Verbenaceae. It is a large genus of shrubs or under shrubs, rarely herbs, distributed chiefly in tropical and subtropical America. A few species are found in the tropics of the old world. Five species occur in India of which one is ornamental. Lippia nodiflora (Linn.) Rich syn. Phyla nodiflora (Linn.) Greene, is a creeping perennial herb, stems rooting at the nodes and is much branched.[2] It is known for its rampant growth, growing in upper Gangetic plains,Ceylon, all tropical, warm temperate and the Mediterranean regions. L nodiflora characteristically grows in maritime areas or near rivers, showing a strong preference for wet grassy places. In the present mini review article multiple pharmacological activity of the plant and the phyto-chemical survey of other species of genus Lippia is presented here.[3] Duration: Perennial Nativity Non-native Lifeform: Forb/Herb General: Creeping, mat- forming perennial herb, 5-15 cm tall; stems decumbent and trailing along the ground, to 90 cm long, rooting at the nodes. Leaves: Opposite along the stems; blades spatulate to obovate, mostly about 2 cm long and 1 cm wide, thick-textured, sharply and regulary serrate near the apex and wedge-shaped at the base, usually tapering into a short petiole; leaf surfaces glabrous or strigollose-puberulent. Flowers: Tiny but attractive and purple, tightly clustered in dense globose

to cylindric spikes, 2 cm tall, spikes located at the tips of ascending axillary peduncles which are longer than the leaves; eachflower subtended by a cuneate-obovate bractlet, corolla bell-shaped, 5-lobed, and obscurely 2- lipped, 2-3 mm long, slightly longer than the subtending bractlet, purple to rose or occasionally white,often with a yellow center. Fruits: Nutlets 2 per flower, enclosed in the persistent calyx[4]. Ecology: Found in moist, typically disturbed soils, from 3,000-6,500 ft, flowers March- September. Distribution: Native to S. Amer; introduced to w N. Amer. from MO and CO to s.[5] It is often grown as an ornamental plant for ground cover, and is often present in yards or disturbed areas as a lawn weed. The inflorescence consists of a purple centre encircled by small white-to- pinkflowers. The flower takes on a match-like look, which is why the plant is sometimes called matchweed.[6] It is similar to the related species Phyla lanceolata, but differs in having much shorterleaves that are often blunt and much more rounded. Both species are common as weeds and in the ornamental environment. Habit: A small, perennial herb with stems creeping and rooting at nodes, more or less square and hairy.[7]

Leaves: Small, opposite, spathulate, base cuneate, apex rounded, upper part deeply serrate,hairy.

- **Inflorescence:** Axillary globose heads which later on elongate and become spicate.
- **Flowers:** Closed, bracteates, bracts elliptic or obovate.
- Calyx deeply bilabiate, compressed, hairy. Corolla white pale-blue, bilabiate, upper lip erect

  bifid, the lower 3-lobed, the central lobe the largest. Stamens 4, didynamous, included.Ovary 2-celled, ovules 2, stigma oblique. Fruits : Drupe dry, splitting into 2, 1-seeded

  pyrenes.
- **Flowering and Fruiting Time:** Throughout the year.
- **Significance:** Generally found near moist places and river beds.

Plant Phylanodiflora

MATERIAL AND METHOD

Herbal Drug and Extraction Process

Herbal Drug

- **Collection of the plant material**

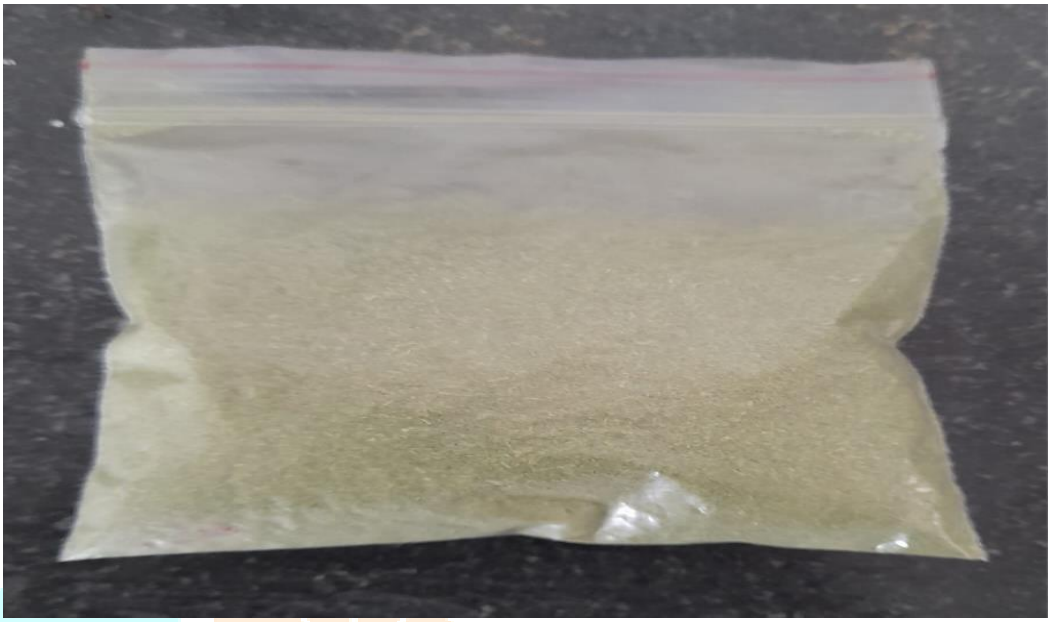
Phyla nodiflora, medicinal plant was collected from the side area of Bhima River.

- **Drying Method**

The whole plant of phyla nodiflora were collected and cleaned properly and then shadedried at room temperature for 15 days.

- **Grinding process**

The plant material was crushed well into fine powder using an electronic grinder.



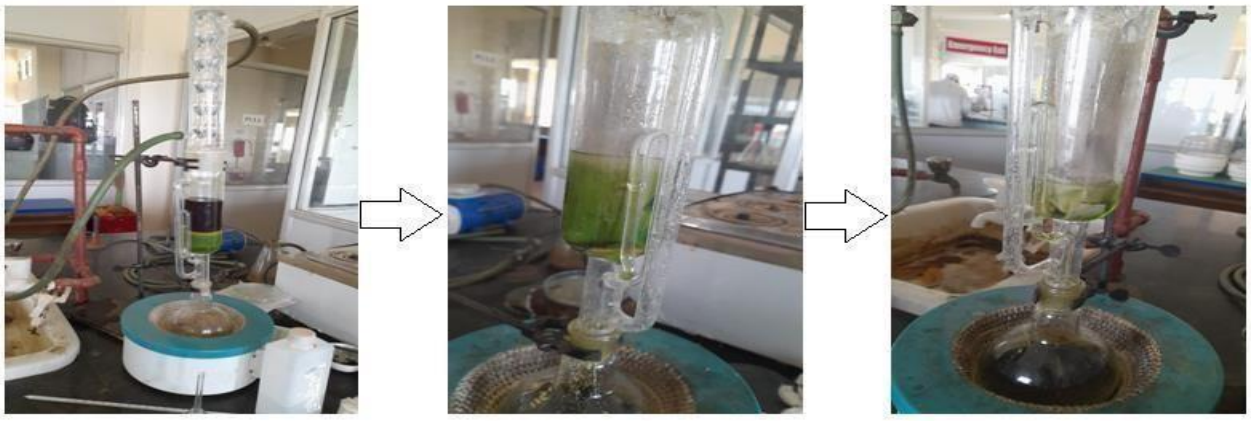
- **Sieving Process**

By using Sieve number 10, we Sieve the herbal drug and kept into air tight polythene bagsand stored at room temperature.

Extraction Process

The herbal powder of Phyla Nodiflora plant was extracted in Methanol in soxhlet apparatus for 3days.

Process



Take 20 gm of Herbal drug powder.

↓

Take 500 ml of Methanol as a solvent.

↓

By using the Soxhlet apparatus, start the extraction process.

↓

Keep the extraction upto deep green colour to colourless liquid.

↓

For occurance of deep colour to colourless liquid it take time 70-80 hours.

↓

Product is collected.

↓

Separate the methanol from the product by using Distillation method.

↓

Extract is collected in the Petri Dish.

↓

Dried the extract.

EXPERIMENTAL WORK

Chemicals & Phytochemical Screening Test

Chemicals and reagents

All chemicals used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy- 2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), Ascorbic acid, 2,4,6-tri(2-pyridyl)-s- triazine (TPTZ), ABTSradical dot+ [(2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], quercetin dehydrate, gallic acid, anhydrous sodium carbonate (Na2CO3), aluminum tri chloride, potassium acetate, sodium acetate, ferric chloride hexahydrate (FeCl3.6H2O),Folin–Ciocalteu reagent, Dragendorff's reagent, mercuric chloride, potassium iodide, iodine were purchased from Sigma–Aldrich. Ethanol, methanol, hydrochloric acid (HCl), sulfuric acid (H2SO4),chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH) were purchased from Merck and potassium peroxodisulfate from Fluka. All chemicals and reagents wereused without further purification.[16]

Phytochemical screening

The crude methanolic extracts of bark and leaves were tested for the presence of alkaloids, steroids, tannins, saponins and glycosides. The qualitative results are expressed as (+) for the presence and (−)for the absence of phytochemicals. Phytochemicals (Greek: phyton = plant) are chemical compoundsnaturally present in the plants attributing to positive or negative health effects. Medicinal plants usedin different diseases and ailments are the richest bio reservoirs of various phytochemicals. The medicinal properties of the plants are determined by the phytochemical constituents. Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed in various parts of the plants 31 Nature is a unique source of structures of high phytochemical diversity representing phenolics (45%). terpenoids and steroids (27%) and alkaloids (18%) as major groups of phytochemicals. Although, these compoundsseem to be non-essential to the plant producing them, they play a vital role in survival by mediation of ecological interactions with competitors, protect them from diseases, pollution, stress, UV rays and also contribute for colour, aroma and flavour with respect to the plant. The metabolites producedby the plants to protect themselves against biotic and abiotic stresses have turned into medicines thatpeople can use to treat various diseases.[20] Phytochemicals can be separated from the plant materialby various extraction techniques. The most commonly used conventional methods include maceration, percolation, infusion, digestion, decoction, hot continuous extraction (Soxhlet extraction) etc., recently, eco- friendly techniques such as Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extractions (SFE) and AcceleratedSolvent Extraction (ASE) have also been introduced. Different types of solvents viz. water, ethanol,methanol, acetone, ether, benzene, chloroform etc. are used in the extraction process 12 Extraction of phytochemicals from the plant materials is affected by pre-extraction factors (plant part used, its origin and particle size, moisture content, method of drying, degree of processing etc.) and extraction-related factors (extraction method adopted, solvent chosen, solvent to sample ratio, pH and temperature of the solvent, and length of extraction).

II. PHARMACOLOGICAL EFFECTS

Pharmacological effects

- Diuretic, antihyperuricemic and antiurolithiatic effects.

Two phenylethanoid glycosides, arenarioside and verbascoside, and three flavonoids, 6- hydroxyluteolin. 6-hydroxyluteolin-7-O-glycoside and nodifloretin were isolated from Lippia nodiflora methanolic extract. The isolated compounds inhibited xanthine oxidase activity, with ICs values between 7.52 +0.01 and 130.00 = 2.25 μM) The antihyperuricemic effects of the Lippia nodiflora methanol extract, fractions, and chemical constituents and their mechanism of action wereinvestigated in potassium oxonate- and hypoxanthine-induced hyperuricemic rats.[22] Oraladministration of methanol extract showed a dose- dependent reduction of the serum uric acid level of hyperuricemic rats. Bioactivity-guided purification of the most antihyperuricemic fraction led to isolation of two phenylethanoid glycosides, arenarioside and verbascoside and three flavonoids, 6- hydroxyluteolin, 6-hydroxyluteolin-7-O-glycoside, and nodifloretin. The serum uric acid reduction effect of the isolated compounds ranged from 22.08% to 66.94%. Flavonoids mainly accountable forthe uric acid lowering effect of Lippia sodiflora through the inhibition of XOD/XDH activities and partially by uricosuric effect.[23] Lippia nodiflora ethanol extract exhibited antiurolithiatic effect. Theextract significantly prevented the formation of the calcium oxonate stone, dissolved the pre- formedcalcium oxolate stone in the kidney of rats induced by gentamycin and calculi producing diet due toits ability to increase the urinary pH and excretion of the calcium and oxolate, and alsoto reduce the urine super-saturation with the calculogenic ions.[24] The diuretic activity of petroleum ether, chloroform, methanol and aqueous extract of the dried aerial parts of Phyla nodiflora (250 and 500 mg/ kg ip), was studied in rats. The volumes of urine, urinary concentration of sodium and potassiumions were estimated. The results showed that methanol and aqueous extract at 500 mg/kgpossessed significant ($p<0.05$) increase in the urine volume and electrolyte excretion ($p<0.001$) when comparedto control. The diuretic potential of methanol extract of Lippia nodiflora (200 and 400 mg/kg bw, ip) was studied. in hydrated rats and their urine output was monitored over a period of 5 and 24 h after drug administration[25]. The extract at doses of 200 and 400 mg kg caused significant increase in volume of urine with increase in Na". Ca and Cl excretion accompanied by the excretion of K' in dose dependent manner.

- Hepatoprotective effects.

The hepatoprotective activity of ethanolic leaf extract of Lippia nodiflora (100 and 200 mg/kg bw/day, orally, for 15 days) was evaluated against CCI, induced hepatic damage in rats. Both doses restored the elevated levels of total bilirubin, aspartate transaminase, alanine transaminase and alkaline phosphatase in CCI, intoxicated rats to normal levels. The hepatoprotective activity was dosedependent The hepatoprotective and antioxidant activity of methanol extract of Lippia nodiflora (200and 400mg/kg, orally, for 7) was evaluated in acute experimental liver injury induced by paracetamol. The methanol extract possessed significant ($p<0.001$) hepatoprotective effect, it decreased the activity of SGOT. SGPT. ALP, decreased bilirubin and lipid peroxidation, while it significantly ($p<0.001$) increased the levels of total proteins, glutathione, catalase and superoxide dismutase in a dose dependent manner. The hepatoprotective effect of crude flavonoid fraction (25, 50 mg/kg for 21 days) of aerial parts of Lippia nodiflora was evaluated in ethanol induced oxidative stress in liver in rats.[26] The crude flavonoid fraction showed significant ($p<0.05$)protective effect by decreasing the elevated liver marker enzymes, total bilirubin, lipid peroxidationmarker and ameliorated the diminished serum total protein as well as antioxidant levels in a dose dependent manner The hepatoprotective effect of methanolic extracts of Lippia nodiflora was studiedin HepG2 cells. The extract reduced reactive oxygen species production against LPS induced toxicityon HepG2 cells, and decreased the apoptotic gene expression and protect the liver cells against toxicity.

- Antimicrobial activity

The antibacterial activity of the stems and leaves extracts of phyla modiflora was studied against Staphylococcus aureus, Micrococcus luteus, Proteus micrococcus luteus and Shigella boydii. The antifungal activity of the extracts was studies against Aspergillus niger and Candida albicans. The ethanol extracts showed significant antibacterial and antifungal activity, they showed zone of inhibition of 3 to 12 mm. The zone of inhibition produced by petroleum ether fraction was 6 to 10 mm against all tested organisms. The zone of inhibition produced by aqueous fraction was 10 to 12 mm against all tested organisms except, Staphylococcus aureus and Micrococcus luteus. Theantimicrobial activity of hexane, chloroform, ethyl acetate and methanol extracts of aerial parts of Phyla nodiflora was evaluated against human pathogenic bacteria [Staphylococcus aureus. Staphylococcus epidermidis, Klebsiella pneumoniae, Enterococcus faecalis, Shigella flexneri, Methicillin resistant Staphylococcus aureus (+) clinical isolate, Micrococcus luteus, Salmonella paratyphi B (-) clinical isolate, Salmonella typhi. Pseudomonas aeruginosa, Escherichia coli, Vibrio cholerae, and fungi [Candida albicans, Candida krussic, Candida tropicalis, Trichophyton mentagrophytes, Microsporum gypseum and Malassezia pachydermatis]. All the extracts possessed inhibitory effects on both bacteria and fungi. Ethyl acetate extract showed the most potent antibacterial and antifungalactivity. The MIC of the ethyl acetate extract of the aerial parts ranged between 0.078 and 0.312mg/ml. Similarly for methanol extract of the roots, the MIC ranged between0.625 and 2.5 mg/ml.[28] The ethyl acetate extract inhibited growth of Staphylococcus aureus, Salmonella typhi and Malassezia pachydermatis at 0.312 mg/ml and Klebsiella pneumoniae at 0.078mg/ml. Methanol extract of the roots inhibited the growth of Staphylococcus aureus at 0.625 mg/mland the growth of K. pneumoniae. Salmonella typhi and M. pachydermatis at 2.5 mg/ml. The minimum bactericidal concentration (MBC) of ethyl acetate extract of the aerial parts against Staphylococcus aureus, Salmonella typhi and Malassezia pachydermatis was at 0.625 mg/ml and Klebsiella pneumoniae was at 0.156 mg/ml. The MBC value of methanol extract of the roots rangedbetween 1.25 mg/ml to 5 mg/ml. The methanolic extract from the leaves and flowers of Lippia nodiflora showed concentrations- dependent antimicrobial activity against Bacillus subtilis, Bacilluscereus. Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiella oxytoca and Esherichia coli. They also possessed antifungal activity against Aspergillus niger and Candida albicans. Bacteria were more sensitive than fungi, and Gram positivebacteria were more sensitive than Gram negative ones.[29] Aqueous extract of Lippia nodiflora Concentration dependent antibacterial activity against Esherichia coli but showed no activity e against Staphylococcus aureus and Pseudomonas aeruginosa. Ethanolic extract possessed antibacterial activityagainst Gram positive (Staphylococcus aureus) and Gram negative (Esherichiacoli) but not effective against Pseudomonas aeruginosa The methanolic extract of the whole Lippia nodiflora showed antibacterial activity against.[30] Pseudomonas aueroginosa, Escherichia coli and Staphylococcus aureuswith inhibition zone of 15, 8 and 7 mm, respectively The antimicrobial activity of the extracts of Lippianodiflora was tested against E. coli, Salmonella typhi, P. alcaligens, Proteus mirabilis and E aerogenes.All extracts showed concentration dependent antimicrobial activity against all the tested bacteria. Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus aureus (MRSA) and Bacillus subtillus. Ethyl acetate and chloroform fractions showed excellent activity against Bacillussubtilis. Staphylococcus epidermidis and Staphylococcus aureus, Then-hexane and n-butanol fractions of plant were active against Escherichia coli and Pseudomonas aeruginosa, ethyl acetate, chloroform, n-butanol and n-hexane fractions showed promising activity against Salmonella and Staphylococcus aureus (MRSA), almost all fractions active against Staphylococcus aureus. The methanolic extract from Lippia nodiflora showed antimicrobial activities against Escherichia coli, Proteus vulgaris, Klebsiella pneumonia.[31] Bacillus cereus, Bacillus subtilis, Staphylococcus aureus. Pseudomonas aeruginosa and B. clausit as well as Aspergillus niger and Candida albicans. Increasing concentrations of extracts increased the antimicrobial activities against allthe tessted microorganisms. Bacteria were more sensitive than fungi. In both Gram negative and Gram positive bacteria tested, Klebsiella pneumonia, Pseudomonas aeruginosa and Bacillus subtilis showed more sensitivity than the other tested species.[32] Thirteen mm zone of inhibition observed against Klebsiella pneumonia, Bacillus cereus and Bacillus subtilis, 12 mm against Escherichia coliand Proteusvulgaris and 11 and 10 mm against Pseudomonas aueroginosa and Staphylococcus aureus respectivelyin 250 µg/dise methanolic extract of Lippia nodiflora. Methanolic extract of Lippia nodiflora in 500 µg/disc showed the highest inhibition zone against P. vulgaris, K. pneumonia, B. cereus and B. subtilisand lowest inhibition zone against B. clausi. Fungi exhibited the same zone of inhibition

(11 at 250 µgper dise) for both Aspergillus niger and Candida albicans.At 500 g per disc, highest zone of inhibition (14 mm) occurred against A. niger and lowest zone of inhibition (11 mm) occurred against Calbicans. The antimicrobial activity of the methanol extract of whole Lippia nodiflora was studied against Staphylococcus aureus, Staphylococcus epidermidis. Proteus vulgaris, Escherichia coli, Salmonella paratyphi A, Salmonella paratyphi B (clinical isolate), Klebsiella pneumonia, Salmonella typhimurium, Candida albicans and Cryptococcus neoformans (Clinical isolate).[33] The methanol extract of Lippia nodiflora possessed antimicrobial activity against Staphylococcus epidermidis, Staphylococcus aureus. Proteus vulgaris, Salmonella paratyphi A, Escherichia coli and Salmonella paratyphi B, at concentrations below 500 ug/ml Klebsiella pneumonia and Salmonella typhimurium did not show response, while, the extract exerted antifungal activity against Candida albicans and Cryptococcus neoformans at 400 and 500µg/ml. Five medicinal plants (Phyla nodiflora, Lawsonia inermis, Cassiafistula, Vernonia cinerea and Aristolochia bracteolate) were tested as antifungal therapyagainst fungal pathogen isolated from infected nail (Candida sp.).[34] Ethanol extracts of Phyla modiflora leaves exhibited complete inhibitory effect against the tested nail fungus, while, aqueous extract showed 75% inhibition after 48 hrs. The crude extracts of Lippia nodiflora were tested for antifungal effects against Aspergillus niger, A. flavus, Paecilomyces varioti, Microsporu gypseum and Trichophyton rubrum. All crude extracts including ethanol, methanol, ethyl acetate, chloroform and aqueous extracts showed high activity against the tested microorganisms. Ethanol and aqueous extracts appeared to be the most effective antifungal agents compared to methanol, chloroform and ethyl acetate).

III. Summary

Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Phytoconstituentsindividually or in the combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the importantphytochemicals with diverse biological activities. The pharmacological activity of a plantcan be predicted by the identification of the phytochemicals. Currently, phytochemicals are determined by various modern techniques, but the conventional qualitative tests are still popular forthe preliminaryphytochemical screening of plants.

IV. Conclusion

Phytochemical screening of the whole plant extracts of Phyla nodiflora indicates the presence of Alkaloids, Diterpenes, Saponins, Phytosterols, Tannins, Flavonoids and Carbohydrates suggesting that it is an important source of bioactive compounds that may supply novel medicine. Phytochemicalanalysis of this plant may be useful in developing new specialized drugs with more efficiency.

V. Future Scope

To fabricate different types of formulations like oil, shampoo, gel, cream, lotion, etc.

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