Phytochemical Properties and Antimicrobial Activities of Some Important Medicinal Plants of Dhemaji District of Assam

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ABSTRACT

Background: Assam along with the other North Eastern States i.e., Manipur, Mizoram, Sikkim, Tripura, Arunachal Pradesh, Meghalaya and Nagaland contribute 50% of India's entire plant biodiversity. Phytochemicals are bioactive chemical compounds obtained from the plants and are widely used as traditional herbal medicine. These herbal medicines are used by the local people to cure various diseases.

Objectives: The objective of the present study is the phytochemical as well as antimicrobial activity of ten medicinal plants i.e., *Vitexnegundo*L., *Oldenlandiacorymbosa*L., *Centellaasiatica*(L.) Urb., *Costusspeciosus*(J. Koenig) Sm., *Ocimumsanctum*L., *Piper nigrum*L., *Mimusapudica*L., *Phlogacanthusthysiflorus*Nees., *Andrographispaniculata*(Burm. F) Neesand *Ipomoea aquatic* Forssk.

Methodology: Taxonomy of the studied plants were confirmed using the existing tools. Plant extracts were subjected to phytochemical and antimicrobial testing based on the standard protocol previously described.

Results: It was found that steroids were present in the leaves of *Costusspeciosus*, *Piper nigrum*, *Mimusapudica*, *Ipomoeaaquat-ica*. Terpenoids were most commonly observed in the leaves of *Oldenlandiacorymbosa*, *Piper nigrum*, *Phlogacanthusthysiflorus*, *Andrographispaniculata*. The flavonoids were absent in the leaves of *Ocimum sanctum*. There were mixed results for the presence of the phytochemicals such as tannins, glycosides, coumarins etc. The antimicrobial study was carried out by the disc diffusion method against the human pathogenic bacteria. It was found that the selected medicinal plants are good sources of antimicrobial against pathogens like *Salmonella typhi*, *E. coli* and *Klebsiella pneumonia*.

Conclusion: It was concluded that the plants studied were rich in phytochemicals with significant pharmacological and medicinal properties.

Key Words: Phytochemicals, *E. coli*, *Oldenlandiacorymbosa*L., *Ipomoea aquatic* Forssk, antimicrobial, Assam

INTRODUCTION

Phytochemicals are chemicals that are present naturally in plants in the form of organic compounds. These phytochemicals synthesized in plant parts are used up by the local people for the healing of certain disorders¹. Primary metabolites are essential for growth and development. Secondary metabolites produced by plants are often colourful and flavoured compounds. In recent years, secondary plant metabolites i.e. phytochemicals have been extensively investigated as a source of medicinal agents². It's a standard process to look for the presence of newer drugs from natural products derived from plant species using pharmacological activity studies³. These are synthesized in almost all parts of the plant

like roots, tubers, stems, leaves, flowers, barks, fruits, seeds etc. These organic compounds provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, coumarins, proteins and glycosides.

In the present work phytochemical analysis and antimicrobial activity were carried out in ten plants i.e., *Vitexnegundo*, *Oldenlandiacorymbosa*, *Centellaasiatica*, *Costusspeciosus*, *Ocimum sanctum*, *Pipernigrum*, *Mimusapudica*, *Phlogacanthusthysiflorus*, *Andrographispaniculata* and *Ipomoeaaquat-ica* of Dhemaji district of Assam. The scientific names, diagram, distribution and medicinal properties of the studied plants has been tabulated below (Table 1).

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Details of the studied plants:

Table 1: Features of selected plants

Serial no	Plants Scientific name/ Common Name	Picture	Distribution	Medicinal use
1	<i>Vitexnegundo</i> L / Pachatia		Assam, particularly in-LakhimpurandDhemaji district.	Leaves are used for the treatment of eye disease, ulcers, fever and jaundice.
2	<i>Oldenlandiacorymbosa</i> L/ Bonjaluk		Assam, particularly in the Dhemaji district.	Gastric irritation, jaundice, nervous depression and boils.
3	<i>Centellaasiatica</i> (L.) Urb/Bormanimoni		Almost all plains districts of Assam.	Wounds, skin irritations, headache, stomach trouble.
4	<i>Costusspeciosus</i> (J. Koenig)Sm/Jomlakhuti		Occurs throughout the foothills of Arunachal Pradesh andDhemaji District.	Leaves and stem sareuses in diarrhoea, wounds, jaundice, leprosy, asthma, Intestinal worms.
5	<i>Ocimum sanctum</i> L / Tulsi		Almost all districts of Assam.	Malaria, periodic fever, asthma, cough, pyorrhea, insect bites, skin disorders, eye disorder.
6	<i>Piper nigrum</i> L /Jaluk		Plains of Assam	Fruits used in cough, tonsillitis.

Table 1: (Continued)

Serial no	Plants Scientific name/ Common Name	Picture	Distribution	Medicinal use
7	<i>Mimusapudica</i> L/Nilajibon		Dhemaji, Kamrup district.	Piles, ulcers, wounds. Dysentery, fever.
8	<i>Phlogacanthus thyrsiflorus</i> Nees/Teetaphool		Lakhimpur, Dhemaji district.	Skin diseases.
9	<i>Andrographis paniculata</i> (Burm. F) Nees/ Sirata		Kamrup, Lakhimpur district.	Diabetes, dysentery, fever.
10	<i>Ipomoea aquatica</i> Forsk/Kolmou		Almost all districts of Assam.	Flower juice is applied in the early morning around the eye to cure the black ring around the eyes.

MATERIALS AND METHODS

Collection of Plants Sample

Fresh plant parts of *Vitex negundo*, *Oldenlandia corymbosa*, *Centella asiatica*, *Costus speciosus*, *Ocimum sanctum*, *Piper nigrum*, *Mimusapudica*, *Phlogacanthus thyrsiflorus*, *Andrographis paniculata*, *Ipomoea aquatica* were collected from different villages of Dhemaji district of Assam. The plant materials were identified using taxonomic tools. The plant materials

were washed under running water, shaded and dried until all the water molecules evaporated and plants become well dried for grinding. After completion of the drying process, the plant materials were ground in a grinder and fine powder was transferred into the sealed container with proper labeling. 50 grams of powder of plant sample was taken to extract with an adequate amount of ethanol (4:1) using soxhlet apparatus. The liquid part is stored at 4°C in a separate container.

Chemicals and reagents

Distilled water, methanol, Di-ethyl ether hexane, Sodium phosphate buffer, DNS, Starch, DPPH, Ethanol, MH agar, Sodium acetate, sodium hydroxide, hydrogen peroxide, 95% ethanol, 1% lead acetate, hydrochloric acid, sulphuric acid, sodium carbonate, Chloroform [Jaldhara and Co.].

Glassware and plastic wares:

Beaker, conical flask, test tubes, measuring cylinder, Pipette, Petri dishes, test tube stand, plastic tray, micropipette tips.

Equipment:

Some of the equipment utilized for the study included-AN-AMED Electronic Balance, spectrophotometer, pipettes, soxhlet apparatus, micropipettes, centrifuge, Hot plate water bath, Laminar air flow, -70°C Temperature Freezer were the major equipment used in the study.

Preparation of plant extracts:

Water extract:

The water extraction was done using the standard method, where ground plant material of 5gm weighed was crushed in 100ml of sterile distilled water. The mixture was boiled at 50-60°C for 30 minutes on the water bath and it was filtered through what-man No.1 filter paper. Then the filtrate was centrifuged at 2500 rpm for 15 minutes. The extract was collected, labelled and stored in sterile bottles at 5°C for further different experimental use.

Ethanol extract:

Ground samples (5gm) were extracted with 100 ml of 95% ethanol on a water bath at 70°C for 2 hours. The extracted samples were centrifuged and the supernatant was transferred into 50 ml volumetric flask and adjust volume to 50 ml with 95% ethanol. The sample was stored at -4°C.

Qualitative analysis of phytochemicals:

Chemical tests were carried by using aqueous and ethanol extracts to identify various phytochemicals using standard methods⁴⁻⁷. The qualitative analysis for the extracts for the presence of chemical constituents was performed by various chemical tests like Steroids (Salkowski test), Terpenoids (Salkowski test), Flavonoids (Alkaline reagent test, sulfuric acid test, Lead acetate test), Tannins (Lead acetate test), Glycosides (Keller kiliani test), Coumarins (NaCl test).

Antimicrobial activity of plant extracts:

Antimicrobial tests using the leaf extracts of *V.negundo*, *O.corymbosa*, *C.asiatica*, *C.speciosus*, *O. sanctum*, *P.nigrum*, *M.pudica*, *P.thyrsiflorus*, *A.paniculata*, *I.aquatica* were carried out against the pure culture of *Salmonellatyphi*, *Klebsiella species* and *Escherichia coli*. Bacteria were

cultured overnight at 37°C for 72 hours. The antibacterial activity of the leaf was determined using the good diffusion method. The bacterial strain was spreading on Muller Hinton Media with the help of the "L" rod. Wells (5mm diameter) were punched in the agar in the petri dish. Then the concentrated leaf extracts were added to the well. The plates were inoculated for 48 hours and the bacterial was assessed by measuring the diameter of the zone of inhibition in mm.

RESULTS

Phytochemical analysis

The phytochemical characteristics of ten medicinal plants tested were summarized in the table-2. The results revealed the presence of medically active compounds in the ten plants studied. The leaves of *V. negundo* have positive results for the test flavonoid, tannin, glycosides, coumarin. For *O.corymbosa*, we have found positive results for terpenoid, flavonoid, tannin, coumarin. In the case of *C.asiatica*, we have found positive results for terpenoid, flavonoid, tannin, coumarin. *C.speciosus*, showed a positive result for steroid, flavonoid, tannin, glycoside. *O.sanctum*, have positive results for tannin, glycoside, coumarin. *P.nigrum*, have a positive result for the tested steroid, tannin, terpenoid, flavonoid, glycoside. *M.pudica* have found positive result for steroid, flavonoid, tannin, coumarin, *P.thyrsiflorus* showed positive results for terpenoid, flavonoid, tannin and glycoside. *A.paniculata* have positive result for terpenoid, tannin, glycoside, a flavonoid. The plant *I.aquatica* showed positive result for steroid, flavonoid and tannins.

Antimicrobial activity

Antimicrobial activity for the leaves of *V.negundo*, *O.corymbosa*, *C.asiatica*, *C.speciosus*, *O. sanctum*, *P. nigrum*, *M. pudica*, *P. thyrsiflorus*, *A. paniculata*, *I.aquatica* against *Salmonella typhi*, *E. coli* and *Klebsiella species* are given below. (Table 3).

DISCUSSION

Analysis of plant extract revealed the presence of phytochemicals like steroids, terpenoids, flavonoids, tannins, glycosides, carbohydrates, proteins and amino acids. The important thing is that except for *O. sanctum* all plant samples contain one common and abundant secondary metabolite flavonoid. The literature survey of a previous study by Yadav et al. revealed the presence of phytochemicals in different solvents extracts and also find total phenolic and flavonoid contents of the selected medicinal plants⁸. Worked on phytochemical evaluation and determination of the antimicrobial activity of the plants like *Ricinus com-*

Table 2: Phytochemical constituents of ten medicinal plants studied.

PLANTS	AQUEOUS EXTRACT	STERIODS	TERPENIDS	FLAVONIDS			TANNINS	GLYCOSIDES	COUMARINS
		SALKOWSKI TEST	SALKOWSKI TEST	ALKALINE REAGENT TEST	H ₂ SO ₄ TEST	LEAD ACETATE TEST	LEAD ACETATE TEST	KELLER-KILIANI TEST	NACL TEST
<i>Vitexnegundo</i>	leaves	-	+	+	-	+	+	+	+
<i>Oldenlandiacorymbosa</i>	leaves	-	+	+	+	-	+	-	+
<i>Centellaasiatica</i>	leaves	-	-	+	+	+	+	-	+
<i>Costusspeciosus</i>	leaves	+	-	+	+	+	+	+	+
<i>Ocimum sanctum</i>	leaves	+	-	-	-	-	+	+	+
<i>Piper nigrum</i>	leaves	+	+	+	-	-	+	+	-
<i>Mimusapudica</i>	leaves	+	-	+	+	-	+	-	+
<i>Phlogacanthusthysiflorus</i>	leaves	-	+	-	-	+	+	+	-
<i>Andrographispaniculata</i>	leaves	-	+	+	-	-	+	+	-
<i>Ipomoea Aquatica</i>	leaves	+	-	+	+	+	+	+	-

Table 3: Antimicrobial activity for ten medicinal plants

Plants name	Aqueous extract	Bacterial pathogen	Diameter of zone of inhibition
<i>Vitexnegundo</i>	Leaves	<i>Salmonella typhi</i>	1.6cm
<i>Oldenlandiacorymbosa</i>	Leaves	<i>E.coli</i>	0.9cm
		<i>Klebsiella species</i>	1.1cm
		absent	-----
<i>Centellaasiatica</i>	Leaves	absent	-----
<i>Costusspeciosus</i>	Leaves	<i>E.coli</i>	0.2cm
<i>Ocimum sanctum</i>	Leaves	absent	-----
<i>Piper nigrum</i>	Leaves	<i>E.coli</i>	0.4cm
<i>Mimusapudica</i>	Leaves	<i>E.coli</i>	0.2cm
<i>Phlogocanthusthysiflorus</i>	Leaves	<i>Klebsiellapnuemonia</i>	0.7cm
<i>Andrographispaniculata</i>	Leaves	<i>E.coli</i>	0.3cm
<i>Ipomoea aquatic</i>	Leaves	<i>E. coli</i>	0.3cm

munis (leaves, stem, roots), *Ipomoea Aquatica* (stem) had shown antimicrobial activity against *E.coli*. *Xanthium strumarium* (leaves, roots) and *Menthapiperita* (stem) had shown strong antimicrobial activity against *Staphylococcus aureus*⁹. *Oldenlandiacorymbosa* had not shown antimicrobial activity against *Staphylococcus aureus* and *E. coli*. In the current study leaves of *Oldenlandiacorymbosa* and *Ipomoea Aquatica* had shown antimicrobial activity. Previous work on the phytochemical analysis of four selected medicinal plants i.e., *Trigonellafoenum –graecum*, *Syzgiumcumini*, *Terminalia chebula* and *Salvadorapersica* revealed the presence of phytochemicals, flavonoid, Saponins and tannins¹⁰. In the present study of these plants also flavonoids and tannins are present in all of them except

Ocimum sanctum, which did not contain flavonoids. Previous work by Sharma et al. on five medicinal plants such as *Tinosporacordifolia*, *Bryophyllumpinnatum*, *Terminalia belerica*, *Xanthium strumarium* and *Oldenlandiacorymbosa* revealed that Glycosides were absent from the leaves of *Tinosporacordifolia*, steroids and terpenoids were absent in the leaves of *Xanthium strumarium*. Alkaloids were absent in the leaves of *Terminalia bellerica* and also in the leaves of *Tinosporacordifolia*¹¹. Tannins bind to proline-rich protein and thereby interfere in the protein synthesis¹². Hydroxylated phenolic substances, Flavonoids are manufacture by plants in response to microbial infections and they are effective antimicrobial substances against a wide range of microorganisms *in vitro*. Their activity is due to their

ability to complex with extracellular and soluble protein and to complex with bacterial cell wall¹³. They are effective antioxidants and show strong anticancer activities also¹⁴⁻¹⁶. Plant extracts were also containing saponins, which are known to produce inhibitory effects on inflammation¹⁷. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones¹⁸. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity¹⁹. Glycosides are known to lower blood pressure according to many reports²⁰. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites²¹.

CONCLUSION

Medicinal plants have had considerable global interest in recent years. The chosen ten medicinal plants are the source of the secondary metabolites i.e. steroids, terpenoids, flavonoids, tannins, glycosides and coumarins. Medicinal plants play an important role in treating various diseases. Due to the presence of secondary metabolites in medicinal plants they can activate in anti-diuretic, anti-inflammatory, anti-cancer, anti-malarial, anti-bacterial, anti-fungal, anti-diabetic and hepato-protective activity.

Nowadays, researchers can develop drugs from selected medicinal plants. But there are much more issues and challenges which have to be solved very effectively for these medicinal plants having antibacterial and phytochemical properties to promote them to the field of application. Since these medicinal plants are used by the traditional people for a long back so proper isolation, identification and mode of action of individual phenolic, as well as other biologically active compounds, would surely open the door for developing drugs from these plant resources. Finally, we will also have to give importance to the proper conservation of these medicinal plant species.

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Author's Contribution

MK: Did the wet lab experiments

PD: Did the survey component

MPS: Designed the study

PB: Expertise in taxonomy and drafting the paper.

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