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SCREENING OF PHYTOCHEMICALS IN CRUDE LEAF EXTRACTS OF FOUR MEDICINAL PLANTS FROM NORTH BENGAL

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ABSTRACT

Plants are source of large amount of drugs comprising to different groups such as antispasmodics, anticancer, antimicrobials etc. A large number of the plants possess the antibiotic properties in the traditional system and are mostly used by the tribal people worldwide. Plants have been known to relieve various disease in ayurveda. The biologically active compounds present in plants are known as phytochemicals. These phytochemicals are secondary metabolites derived from various parts of the plants such as leaves, roots, bark, flowers, seeds and pulps. The aim of present study was to investigate the presence of phytochemical groups in leaf extracts of four plants such as *Rauvolfia serpentina*, *Diplazium esculantum*, *Nictanthus arbortristis* and *Moringa olifera*. These phytochemicals used in the pharmacognostic drug development and treatment of various diseases. Our findings provided evidence that crude aqueous and organic solvent extracts of these tested leaf extracts contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

KEYWORDS: *Medicinal plants, leaf extract, Phytochemical analysis.*

INTRODUCTION

Herbs have been always the main principle form of medicine since traditions in India and now-a-days, it becomes most popular throughout the world. Herbal medicine, rather than merely curing a particular disease, aims at returning the body back to its natural state of health.[1] The phytochemical components of medicinal plants often act individually, additively or synergistically in improvement of health. They are not only providing traditional and ethnic medicine but also promising for highly efficient novel bioactive molecules. Since ages, man has been dependent on nature for curing various body diseases. Drugs obtained from the plants are easily available, less expensive, safe and efficient and rarely have side effects. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety and efficiency.[2] Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include secondary compounds. Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds.[3] Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances.[4,5,6] Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. The systematic screening of plant species with the purpose of discovering new bioactive compounds can help us to cure many fungal and bacterial diseases of economically important crops and animals including human being.

In this study, qualitative phytochemical analysis were carried out using leaves of four plants namely *Rauvolfia serpentina*, *Diplazium esculantum*, *Nyctanthes arbortristis* and *Moringa olifera* in North Bengal of India.

MATERIALS AND METHODS

Materials

Leaves of the plants like *Rauvolfia serpentina*, *Diplazium esculentum*, *Nictanthus arbortristis* and *Moringa olifera*, petriplates, whatman filter paper, water bath, test tubes, beaker, TLC plate.

Collection of leaf material

Leaves of four plants were collected from Naxalbari region under the jurisdiction of Siliguri subdivision of Darjeeling district. Collected leaves were to be processed for cleaning in order to prevent the deterioration of phytochemicals present in leaf parts.

Qualitative analysis of collected four leaf extracts

Cleaning

After collection of plant parts, they were cleaned properly. The cleaning process include peeling or stripping leaves from stems. Cleaning was done by hands in order to get better results.

Drying

The main purpose of drying was to remove the water content from plant parts so that they could be stored. Plants were dried immediately as soon as the plants collection to prevent spoilage of plant materials. Drying was done by natural process that included sun-drying and air-drying. While drying, proper care was taken to avoid direct sunlight, as it might damage the plant components.

Powdering

After complete drying of leaves, they were powdered well for further analysis.

Preparation of aqueous extract: 2 gm of leaves of *Rauvolfia serpentina*, *Diplazium esculentum*, *Nyctanthus arbortristis* and *Moringa olifera* leaf powder was taken in a separate beaker and water was added until total powder was dipped within the water. It was boiled for 10-15min. Water extract was filtered through Whattmann filter paper separately and the filtrate was used for further analysis.

Test for Tannins

Ferric chloride test: Filtrate was taken in a test tube and 0.1% ferric chloride was added. Brownish green or blue-black coloration appeared.

Test for Phlobotannins

Hydrochloric acid test: Deposition of a red precipitate occurred when an aqueous extract of each sample was boiled with 1% aqueous HCl and was taken as evidence for the presence of phlobotannins.

Test for saponin

Froth test: About 2 gm of the powdered sample was boiled in 20 ml of distilled water in a water bath and then filtered. To 10 ml of the filtrate, 5 ml of distilled water was added and the mixture was shaken vigorously to obtain a stable, persistent froth. The froth was then mixed with olive oil and shaken vigorously, and the mixture was observed for the formation of an emulsion. After waiting for 10 minutes, the presence of saponin was indicated by the formation of froth.

Test for Carbohydrates

Molisch Test: 1 ml of the test solution was treated with a few drops of alcoholic α-naphthol. Then, a few drops of 0.2 ml concentrated H_2SO_4 were carefully added along the sides of the test tube. A purple to violet-colored ring was observed at the junction, indicating the presence of carbohydrates.

Fehling's Test

Equal volumes of Fehling's A (copper sulfate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) were mixed. A few drops of the sample were then added and the mixture was boiled. A brick-red precipitate of cuprous oxide was formed, indicating the presence of reducing sugars.

Benedict's Test

The filtrate was treated with Benedict's reagent and gently heated. The formation of an orange-red precipitate indicated the presence of reducing sugars.

Iodine Test

The crude extract was mixed with 2 ml of iodine solution. A dark blue or purple coloration was observed, indicating the presence of carbohydrates.

Test for Proteins

Millon's Test: To the test solution, 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid) was added. A white precipitate was formed, which turned red upon gentle heating.

Biuret Test

To the test solution, 4% NaOH and a few drops of 1% $CuSO_4$ solution were added. A violet color was observed, indicating the presence of proteins.

Test for Amino Acids

Ninhydrin Test: About 0.5 mg of the extract was taken and a few drops of freshly prepared 0.2% ninhydrin reagent were added. The mixture was then heated. The appearance of a pink or purple color indicated the presence of proteins, peptides, or amino acids.

Test for Diterpenes

Copper Acetate Test: The extract was dissolved in water and treated with 3–4 drops of copper acetate solution. The formation of an emerald green color indicated the presence of diterpenes.

Preparation of Acid Extract

A small amount of powdered plant leaf was taken in a beaker and acid was added until the powder was completely submerged. After 20 minutes, the mixture was filtered using Whatman filter paper. The filtered sample was then used for further analysis.

Tests for Alkaloids

Mayer's Test: The filtrate was treated with Mayer's reagent (1.35 g mercuric chloride in 60 ml water and 5 g potassium iodide in 40 ml water). A yellow-colored precipitate was formed, indicating the presence of alkaloids.

Wagner's Test

The filtrate was treated with Wagner's reagent (1.27 g iodine and 2 g potassium iodide in 100 ml water). A

brown or reddish precipitate was formed, indicating the presence of alkaloids.

Hager's Test
The filtrate was treated with Hager's reagent (saturated picric acid solution). The formation of a yellow-colored precipitate confirmed the presence of alkaloids.

Test for Organic Acids
The powdered extract was dissolved in hydrochloric acid and filtered after some time. The filtrate was then used for the following tests:

Oxalic Acid Test
To the test solution, a few drops of 1% $KMnO_4$ and dilute H_2SO_4 were added. The disappearance of color indicated the presence of oxalic acid.

Malic Acid Test
To the test solution, 2–3 drops of 40% $FeCl_3$ solution were added. A yellowish color appeared, indicating the presence of malic acid.

Test for Inorganic Acid
Sulphate Test: To the test solution, lead acetate reagent was added. A white precipitate appeared, which was soluble in NaOH, indicating the presence of sulphates.

Test for Flavonoids
Alkaline Reagent Test: The extract was treated with a few drops of sodium hydroxide solution. An intense yellow color was formed, which became colorless upon the addition of dilute acid, indicating the presence of flavonoids.

Lead Acetate Test
The extract was treated with a few drops of lead acetate solution. A yellow-colored precipitate was formed, indicating the presence of flavonoids.

Test for Cardiac Glycosides
Keller-Killiani Test 0.5 g of the methanolic extract was taken and treated with 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride. The mixture was transferred to a test tube and 0.5 ml of concentrated H_2SO_4 was added along the side. A blue color appeared in the acetic acid layer, indicating the presence of cardiac glycosides.

Test for Sterols and Triterpenoids
***Liebermann–Burchard Test*:** A small amount of methanolic extract was treated with a few drops of acetic anhydride, boiled and cooled. Concentrated H_2SO_4 was added along the side of the test tube. A brown ring appeared at the junction of the two layers and the upper layer turned green, indicating the presence of sterols. A deep red color indicated the presence of triterpenoids.

Salkowski's Test: A small amount of methanolic extract was treated with 2 ml of chloroform. Then, 3 ml of concentrated H_2SO_4 was carefully added to form a separate layer. The mixture was shaken and allowed to stand. A red color in the lower layer indicated the presence of sterols, while a yellow-colored lower layer indicated the presence of triterpenoids.

RESULTS
The results of phytochemical screening of leaf extracts of *Rauvolfia serpentina*, *Diplazium esculentum*, *Nictanthus arbortristis* and *Moringa olifera* is presented in Table 1. These leaf extract tested for the presence of alkaloids, flavonoids, tannins, phlobatannins, saponins, steroids, triterpenoids, glycosides, carbohydrates, proteins, organic acid, inorganic acid and diterpenes.

In this study, *Rauvolfia serpentina*, *Moringa olifera and Nictanthus arbortristis* showed positive result for the alkaloids, flavonoids, tannins, phlobatannins, saponins, steroids, glycosides, carbohydrates, proteins, organic acid and negative result for triterpenoids, inorganic acid and diterpenes. In case of *Diplazium esculentum*, all phytochemicals present as observed in remaining three leaf extracts except phlobatanins, inorganic acid and triterpenoids.

Table – 1.

Sl. No	Photochemical constituents	*Rauvolfia sepentina*	*Moringa olifera*	*Nyctanthes arbortristis*	*Diplazium esculentum*
1.	Tannins: Ferric chloride test	 +	 +	 +	 +
2.	Phlobatannins: Hydrochloric acid test	 ++	 ++	 +	 -
3.	Saponin: Froth test	 +	 ++	 +	 +
4.	Flavonoids: Alkaline reagent test Lead acetate test	 + +	 + +	 + +	 - +
5.	Sterols: Leibermann-Burchard test	 +	 ++	 ++	 +

	Salkowski's test	+	+	++	+
6	Triterpenoids: Liebermann-Burchard test Salkowski's test	 - -	 - -	 - -	 - -
7	Cardiac glycosides: Keller-killiani test	 +	 ++	 ++	 +
8	Alkaloids: Mayer's test Wagner's test Hager's test	 ++ + +	 + ++ +	 + ++ +	 + + +
9	Organic acid test: Oxalic acid test Malic acid test	 + +	 + +	 + +	 + +
10	Inorganic acid test: Sulphate test	 -	 -	 -	 -
11	Diterpenes: Copper-acetate test	 -	 -	 +	 +
12	Carbohydrates: Molischs test Fehling's test Benedict's test	 + + +	 + + +	 + + +	 + + +
13	Protein: Millions test	 +	 +	 +	 +

++ indicates strong positive result, + indicates positive result, - indicates negative result

DISCUSSION

Phytochemical screening of the plants revealed some differences in the constituents of the two plants tested. *Nyctanthus arbortristis* tested positive for all the phytochemicals except triterpenoids and *Diplazium esculentum* showed the presence of alkaloids, carbohydrates, tannins, saponin, protein glycosides except triterpenoids. The presence of alkaloids in plant extract may be participated in plant metabolism sequences[7] and one of their common biological property is their cytotoxicity.[8] Saponins known to produce inhibitory effect on inflammation.[9] It has the property of precipitating and coagulating red blood cells.[10,11] Steroids have been reported to have antibacterial properties[12] and they are very important compounds such as sex hormones.[13] Several workers have reported the analgesic[14,15], antispasmodic and antibacterial[16,17] properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports.[18] Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[19] They also are effective antioxidant and show strong anticancer activities.[20,21,22] Glycosides are known to lower the blood pressure according to many reports.[23] The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. In the present study, it has been concluded that *Rauvolfia serpentina*, *Diplazium esculentum*, *Nictanthus arbortristis* and *Moringa olifera* have the potential to act as a source of useful drugs because of presence of various phytochemical constituents such as tannins, saponin, alkaloids, flavonoids, sterols and carbohydrates. These phytoconstituents has the potential to improve the health status of the consumers. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate , purify and characterize the active constituents responsible for the activity of these plants.

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