International Journal of scientific research and management (IJSRM)
||Volume||4||Issue||09||Pages||4578-4585||2016||
Website: www.ijsrm.in ISSN (e): 2321-3418

Phytochemical Analysis of Aqueous and Alcohlic Extract Of Some Medicinal Plants

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Abstract

Medicinal plants have bioactive compounds which are used for curing of various human diseases as well as animal diseases and also play an important role in healing. Phytochemicals have two categories i.e. primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal Plants have antifungal, antibacterial and anti-inflammatory activities. The present study involves three different medicinal plants *Moringa oleifera, Dalbergia sissoo and Saraca indica.* The leaves of the selected medicinal plants were washed, air dried and then powdered. The aqueous and alcoholic extract of leaf samples were used for the phytochemical analysis to find out the phytochemical constituents in the plant. The aim of the present study was to carried out the phytochemical analysis of aqueous and alcoholic extract of *Moringa oleifera, Dalbergia sissoo and Saraca indica.* Moringa oleifera is one of the species of family Moringaceae. Moringa oleifera is commonly known as "Drumstick". Ashoka is the most ancient tree of India, known as a Saraca asoca (Roxb.), De.wild or Saraca indica belonging to family Caesalpinaceae. *Dalbergia sissoo* belonging to family *Fabaceae* and commonly known as Shisham or Sisam. The present study reveals the medicinal values of *Moringa oleifera, Dalbergia sissoo, Saraca indica* after reviewing their Phytotochemical analysis.

Keywords: Phytotochemical analysis, Medicinal Plants, Primary constituents, Secondary constituents.

INTRODUCTION

The medicinal plants are useful for healing as well as for curing of animal as well as human diseases because of the presence of phytochemical constituents. Several types of medicinal plants are existing in the nature and are effective in treating different type of diseases. Herbal medicine is a triumph of popular therapeutic diversity. The use of plants as a source of medicine has been inherited from the onset of human civilization and is an important component of the healthcare system. . In recent times there has been a tremendous increase in the use of plant based health products. In recent years, phytochemical analysis has begun to play an important role in ethnobotanical studies [1]. In several cases, such analyses have led to the discovery of novel bioactive phytochemicals. The present study is to evaluate the preliminary phytochemical characteristics such as determination of pharmacognostic principals of some medicinal plants of different families.

In the present investigation, an attempt was made by some phytochemical tests of medicinally important plants like *Moringa oleifera* is one of the species of family *Moringaceae,* native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the pacific and Caribbean Islands. *Moringa oleifera* is commonly known as "Drumstick and also named as horseradish tree, oil tree, miracle tree, and "Mothers best friend"[2].The leaves are outstanding as a source of vitamins A when raw as a

source of vitamin C. They are also good sources of vitamin B and are among the best plant sources of minerals *Moringa oleifera* is used as an extract (aqueous and ethanolic) for further phytochemical analysis.

Ashoka is one of the most legendary and sacred trees of India. Ashoka tree, universally known by its binomial Latin name *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging to family Caesalpinaceae [3]. The Ashoka tree's dried bark contains tannins, sterol, catechol, and other organic calcium compounds. The powered bark of the tree also contains Aluminum, strontium, calcium, iron, magnesium, phosphate, potassium, sodium, and silica.

Dalbergia sissoo Roxb. belongs to Leguminoseae plant family which is native to India and had been long cultivated in Egypt has shade tree on the banks of irrigation canals [4]. It is a large deciduous tree, often with crooked trunk and light crown. *Dalbergia sissoo* includes many members which are broadly used in folk medicine for several diseases [4-9].

MATERIALS AND METHODS

Collection of plant materials

The present study was carried out in the Department of Pharmacology and Toxicology, and Dept. of Veterinary Biochemistry, Nagpur Veterinary College, Nagpur. Leaves were collected from the *Moringa oleifera, Dalbergia Sissoo, Saraca indica* plant from the herbal garden. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.

Collection and identification of plant material

The *Moringa oleifera, Dalbergia Sissoo, Saraca indica* leaves were procured from Nagpur region and were authenticated from Department of Botany, Institute of Science, RTM Nagpur University, Nagpur. The seeds were dried at room temperature and powdered. The powder was stored in glass bottle in a cool and dry place away from direct sunlight and used for preparation of aqueous extract.

Preparation of leaf extracts

Weigh 50gms of powder of dried leaves of *Moringa oleifera, Saraca indica* and bark of *Dalbergia Sissoo* were dried at room temperature (32-35 ^{0}C) to constant weight to over a period of five days. The dried leaves and bark were ground into powered using a mortar and pestle. Fifty grams of powdered leaves and bark were separately extracted in 500 ml conical flask with 90% ethanol (Ethanolic extraction) and in water (Aqueous Extraction). The conical flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional manual agitation of the flask using a sterile glass rod at every 24 hour. The extracts were separately filtered using sterile Whatman no. 1 filter paper. These extracts (ethanolic and aqueous) were used in further process.

Alcoholic extract of dried Leaves powder dried leaves of *Moringa oleifera, Saraca indica* and bark of *Dalbergia Sissoo* was prepared. A little extract was taken in 5 ml of 1.5 % hydrochloric acid (V/V) and filtered; the filtrate was used for testing active principle of plants.

The Aqueous and Alcoholic extract was prepared by the method described by Rosenthaler (1930).

Phytochemical study for the qualitative analysis of certain active principles

The phytochemical study was undertaken for determination of various active constituents of aqueous extract of the dried leaves of *Moringa oleifera, Saraca indica* and bark of *Dalbergia Sissoo* by the various tests described by Rosenthaler (1930).

1. Test for Sterols

A. Salkowski's reaction

A few mg residues of extract were taken in 2 ml of chloroform and sulphuric acid was added by the side of the test tube. The test tube was shaken for a few minutes, red colour development in the chloroform layer and greenish yellow fluorescence in the lower layer indicated the presence of sterols and terpenoids in the extract.

B. Libermann Buchard Reaction

A few mg of residues of extract was dissolved in chloroform and a few drops of acetic anhydride added followed by concentrated sulphuric acid by the side of the test tube. Transient colour development from red to blue and finally green indicated the presence of sterols.

2. Test for Alkaloids

A little extract was taken in 5 ml of 1.5 % hydrochloric acid (V/V) and filtered; the filtrate was used for testing alkaloids.

A. Dragendroff's reagent

It was prepared by mixing solution A (17 gm of Bismuth subnitrate +200 gm of tartaric acid+ 800 ml of distilled water) and solution B (160 gm potassium iodide + 400 ml of distilled water) in 1:1 proportion (V/V). From this solution a working standard was prepared by taking 50 ml of this solution and adding 100 gm of tartaric acid and making its volume up to 500 ml with distilled water.

This reagent was sprayed on a filter paper and the paper was dried. The sample solution was applied on the paper using a capillary tube. Development of an orange-red colour indicated the presence of alkaloids.

B. Wagner's reagent:

1.27 gm iodine and 2 gm potassium iodide were dissolved in 5 ml of distilled water and solution further divided in 100 ml distilled water. To this a little acid solution of the extract was added. Appearance of brown flocculent precipitate indicated the presence of alkaloids.

3. Test for Amino acids

A. Ninhydrin Test

A few ml of 0.1 % solution of ninhydrin in alcohol, when added to the extract, violet or purple colour development indicates the presence of amino acids.

4. Test for Proteins

A. Xanthoproteic Test

A little residue was taken in 2 ml of water and to it 0.5 ml of concentrated nitric acid was added. The appearance of (white or yellow) precipitate indicated the presence of proteins.

B. Biuret Test

A few mg of residue was taken in water and 1 ml of 1 % solution of sodium hydroxide was added followed by a drop of 1 % solution of copper sulphate. Violet – pink colour development indicates the presence of proteins.

5. Test for Reducing sugars

The presence of reducing sugars was tested with extract. The extract was dissolved in warm distilled water and tested with Benedicts and Fehling's reagent.

A. Benedict's reagent

Five ml of extract solution was taken and equal quantity of Benedict's reagent was added to it and heated. The appearance of brownish red precipitate (reduction) indicated the presence of reducing sugars.

B. Fehling's reagent

Two ml of solution was added to 0.5 ml of Fehling's reagent (Fehling's solution A and B mixed immediately before use) and 2 ml of 10 5 sodium hydroxide solution. The mixture was then heated on a water bath for 10 minutes. The appearance of red precipitate indicated the presence of reducing sugars.

6. Test for Glycosides

A. Benedict's reagent

The above tests for reducing sugars were repeated with extract. The solution obtained in Benedict's test was filtered and to this solution, dilute hydrochloric acid was added for hydrolyzing the glycosides. The pH of the solution was alkaline. Equal quantity of Benedict's reagent was added and boiled. The appearance of brownish precipitate indicated the presence of glycosides.

B. Fehling's reagent

This test is performed with the solution obtained in Fehling's test. To the clear solution a few drops of dilute HCL was added and boiled for 5 minutes for hydrolyzing the glycosides. Fehling's reagent was again added to note any further reduction, which indicated the presence of glycosides.

7. Test for Saponins

A. Foam test

A few mg residue taken in a test tube with small amount of sodium bicarbonate and distilled water, it was shaken vigorously. Formation of froth indicates the presence of saponins.

8. Test for Tannins

DOI: 10.18535/ijsrm/v4i9.17

A residue of the extract taken up separately in the ethanol. It was warmed and filtered. Tests were carried out with this filtrate using following reagents.

A. Lead acetate test

A few drops of lead acetate solution were added to the extract. The formation of precipitate indicated the presence of tannins.

B. Ferric chloride Test

A few drops of ferric chloride solution were added to the little of the above filtrate. Green colouration in the filtrate of extract indicated the presence of tannins.

9. Test for Flavonoids.

A small quantity of residue was dissolved in 5 ml of ethanol (95%) and treated with a few drops of concentrated hydrochloric acid and 0.5 gm of magnesium turnings. Development of either pink or magnet colour indicated the presence of flavonoids.

10. Test for Resins

The alcoholic extract was dissolved in alcohol. To this, a few drops of distilled water was added. The appearance of turbidity considered as a positive test for resins

Table -1 Qualitative Phytochemical Analysis of Aqueous Extract of leaves of *Moringa oleifera, Saraca indica* and bark of *Dalbergia Sissoo*

Sr. No.	Active principle	Test Applied	Observation	Aqueous Extract			Alcoholic Extract		
				Result of *Moringa oleifera*	Result of *Saraca indica*	Result of *Dalbergia Sissoo*	Result of *Moringa oleifera*	Result of *Saraca indica*	Result of *Dalbergia Sissoo*
1	Sterols	A.Salkowskis reaction	Development of red colour in chloroform layer	Present	Present	Present	Present	Present	Present
		B. Liberman Buchard reaction	Development of transient colour	Present	Present	Present	Present	Present	Present
2	Alkaloids	A. Dragon-droffs reagent	Development of orange red colour	Present	Absent	Present	Present	Absent	Absent

		B. Wagners reaction	Appearance of brown flocculent precipitation	Present	Absent	Absent	Present	Absent	Absent
3	Amino acids	Ninhydrin test	Development of violet colour.	Absent	Absent	Present	Absent	Present	Present
4	Proteins	A. Xanthoproteic test	White precipitate was formed	Absent	Absent	Present	Absent	Present	Present
		B. Biuret test	Violet pink colour developed	Absent	Absent	Present	Absent	Present	Present
5	Reducing sugars	A. Benedicts reagent	Development of brownish red precipitate	Absent	Present	Present	Absent	Present	Present
		B. Fehlings reagent	Appearance of red precipitate	Absent	Present	Present	Absent	Present	Present
6	Glycosides	A. Benedicts reagent	Development of brownish red precipitate	Absent	Absent	Absent	Absent	Present	Absent
		B. Fehlings reagent	Appearance of red precipitate	Absent	Absent	Absent	Absent	Present	Absent
7	Saponins	Foam test	Formation of froth	Present	Present	Present	Present	Present	Present
8	Tannins	A. Lead acetate test	Formation of precipitate	Present	Absent	Present	Absent	Present	Present
		B. Ferric chloride test	Occurrence of green colouration of filtrate	Present	Absent	Present	Absent	Present	Present
9	Flavonoids	Test for flavonoids	Development of red or pink colour	Present	Present	Present	Present	Present	Present
10	Terpenoids	Salkowski's reaction	Greenish yellow fluorescenc	Present	Present	Present	Present	Present	Present

			e in the lower layer						
11	Resins	Test for resins	No turbidity was appeared	Present	Present	Present	Present	Present	Present

(+ indicates presence) (- indicates absence)

RESULTS

In this study, preliminary phytochemical screening was conducted on Aqueous and Alcoholic extract of *Moringa oleifera, Dalbergia Sissoo, Saraca indica* plant parts (leaf and bark). The results are presented in the table (Table 1).

DISCUSSION

The present study reveals that *Moringa oleifera, Dalbergia Sissoo, Saraca indica* plant shows the presence of phytochemical constituents like Sterols, Alkaloids, Amino acids, Proteins, Reducing sugars, Glycosides, Saponins, Tannins, Flavonoids, Resins, Terpenoids in different solvent extracts as shown in Table1. In this study, three extracts were phytochemically analysed.

Phytochemical investigation of *Moringa oleifera* is studied and found to contain alkaloids that are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs [11]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [12].Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical activities [13]. Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells 14].

Preliminary phytochemical investigation of the ethanolic bark extract of *D. sissoo* showed that it contained carbohydrates, proteins, amino acids, phenolic compounds and flavanoids. Leaf juice for eye ailments, considering the wood and bark as abortifacient, anthelmintic, antipyretic, aphrodisiac, expectorant, and refrigerant. The wood and bark for anal disorders, blood diseases, burning sensations, dysentery, dyspepsia, leucoderma, and skin ailments.

Saraca indica is reported to contain glycoside, flavonoids, tannins, and saponins. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-tumour, antiprogestational, antiestrogenic activity against menorrhagia and anti-cancer.

ACKNOWLEDGEMENT

The authors are thankful to the departments of Vet. Pharmacology and Toxicology and Department of Veterinary Biochemistry for help during the course of the practical work of the pharmacological study.

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