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Research article

A comparative phytochemical screening of various plants of India

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ABSTRACT

The medicinal value of plants generally depends upon its phytoconstituents which are present in various parts of plants, the present article attempt to compare the phytochemical screening of the Moringa Oleifera, Rubia Cardifolia, Zizyphus Jujuba melia azederach and Brassica Rapa plants which belongs the different families of plants. For phytochemical screening different type of extracts were prepared and tested for the presence of secondary metabolites. Phytochemicals such as carbohydrates, alkaloids, sterols, tannins, volatile oils, saponins, anthroquinone glycosides and flavanoids are reported. Phytoconstituents in various extracts gives us clue for further investigation.

Keywords: Moringa oleifera, melia azederach, rubia cardifolia, flavonoids, diuretic antilithic, Terpinoides.

INTRODUCTION

The gastro retentive drug delivery system can be retained in the stomach and assist in improving the oral control delivery of drug that have an absorption window in a particular region of the gastrointestinal tract. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals. These are non- nutritive chemicals that have protective or disease preventive property[1].the plant for phytochemical study are **1**. *Rubia Cardiofolia* Linn. Belongs to family rubiaceae It is a climbing plant growing in the northwest Himalayas ,nilgiris and other hilly districts of India .Roots contains resinous and extractive matter ,gum ,sugar colouring matter and salts of lime colouring matter consists of a red crystalline principle purpurine a yellow principle glucoside manjistine **2.** *Moringa Oleifera* lam. belongs to family moringaceae It is a beautiful tree plant wild in the sub Himalayan range and commonly cultivated in India and Burma bark contain a white crystalline alkaloid 2resins, an inorganic acid mucilage ,mysristic acid ,7.3 %, palmitic acid, oleic acid ,stearic acid, behenic acid and lignoceric acid 3%, diuretic and antilithic. **3.** *Melia Azedarach* belongs to family meliaceae the active principle is a light yellow non crystalline, bitter resinous substance without alkaloids properties sugar is present s tannins occurs in the outer portion of bark activity resides in the liver are inner bark anthelmintic, insecticidal properties **4**. *Zizyphus Jujuba* family rhamnaceae its fruit contain mucilage and sugar in addition to fruit acid Bark contains much tannins and a crystalizable principle zizyphic acid. fruit is eaten often with vegetable it is also made in to a preserve by removing the stone and adding chilies and salt anodyne, digestive, blood purifier, tonic, cough and colds. ***5***. *Brassica Rapa B. campestris rapa* (shaljam) belongs to family cruciferae A decoction of the leaves or stems is used in the treatment of cancer. The powdered seed is said to be a folk remedy for cancer. The comparative physiochemical study of these plant show the information about the various constituents present in the extracts of these plantsvertigo [1, 2].

MATERIALS AND METHODS

The plants were identified and collected from the local market of Bhopal. The plants were authenticated from safia college Department of Botany Bhopal, The selected parts were washed, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of plant drug

The powdered plant material was subjected to hot

continuous extraction in a soxhlet apparatus.The powdered plant drug was successively extracted with methanol, ethanol as solvent. The liquid extracts were collected in a tarred conical flask. The solvent was removed by distillation. The extracts obtained with solvent were weighed to a constant weight and percentage of yield (w/w) was calculated.

Phytochemical screening

Phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of plants, these were subjected to the qualitative test analysis using standard methods

Preliminary Phytochemical investigation The extracts were subjected to preliminary qualitative phytochemical investigation. The various tests and reagents used are given below and observations are recorded [3, 4].

Alkaloids

Preparation of test solution: The test solution was prepared by dissolving extracts in the dilute hydrochloric acid solution.

Mayer's test: The acidic test solution with Mayer's reagent (Pot. Mercuric iodide) gave cream coloured precipitate.

Hager's test: The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

Dragendorff's test: The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

Wagner's test: The acidic test solution treated with Wagner's reagent (Iodine in Potassium iodide) gave brown precipitate.

Amino acids

Preparation of test solution: The test solution was prepared by dissolving the extract in water.

Ninhydrin test: Test solution when treated with Ninhydrin reagent gave blue colour.

Test for Tyrosine: 3 ml of test solution when heated with 3 drops Millon's reagent solution showed dark red colour.

Test for Tryptophan: To 3 ml test solution added few drops of glyoxalic acid and conc. H2SO4. Reddish violet ringappeared at junction of two layers.

Test for Cysteine: To 5 ml test solution added few drops of 40%NaOH and 10% lead acetate solution and Boiled black precipitate of lead sulphate formed.

Carbohydrates

Preparation of test solution: The test solution was prepared by dissolving the extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

Molish's test: Test solution with few drops of Molish's reagent and 2 ml of conc. H_2SO_4 added slowly from the sides of the test tube. It showed a purple ring at the junction of two liquids.

Barfoed's test: Test solution treated with Barfoed's reagent and after boiling on a water bath, it showed brick red colour precipitate.

Benedict's test: Test solution treated with Benedict's reagent and after boiling on water bath, it showed reddish brown precipitate.

Fehling's test: The test solution when heated with equal volume of Fehling's A and B solutions, gave orange red precipitate, indicating the presence of reducing sugars.

Selivanoff's test: To a crystal of resorcinol was added to the test solution and warmed on a water bath, then added equal volume of conc. HCl. A rose red colour appeared indicating presence of ketone.

Fats and oils

Solubility test: Oils are soluble in petroleum ether, benzene and chloroform but insoluble in 90% ethanol and in water.

Filter paper gets permanently stained with oils [5, 6].

Flavonoids

The flavonoids are all structurally derived from the parent substance called flavone. The flavonoids occur in the free form as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

Preparation of test solution

To a small amount of extract added equal volume of 2M HCl and heated in a test tube for 30 to 40 min. at 100°C.

The cooled extract was filtered, and extracted with ethyl acetate.

The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

Shinoda test: Test solution with few fragments of magnesium ribbon and conc. HCl showed pink to magenta red colour.

To a small quantity of test solution when lead acetate solution was added, it formed yellow coloured precipitate.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colourless on addition of few drops of dilute acid.

Glycosides

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

Test for Cardiac Glycosides

Baljet's test: The test solution treated with sodium picrate gave yellow to orange colour.

Raymond's test: Test solution treated with dinitrobenzene in hot methanolic alkali gave violet colour.

Bromine water test: Test solution dissolved in bromine water giving yellow precipitate.

Keller-Killiani test for digitoxose: The test solution treated with few drops of $Fecl_3$ solution and mixed, then H_2SO_4 containing

$FeCl_3$ solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

Legal's test: Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

Test for anthraquinone glycosides

Borntrager's test: Boiled powdered drug with 5 ml of 10% sulphuric acid for 5 mins. Filtered while hot, cool the filtrate, shake gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution of ammonia (10%). Allowed to separate it. The ammonical layer acquired rose pink colour due to the presence of anthraquinones.

Modified Borntrager's test: C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute HCl and 5 ml of 5% solution of $FeCl_3$. For hydrolyzed extract procedure was carried out as described under Borntrager's test.

Cyanogenetic glycosides

Grignard's test: Strips of sodium picrate filter paper were inserted between split cork stoppers which were fitted in to the neck of the test tube containing a small amount of powdered drug in water. Care was exercised that the paper didn't touch the inner side of the test tube. The content was warmed for half an hour. The red colour of the strips indicated the presence of cyanogenetic glycosides.

Coumarin Glycosides

Alcoholic extract when made alkaline show blue or green fluorescence.

Gums and mucilages

Hydrolyzed the test solution using dilute HCl and performed Fehling's or Benedict's test by reagent as red colour was developed gums and mucilage were present.

Proteins

Preparation of Test Solution: The test solution was prepared by dissolving the extract in water.

Millon's test: Test solution was treated with Million's reagent and heated on a water bath. The proteins were stained red.

Biuret test: Test solution when treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

Xanthoproteic test: Test solution was treated with conc. HNO_3 and boiled which gave yellow precipitate.

Precipitation test: The test solution gave white colloidal precipitate with 5% $CuSO_4$ solution and 5% lead acetate solution.

Saponins

Preparation of test solution: The test solution was prepared by dissolving extract in the water.

Foam test: Test solution when shaken showed the formation of foam, which was stable for at least 15 min.

Haemolysis test: 2 ml of 18% sodium chloride in 2 test tubes to one test tube distilled water was added and to other 2 ml of test solution. Few drops of blood were added to both the test tubes. Mixed and observed for haemolysis under microscope.

Steroids

Preparation of test extract solution: The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.

Salkowski test: To the test extract solution add few drops of conc. H_2SO_4, shake and allowed to stand for few minutes lower layer turned red indicating the presence of sterols.

Libermann - Burchard test: The test solution treated with few drops of acetic anhydride and mixed then conc. H_2SO_4 was added from the sides of the test tube, it showed a brown ring at the junction of the two layers and the upper layer turned green.

Libermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride, heated, cooled. Add few drops of concentrated H2SO4. Blue colour appeared.

Sulphur test: Sulphur when added in to the test solution, it sank in it [7, 8].

Tannins and phenol compounds

To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents:

5% FeCl3 solution: Deep blue-black colour.

Lead acetate solution: White precipitate.

Bromine water: Discoloration of bromine water.

Acetic acid solution: Red colour solution.

Dilute iodine solution: Transient red colour.

One drop NH4OH, excess 10% AgNO3 solution. Heated for 20 min in boiling water bath. White precipitate was observed, then dark silver mirror deposited on wall of test tube.

Triterpenoids

Preparation of test extract solution:

The test extract solution was prepared by dissolving extract in the chloroform.

Salkowski test: Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow.

Libermann - Burchard Test: To the test solution of the extract, few drops of acetic anhydride was added and mixed well. Then 1 ml of concentrated sulphuric acid added from the sides of the test tube, a red colour was produced in the lower layer indicating presence of triterpenes.

Vitamins

- Test for Vitamin A: Dissolved a quantity

equivalent to 10 to 15 units in 1 ml of chloroform and add 5 ml of antimony (III) chloride solution, a transient blue colour was produced immediately.

Tests for B complex

Test for B1 (Thiamine HCl): Dissolved 20 mg in 10 ml of water, add 1 ml of 2M acetic acid and 1.6 ml of 1M NaOH, heated on water bath for 30 min and allowed to cool. Added 5 ml of 2M NaOH, 10 ml of potassium ferricyanide solution and 10 ml of n-butanol and shaken for 2 min. The upper layer exhibited an intense light blue fluorescence in UV 365 nm.

Test for B2 (Riboflavin): Dissolved about 1 mg in 100 ml of water. The solution has a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence by reflected light, which disappeared on addition of mineral acids or alkalis.

Tests for Vitamin C

Diluted 1 ml of 2% w/v solution with 5 ml of water and added 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of diluted sodium hydroxide solution. Added 0.6 ml of conc. HCL drop wise and stirred. The yellow color turned blue.

Test for Vitamin D: Dissolved a quantity equivalent to about 1000 units of vitamin D activity in chloroform and added 10 ml of antimony (III) chloride solution, a pinkish- red color appeared.

Organic acids

Neutralized aqueous drug extract with dilute NH4OH solution then performed following tests.

Calcium chloride test

To 2 ml test solution added few drops of 5% $CaCl_2$ solution. If white precipitate formed immediately oxalic acid are present when Precipitate observed on shaking tartaric acid and precipitate observed on boiling and then cooling citric acid are present.

Confirmatory test for Oxalic acid: To 2 ml test solution added few drops of 5% lead acetate solution. White precipitate was formed.

Confirmatory test for Citric acid: To 2 ml test solution, added 1 drop of dilute NH_4OH and excess $AgNO_3$ solution. Boiled for 15 min. Blackish silver mirror was form [9, 10].

RESULTS

The results of phytochemical screening of different extracts of plant were reported in table 1. In the present study the dried powder of rubia cardifolia, moringa oleifera, melia azedarach, zizyphus jujube, brassica rapa were extracted with alcohol and the extracts was formed by soxhelet extraction method then this extracts was used for the preliminary phytochemical investigation.In phytochemical investigation we have performed the various chemical test for the presence of phytoconstituents. Phytochemical investigation of different extracts showed the presence of alkaloids, glycosides, tannins and proteins, vitamins etc. in ethanolic extracts [3, 4].

DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins and steroidsetc. in the extracts of different plants like rubia cardifolia, moringa oleifera, melia brassica rapa. These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids. These phytoconstituents may be responsible for the many pharmacological actions.The phytochemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. It can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future study or application. . Chemical studies reveal 2' -2' methyl cajanone, 2' -hydroxy genistein, isoflavones, cajanin, and cahanones etc., which impart antioxidant properties [11-14].

CONCLUSION

The presence of these phytochemicals make the plant useful for treating different ailments and have a potential of providing useful drugs of human use and further work aiming towards tracing out of phytochemicals present in it and pharmacological activities are in progress.

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