Phytochemical screening and analysis of different medicinal plants

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Abstract:
Phytochemicals are secondary metabolites produced by all plants in which some have medicinal use. The present study involves seven different medicinal plants ie. Curculigo orchioides, Nelumbo nucifera, Psoralea corylifolia, Punica granatum, Sitopaladi churna, Symplocos racemosa, Syzygium cumini. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, phenols in varying concentrations. The main objective of the work was to check the presence of the phytochemical constituents and their amount in all the selected medicinal plants. All the selected medicinal plants were found to contain steroids, alkaloids, carbohydrates and flavanoids. Moreover, triterpenoids are also present in Curculigo orchioides. On the other hand, alkaloids were absent in all plants except Punica granatum and saponins were absent in all plants except Symplocos racemosa. These phytochemicals are responsible for the medicinal properties of these plants.

Keywords:
Curculigo orchioides, Nelumbo nucifera, Psoralea corylifolia, Punica granatum, Sitopaladi churna, Symplocos racemosa, Syzygium cumini, Medicinal plants, phytochemicals, extraction, solvent, screening, estimation.

INTRODUCTION:

Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity [1,2]. These are the reservoirs of potentially useful chemical compounds. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [3, 5].

Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficiency. Thus, the present study deals with the screening based on phytochemical tests of six medicinal plants viz., Curculigo orchioides, Nelumbo nucifera, Psoralea corylifolia, Punica granatum, Sitopaladi churna, Symplocos racemosa, Syzygium cumini for identifying their chemical constituents [6-9]. All these plants possess different bioactivities which were later correlated with the presence of some specific phytoconstituents [10-20].

MATERIALS AND METHODS

Plant materials:

Fresh leaves of Curculigo orchioides(Family: Hypoxidaceae) and Syzygium cumini(Family: Myrtaceae), bark of Symplocos racemosa(Family: Symplocaceae) & Psoralea corylifolia(Family: Papilonaceae), seeds of

Nelumbo nucifera(Family: Nelumbonaceae), fruits of Punica granatum(Family: Punicaceae) and powder of Sitopaladi churna were collected from nearby areas.

Preparation of extracts:
The collected leaves, seeds, bark and fruits of different plants were washed well, shade dried and powdered. This powdered material was weighed in a selected quantity and is subjected to soxhlet extraction using various solvents in successive mode respectively for 48 hours. The solvent was then recovered using Rotary Vacuum Evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an air tight bottle. The crude extracts thus obtained were used for further investigation of Phytochemical screening.

Phytochemical tests:
The extracts of the dry powders were analyzed for the presence of various phytoconstituents like carbohydrates, reducing sugars, monosaccharide, tannins, saponnins flavonoids, terpenes /steroids, alkaloids, anthraquinones, cardiac glucosides and amino acids were identified using standard phytochemical procedures as described below.

1. **Test for steroids:**

Salkowski Test:
Few drops of concentrated sulphuric acid is added to the chloroform extract, shaken and on standing lower layer turns red in color.
Liebermann Burchard's Test:
To the chloroform solution of the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.
2. **Tests for triterpenoids:**
Salkowski Test:
Few drops of concentrated sulphuric acid is added to the chloroform extract, shaken and on standing, lower part turns golden yellow colour.

Lieberman Burchard's Test:
To the chloroform solution of the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

Ischugajiu Test:
Excess of acetylchloride and pinch of zinc chloride are added to the chloroform solution, Kept aside for reaction to subside and warmed on acetonitrile bath, cosinred color is produced.

Brickorn and BrinarTest:
To the chloroform solution, few drops of chlorosulfonic acid in glacial acetic acid (7:3) is added, red color is produced.

3. Test for saponins:

Foam Test:

Small amount of extract is shaken with little quantity of acetonitrile, the foam produced persisted for 10 minutes. It confirms the presence of saponins.

Haemolysis Test:

To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled acetonitrile is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence of saponins

4. Test for steroidal saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

5. Tests for triterpenoidal saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

6. Tests for Alkaloids:

Mayer's Test:

The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives creamy white precipitate.

Dragendroff's Test:

The acid layer with few drops of Dragendroff's reagent (Potassium bismuth iodide) gives reddish brown precipitate.

Wagner's Test:

The acid layer when mixed with few drops of Wagner's reagent(solution of iodide in potassium iodide) gives brown to red precipitate.

Hager's Test:

The acid layer when mixed with few drops of Hager's reagent(Saturated solution of pricric acid)gives yellow coloured precipitate.

7 Tests for Carbohydrates:

Molischs's Test:

The extract is treated with Molisch's reagent and conc .sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

Fehlings's Test:

The extract when heated with Fehling's A and B solutions gives an orange red precipitate showing the presence of reducing sugar.

Benedict's test:

The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

Barfoed'sTest:

Barfoed's reagent is added and boiled on acetonitrile bath for few minutes, reddish precipitate is observed for the presence of carbohydrate.

8. Test for Flavonoids:

Shinoda Test:

The alcoholic solution with few fragments of magnesium ribbon and concentrated hydrochloric acid produced magenta color after few minutes.

Ferric chloride test:

Alcoholic solution of root extract reacts with freshly prepared ferric chloride solution and gave blackfish green color.

Lead Acetate Test:

Alcoholic solution of root extract reacts with 10% lead acetate solution and gave yellow precipitate.

9. Test for Glycosides:

Anthraquinone test:

The plant powder was extracted with either ammonia or caustic soda. The aqueous layer shows pink color

Keller-killiani test:

This is for cardiac glycosides. The plant extract was mixed with 0.4 glacial acetic acid, ferrous chloride and 0.5ml of concentrated sulphuric acid. The acetic acid layer shows blue color.

***10.* Test for Phenolic compounds:-**

Ferric chloride test:-

Treat the extract with ferric chloride solution then blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Gelatin test:-

To the test solution add 1% gelatin solution containing 10% NaCl, and then ppt is formed.

Test for chlorogenic acid:-

Treat the test solution with aqueous ammonia and expose to air gradually, green color is developed.

Quantitative estimation of alkaloids:

Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled acetonitrile until completely dissolved and the solution was diluted to 1000 ml with distilled Acetonitrile. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled acetonitrile) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled acetonitrile). Atropine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, USA) in 10 ml distilled acetonitrile.

To 1ml of extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with4 ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank

prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative estimation of saponins:
Measured quantity of extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a acetonitrile bath at 60°c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with Diosgenin equivalents.

Quantitative estimation of steroids:
1ml of extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a acetonitrile-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled acetonitrile. The absorbance was measured at 780 nm against the reagent blank.

Quantitative estimation of phenoilc compounds:
The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.**In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled acetonitrile and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph was shown in graph A and table 2.

Quantitative Estimation of Flavanoids Compounds
In the procedure, different concentrations of the extracts were mixed with 0.5ml of 5% $NaNO_2$ and allowed to stand for 5 minutes and add 0.5ml of 10% aluminium chloride. Then stand for another 5 minutes to react, add sodium hydroxide solution. Finally make upto 10 ml with distilled water. Absorbance of the sample was measured against the blank at 510nm using a spectrophotometer. A calibration curve was constructed using Quercetin solutions as standard and total flavanoids content of the extract was expressed in terms of milligrams of Quercetin per gram of dry weight and the standard graph was shown in graph A and Table 2

Quantitative estimation of Carbohydrates:
One gram (1g) of extracted sample was placed in 25ml bottle, 10ml of distilled water was then added and shaken vigorously followed by addition of 15cm3 of 52% perchloric acid. This was stirred continuously for 30minutes and the mixture was later filtered using Whatman filter paper. One milliliter (1ml) of the filtrate was mixed with 4 Cm^3 of Anthrone reagent in a test tube and the absorbance of the mixture was measured using spectrophotometer at a wavelength of 620nm. The total soluble carbohydrate was then estimated using the standard curve of Glucose.

Quantitative estimation of iron:

To 1ml of ethyl acetate extract add small amount of hydroxylamine hydrochloride and heat for 5 minutes. Then 1,10 phenanthralene solution was added. Orange red color solution indicates positive for this test. Absorbance of sample was measured against the blank at 529 nm using a spectrophotometer.

Results and discussion:

The data shown in Table 1 shows screening of aqueous extracts of different parts of six medicinal plants viz., Curculigo orchioides, Nelumbo nucifera, Psoralea corylifolia, Punica granatum, Sitopaladi churna, Symplocos racemosa, Syzygium cumini based on phytochemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. The observations and inferences made in the phytochemical tests are presented in table 1. Quantitative analysis of positive compounds in the all selected plant extracts was conducted. Total steroid content present in the extract are expressed in terms of cycloartenol equivalent units by considering cycloartenol as a standard Steroid. Total flavanoid content present in the extract are expressed in terms of quercetin equivalent units by considering quercetin as a standard flavanoid. Total carbohydrate content present in the extract are expressed in terms of glucose equivalent units by considering glucose as a standard. Total saponins content present in the extract are expressed in terms of diosogenin equivalent units by considering diosogenin as a standard saponin.

Psoralea corylifolia contains steroids, flavanoids and total steroid content present in the ethyl acetate extract was found to be 25.53µg equivalent to Cycloartenol units. The total Steroid content in the water extract was found to be 49.37µg equivalents to Cycloartenol units. Total flavanoid content present in the water extract was found to be 86.77µg equivalent to Quercetin units. In *Curculigo orchioides,* Triterpenoids were present in chloroform and water extracts and carbohydrates were found in chloroform extract. On quantitative study, 269micrograms of carbohydrates were found in every 10mg of the dry plant extract. This confirms that the plant has source of carbohydrates. *Symplocos racemosa contains the steroid and saphonins in the extracts.* Total saponins content present in the Ethyl acetate extract were found to be 13.62µg equivalent to diosogenin units. The total content of triterpenoidal saponins in the ethyl acetate extract was found to be 11.86 µg equivalents to diosogenin units. Total Steroid content present in the Ethyl acetate extract was found to be 3.14µg equivalent to cycloartenol units. The total content in the methanol extract was found to be 3.77 µg equivalents to cycloartenol units. In *Nelumbo nucifera,* flavanoids and carbohydrates were reported. Total flavanoid content present in the water extract was found to be 123.5µg equivalent to quercetin units. Total carbohydrate content present in the water extract was found to be 47.31µg equivalent to glucose units.

In *Punica granatum* flavanoids and steroids were identified and estimated. Total flavanoid content present in the water and acetone extract were found to be 6.9µg and 11.5µg equivalent to quercetin units respectively. Total steroid content present in the acetone and water extract were found to be 3.66µg and 5 µg equivalent to cycloartenol units respectively. Flavanoid and carbohydrates were reported in *Sitopaladi churna* plant. Total flavanoid content present in the water extract was found to be 123.5µg equivalent to quercetin units. Total carbohydrates present in the water extract were found to be 47.31µg equivalent to glucose units. Steroid and carbohydrates are observed in *Syzygium cumini* plant extracts. Total steroid content present in the chloroform and acetonitrile extract was found to be 2.36 µg and 3.63 µg equivalent to

cycloartenol units respectively. Total carbohydrates content present in the chloroform was found to be 1.44µg equivalent to glucose units.

Conclusion:

Results reveal that selected seven different medicinal plants ie. *Curculigo orchioides, Nelumbo nucifera, Psoralea corylifolia, Punica granatum, Sitopaladi churna, Symplocos racemosa, Syzygium cumini* contain various phytochemicals like alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, phenols. Estimated amount of the reported phytochemicals reveals the significance of the above selected plants for their medicinal activity.

S.no	***Sec.* Metabolites**	***Curculigo orchioides***		***Nelumbo nucifera***		***Psoralea corylifolia***		***Punica granatum***	
		Chloroform	**Water**	**Ethyl Acetate**	**Water**	**Ethyl acetate**	**Water**	**Acetone**	**Water**
1	Steroids	-----	-----	-----	-----	+	+	+	+
2	Triterpenes	+	+	-----	-----	-----	-----	-----	-----
3	Saponins	-----	-----	-----	-----	-----	-----	-----	-----
4	Alkaloids	-----	-----	-----	-----	-----	-----	-----	-----
5	Carbohydrates	+	-----	-----	+	-----	-----	-----	-----
6	Flavonoids	-----	-----	-----	+	-----	+	+	+
7	Phenolic compounds	-----	-----	-----	-----	-----	-----	-----	-----
8	Iron	-----	-----	+	-----	-----	-----	-----	-----

S.no	***Sec.* Metabolites**	***Sitopaladi churna***		***Symplocos racemosa***		***Syzygium cumini***	
		Ethyl Acetate	**Water**	**Ethyl acetate**	**Methanol**	**Ethyl acetate**	**Acetonitrile**
1	Steroids	--	--	+	+	+	+
2	Triterpenes	--	--	--	--	--	--
3	Saponins	--	--	--	+	--	--
4	Alkaloids	--	--	--	--	--	--

5	Carbohydrates	--	+	--	--	+	--
6	Flavonoids	--	+	--	+	--	--
7	Phenolic compounds	--	--	--	--	--	--
8	Steroidal saponins	--	--	+	--	--	--
9	Triterpenoidal saponins	--	--	+	--	--	--

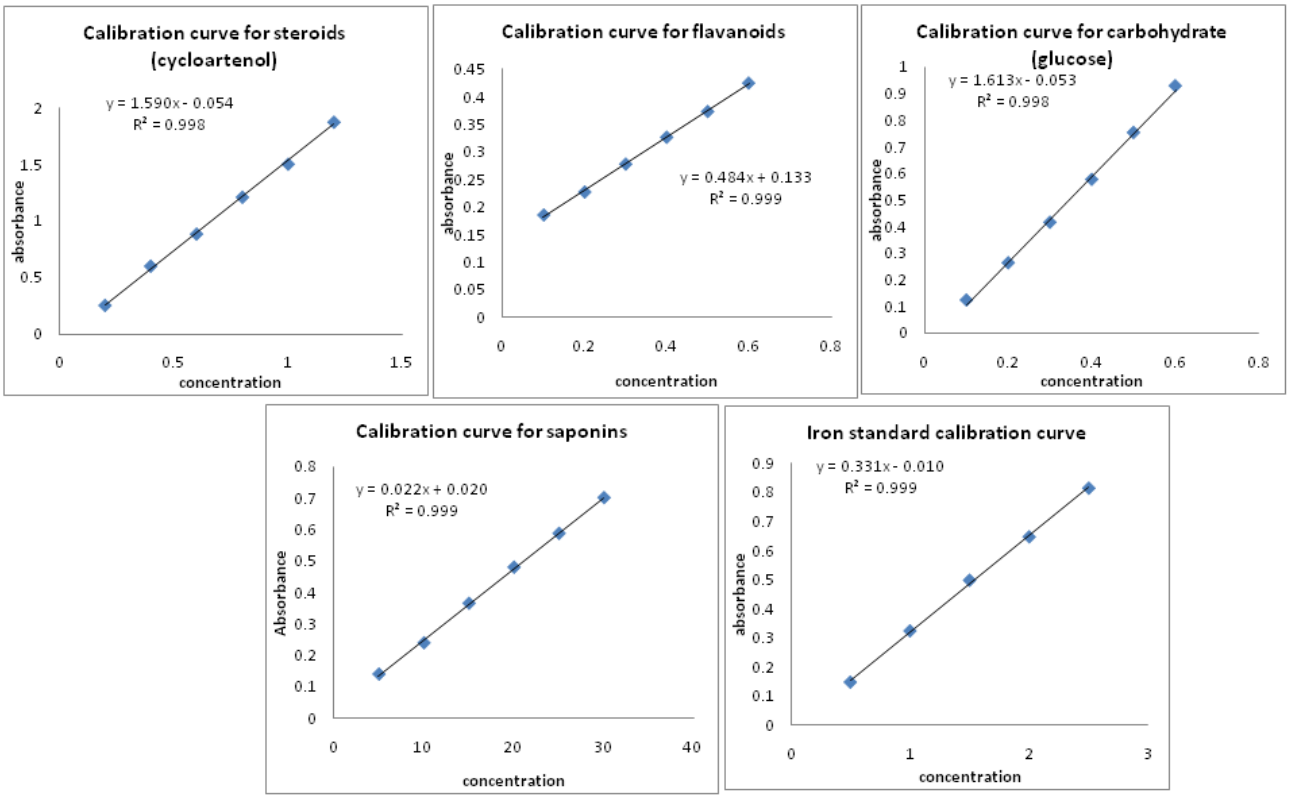
(+) = Presence, (-) = Absence

Table 1: Results of phytochemical analyses of the selected seven medicinal plants

Steroids		**Flavanoids**		**Carbohydrates**	
Concentration	**Absorbance**	**Concentration**	**Absorbance**	**Concentration**	**Absorbance**
0.2	0.258	0.2	0.185	0.1	0.235
0.4	0.605	0.4	0.227	0.2	0.318
0.6	0.889	0.6	0.278	0.3	0.405
0.8	1.215	0.8	0.327	0.4	0.498
1.0	1.507	1.0	0.374	0.5	0.584
1.2	1.878	1.2	0.426	0.6	0.678
Correlation coefficient: 0.998 Slope: 1.590 Intercept: -0.054		Correlation coefficient: 0.999 Slope: 0.242 Intercept: 0.133		Correlation coefficient: 0.999 Slope: 0.242 Intercept: 0.133	

Saponins		**Iron**	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
5	0.138	0.5	0.148
10	0.238	1	0.324
15	0.364	1.5	0.498
20	0.479	2	0.647
25	0.587	2.5	0.814
30	0.701		
Correlation coefficient: 0.999 Slope: 0.022 Intercept: 0.020		Correlation coefficient: 0.999 Slope: 0.331 Intercept: 0.010	

Table 2: Standard curve results for secondary metabolites:



Graph A: Calibration graphs of standard steroids, flavanoids, carbohydrates, saphonins, Iron

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