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Research Article

Phytochemical Analysis and *Invitro* Antioxidant Activity of Indigenous Medicinal Plants

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Abstract:

Objective: This objective of study to evaluate the polyherbal formulation of ethanolic extracts of *Swertia chirayita*and *Ficus glomerata* for their potential antioxidant properties.

Methods: The ethanolic extracts of *Swertia chirayita* and *Ficus glomerata* were compounds were done by the method of soxhlation. In vitro antioxidant activity was evaluated by DPPH scavenging activity.

Result: Results obtained of antioxidant activity using DPPH method, the effect of antioxidant on DPPH isbelieved to be due to their hydrogen-donating ability.The dose-response curve of DPPH radical scavenging activity of hydroalcoholic extracts of *Ficus glomerata.* It was observed that the methanol extract showed IC 50 value of 72.23 µg/ml ascompare to standard ascorbic acid 17.89 µg/ml.

Conclusion: It was concluded that the dose-response curve of DPPH radical scavenging activity of various methanolic extracts of *Swertia chirayita*and*Ficus glomerata* having promising in vitro antioxidant activity.

Keywords: Anti-oxidant effect, *Swertia chirayita*, *Ficus glomerata*, DPPH, soxhlation

Introduction

Medicinal plants are still a valuable source of new pharmaceutical products and natural products still remain as one of the best reservoir of new structural-typed bioactive compounds[1]. More than 25% of modern medicine comes from natural products and another 25% are structural modification of the lead compounds from natural source[2]. It is estimated that only 15% of higher plants have been investigated for potentially useful biological activity. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Many traditional plant treatments for diabetes are used throughout the world. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one[3].

Herbal medicines were considered to be less toxic with fewer side effects than synthetic drugs. In the Ayurvedic system of medicine, as mentioned in ancient Indian books like

Charak, Samhita, Mahdhav Nidan and AstangSanghra, there are about 600 plants, which are stated to have antioxident property[4]. Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent their possible use in the treatment of Antioxdent activity[5].

*Ficus glomerata*is found almost in all parts of India as a naturally occurring and eatable fruit bearing plant. The plant is a large deciduous tree distributed all over India from outer Himalayan ranges, Punjab, Maharashtra, Bihar, Orissa, West Bengal, Rajasthan, Deccan and up to South India.

Swertia chirayita (Gentianaceae), a popular medicinal herb indigenous to the temperate Himalayas is used in traditional medicine to treat numerous ailments such as liver disorders, malaria, and diabetes and are reported to have a wide spectrum of pharmacological properties. Its medicinal usage is well-documented in Indian pharmaceutical codex, the British, and the American pharmacopeias and in different traditional medicine such as the Ayurveda, Unani, Siddha, and other conventional medical systems. The wide range of medicinal uses include the treatment of chronic fever, malaria, anemia, bronchial asthma, hepatotoxic disorders, liver disorders, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, ulcers, scanty urine, hypertension, melancholia, and certain types of mental disorders, secretion of bile, blood purification, antioxidant and diabetes[7,8].

Hence, in the present study it has been planned to investigate the phytochemical and invitro antioxidant activity of the leaves extracts of *Swertia chirayita* and fruit extract of *Ficus glomerata*to verify potentials of the plants with scientific approach.

Materials and Methods:

Materials:

The parts of the plants were collected and authenticated. The identified and authenticated materials or spices were collected in sufficient quantity, dried and powdered for further studies.

Drying and Size Reduction of Plant Material

The *Swertia chirayita* and *Ficus glomerata* were pulverized to moderately coarse powder. The coarse powder of rhizomes was passed through sieve No. 16 to maintain uniformity and stored in cool and dry place for further study.

Preparation of *Swertia chirayita* and *Ficus glomerata* Extract

a) Extraction of *Swertia chirayita*

Soxhlation method was used for extraction of *swaritachirayta*using hydroalcohol (ethanol: water; 75:25) as the solvent for extraction, 250 ml of solvent was placed in thimble with crushed leaves and inserted in soxhlet extractor. The side arm was covered with glass wool.The process should run for a total of 16 hours. Extract was dried on Rota evaporator then the extract was collected[9].

b) Extraction of *Ficus glomerata*

Extraction of *Ficus glomerata* was carried out in the similar method using hydroalcohol (ethanol: water; 75:25) as the solvent and soxhlation was carried out for 16 hours depicted in **Figure 1**. Finally the extract was dried on Rota evaporator then the extract was collected[9].

Figure 1: Soxhlet extraction of Plant Materials

Physicochemical evaluation

- **Determination of foreign matter**

Under the shade, plant materials were spread and dried. Plant materials were inspected with a lens for the presence of foreign material after proper drying. Foreign materials were manually removed and weighed to determine the percentage of foreign material present in dried crude drug[10].

Determination of ash values

Total ash, acid insoluble ash, water-soluble ash, and sulphated ash are the four methods for determining ash values[11].

a) Total ash

Total ash value denotes the amount of material that remains after ignition, including both physiological and non-physiological ash content. Plant materials were properly dried in the shade and ground into fine powder before being weighed and placed on a previously ignited and tarred silica crucible.The temperature was gradually raised to 500-600 °C until the powdered plant material became white, indicating complete combustion of the carbon present. The remaining material is cooled and weighed. If carbon-free material cannot be obtained, the crucible is cooled and a saturated ammonium nitrate solution is added to moisten the residue.The moistened residue is then ignited to constant weight, cooled in a desiccator, and reweighed. The ash percentage in relation to the air dried drug was calculated.

b) Acid-insoluble ash

After boiling the total ash content in dilute hydrochloric acid and igniting the remaining insoluble matter, the acid soluble content is calculated. To the total ash content in the crucible, 25 mL of hydrochloric acid was added. 5 mL of hot water was added to the crucible, and insoluble matter was collected and washed on ash-free filter paper until the filtrate became neutral. The insoluble matter was returned to the original crucible and ignited to constant weight. The residue was weighed after cooling in a vacuum desiccator for 30 minutes. The percentage of acid-insoluble ash was calculated with reference to air dried drug.

c) Water-soluble ash

Water soluble ash content is the difference in ash content between total ash and the residue remaining following total ash treatment with water. The total ash content obtained in the crucible was heated with 25 cc of distilled water. The insoluble particles was collected using ash-free filter paper and washed with

hot water. After rinsing, it was burnt in a crucible for 15 minutes at 450 °C, and the ash was collected. To calculate the water soluble ash, the weight of the resulting residue was deducted from the weight of the total ash content.

Method

25 milliliter of distilled water was added to the crucible containing the complete ash and heated for 5 minutes. The insoluble materials was collected in an ash-free filter paper or a sintered glass crucible. After being washed with hot water, the ash was burned in a crucible for 15 minutes at a temperature not exceeding 450 °C and weighed. The weight of this residue was deducted from the overall ash weight. The ash percentage in relation to air dried medication was calculated[12].

d) Sulphated ash

After heating a silica crucible to redness for 10 minutes, it was weighed after cooling in desiccators. 1 g of the substance under study was placed to the crucible, which was then more precisely studied. In a Muffle furnace, the crucible was then gradually ignited until thoroughly scorched. The residue was cooled, soaked with 1 cc of concentrated sulphuric acid, and gradually heated until free of white vapours, then ignited at 800 + 25 °C until free of all black particles. The ignition occurred in an area free of air currents. The crucible was allowed to cool after a few drops of sulfuric acid were added before being heated.

Phytochemical screening

Phytochemical screening activity of leaves powders of the *swaritachirayita* and fruits powder *ficus glomerata*were carriedout to analysed the presence of the following compounds present in the plants such as[13, 14]:

Test for Alkaloids:

Alkaloids exist as the salts which are organic acids forms. They are easily soluble in water andalcohols. Hence for the study the solvent chemicals which were used were Hydroalcohol(ethanol: water; 75:25). Various tests are there to determine the alkaloid content in the plantextract.

- **Meyer's Test**

Few drops of the Meyer is reagent, 5mg of Hydroalcoholic extract was added. White and pale-yellow precipitate formation indicates the presence of Alkaloids.

- **Dragendorff'sTest**

To 5 mg of the Hydroalcoholic extract 5ml of distilled water was added, few drop ofhydrochloric acid was added until the acid reaction occurs. To this few drop of Dragendorff'sisreagent was added, formation of orange or orange red precipitate shows the presence of alkaloid.

- **Wagner's test**

5mg of Hydroalcoholic extract with 1.5% of hydrochloric acid. To this few drop of Wagner's reagent was added. Brown ppt indicates the presence of alkaloid.

Tests for Glycosides

Glycosides are the group of the plant secondary metabolites which are present in the form ofsugars. They are the molecules which bound to another functional group with glycosidic bonds.They play important roles in living organisms. Many of the plants stores this chemicalscompounds in inactive glycoside which can be made active by enzyme hydrolysis, Test todetermine the glycosides as follows.

- **Killer-killani test**

Dissolved2 ml of extract in Glacial acetic acid and then added one drop of 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown color appeared at the junction of the two liquid layers and upper appeared bluish green indicated the presence of glycosides.

- **Baljet's test**

To 1ml of the test extract, 1ml of sodium picrate solution was added and the yellow to orange color revealed the presence of glycoside.

- **Foam test**

0.5gm extract vigorously shaken with water than formation of a layer of foam. It's indicated the presence of glycosides

Test for Carbohydrates

Carbohydrates are the group of the plant secondary metabolites which are present in the form ofsugars. They may be present in the form of monosaccharide, disaccharide and oligosaccharide.

There are various tests to determine the carbohydrates in plant extract.

- **Benedict's test**

To 5ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of red precipitate indicated the presence of sugars.

- **Molisch's test**

A small fraction of extract was taken in ethanol separately and a few drops of 20% w/v solution of α-napthol in ethanol (90%) were added to it. After shaking well, about 1 ml of concentrated sulphuric acid was allowed to flow carefully by the side of the test tube. A reddish violet ring at the junction of the two layers indicated the presence of carbohydrates.

- **Fehling's test**

Extract heated with dil. HCL than neutralized with NaOH than added fehling's solution A & B. Brick red precipitate was formed. It's indicated the presence of carbohydrates.

Test for Phenolic Compounds and Tannins

- **Ferric chloride test**

1ml of extract was taken in a test tube and to it 2 ml of 5% neutral ferric chloride solution was added. Appearance of dark blue colour indicates the presence of phenolic compounds and tannins.

- **Lead tetra acetic acid test:**

1 ml of extract was dissolved with 2 ml of lead tetra acetate solution and formation of precipitate indicates the presence of phenolic compounds and tannins.

- **Bromine water test**

10 ml of bromine water was added to 2 ml of extract taken in test tube. The decoration of bromine water indicates the presence of tannins.

Test for Flavonoids:

Flavonoids are the group of secondary compounds which are present in plants and playsimportant role in various plant metabolic activities. Qualitative detection of alkaloids can bedone by different methods.

- **Alkaline reagent test**

To 10mg of Hydroalcoholic extract 2ml of sodium hydroxide solution is added. Formation ofintense yellow colour which on addition of 0.1% HCL gets colorless which indicated thepresence of Flavonoid.

- **Shinoda test**

10mg of the Hydroalcoholic extract was dissolved in the irrespective diluents. To this10 drops ofdilute HCL was added and small pieces of magnesium was added. Formation of pink, brown orreddish color ppt indicates the presence of Flavonoid.

- **Lead acetate Test**

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Tests for Steroids

- **Salkowski test**

The extract was dissolved in chloroform and equal volume of conc. H_2SO_4 was added. Formation of bluish red to cherry color in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

- **Liebermann-Burchard test**

A small portion from extract was taken with about 1 ml of acetic anhydride and dissolved by warming. The contents were cooled and a few drops of concentrated sulphuric acid were added in each case by the sides of the test tube. Appearance of blue colour indicated the presence of sterols.

Test for Proteins

- **Biuret test**

Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

Tests for Saponins

- **Froth test**

A little fraction of extract was boiled with about 1 ml of distilled water and shaken. Appearance of a characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extract were tested directly.

- **Foam test**

A little fraction of extract was taken with about 2 ml of distilled water. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of Saponins. Aqueous and alcoholic extract were tested directly.

Estimation of Total polyphenol content (TPC)

The total polyphenol content of the extract was estimated using the FolinCiocalteu reagent based assay. 5-50 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. Mixture was administered with 1ml of Folin-Ciocalteu's phenol reagent was shaken for 5 min. Folin-Ciocalteureagent is a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic ($H_3PMo_{12}O_{40}$) acids, which is reduced to blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) during polyphenol oxidation[15].To the above obtained mixture 10 ml of 7% Na_2CO_2 solution was added and the volume was diluted up-to 25ml with methanol. The absorbance of the developed colour was recorded at 765 nm using a UV-Vis spectrophotometer. All determinations, for gallic acid as well as the plant extract, were carried out in triplicate. Data are represented as an average of the three determinations. Using these readings, a calibrated gallic acid standard curve was made. Based on the measured absorbance of the plant extract, the concentration of phenolic was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material)[16].

Estimation of Total flavonoids content (TFC)

Total flavonoid content estimation was done by the help of colorimetric assay based on aluminum chloride method. The 10 mg quercetin was dissolved in 10 ml methanol and various aliquots of 5, 10, 15, 20, and 25µg/ml were prepared in methanol. And the 10 mg of dried extract of were dissolved in 10 ml methanol and filter. 3 ml (1 mg/ml) of this solution was used for the evaluation of flavonoid. In addition, 1 ml of 2**%** $AlCl_3$ methanolic solution was added to 3 ml of extract or normal and allowed to stand at

room temp. for 15 min. absorption was measured at 420 nm[16].

In-Vitro Antioxidant Activity:

In-vitro antioxidant activity of ethanolic extract of *Swertia chirayita*and *Ficus glomerata* using DPPH method

DPPH scavenging activity was measured by the spectrophotometer [17]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Results and Discussion:

Physico-chemical evaluation:

Foreign matter of selected plants*Swertia chirayita* showed highest foreign content among the selected plants.Ash value obtained from the extract were summarized in Table 1.*Swertia chirayita*showed highest ash content (Total ash, water soluble, acid insoluble and sulphated ash value).

Table 1: Foreign matter of the selected plants

Plants	*Swertia chirata*	*Ficus glomerata*
Foreign matter	**2.8 ± 0.16**	**2.3 ± 0.73**
Total ash	8 ± 0.14	6.5 ± 0.64
Water soluble	2.91± 0.042	2.45 ± 0.73
Acid insoluble	0.61 ± 0.27	0.38 ± 0.52
Sulphated	0.81 ± 0.42	0.98 ± 0.63

n=3

Phytochemical analysis of crude extracts:

Various phytochemical analysis performed to determine the presence of various biological component in the extract of *Ficus glomerata*and *Swertia chirayita* proved the presence of component like alkaloids, glycosides, steroid, phenolic compound, flavonoids, and absence of component like carbohydrate was found shown in table no 2.

Table 2: Phytochemical screening of *Ficus glomerata and Swertia chirata*

S. No.	Constituents	*Ficus glomerata*	*Swertia chirata*
1.	**Alkaloids**		
	Mayers's Test:	+ve	+ve
	Dragendorff's test	+ve	+ve
	Wagner's test	-ve	+ve
2.	**Glycosides**		
	Killer-killani Test:	-ve	-ve

	Baljet test Foam test	+ve -ve	-ve +ve
3.	**Carbohydrates** Molisch's test Fehling test Benidict test	 -ve -ve +ve	 +ve +ve -ve
3.	**Flavonoids** Lead acetate Test: Shinoda test	 +ve +ve	 +ve +ve
4.	**Steroids** Lebermamm-Burcard Test: Salkowski test	 -ve -ve	 +ve -ve
5.	**Phenol** Ferric Chloride Test:	 +ve	 +ve
6.	**Proteins** Biuret Test:	 -ve	 +ve
8.	**Saponins** Froth Test:	 +ve	 +ve

Quantitative estimation of total polyphenol content:

Total phenolic content estimation was performed using FolinCiocalteu reagent. The polyphenolic content was expressed in the terms of gallic acid with *ficus glomerata* and *Swertia chirata*equivalent and was found to be 16.63 GAE mg/gm and 23.64 GAE mg/gmrespectively.

Quantitative estimation of Flavonoid content:

Total flavonoid content estimation performed using colorimetric assay by Aluminum chloride method. Flavonoid content was expressed in terms of standard quercetin with *ficusglomerata* and*Swertia chirata*was found to be 20.33 ± 0.63QE mg/g and 23.64 ± 0.63 QE mg/g respectively.

Table No. 3: Total Phenolic and flavonoids content in different Plant extract

S. No.	Different plant Extracts	Total Phenol (mg/gm)	Total Flavonoids (mg/gm)
1.	***Ficus glomerata***	16.63 ± 0.48	20.33 ± 0.63
2.	***Swertia chirata***	23.64 ± 0.54	23.64 ± 0.63

Antioxidant activity

Antioxidant activity of extract from *Ficus glomerata* was determined using DPPH method. The antioxidant property is expressed as the % inhibition of the free radicals present in 1,1-diphenyl-2-picrylhydrazyl(DPPH) which was used as a reagent. Percentage inhibition of free radicals by the extracts of fruits extract of *Ficus glomerata*and leaf extract of *Swarita Chirayta*compared with standard ascorbic acid are shown in table no 5. It was observed that the fruit extract and leaf extract showed IC 50 value of 130.99µg/ml ascompare to standard ascorbic acid 21.74 µg/ml. The extracts clearly demonstrated protondonating capacity, which might function as free

radical inhibitors or scavengers, perhaps acting as major antioxidants. As a result, the methanol extract of this plant has the greatest potential to scavenge DPPH radicals.

Table 4:% Inhibition of ascorbic acid,*Ficus glomerata*and*Swertia chirayta* hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition		
		Ascorbic acid	*Ficus glomerata*	*Swertia chirata*
1	10	28.33±0.48	10.08±0.27	26.29±0.34
2	20	38.17±0.54	19.08±0.19	31.21±0.46
3	40	43.33±0.61	33.13±0.22	37.21±0.18
4	60	58.10±0.18	44.41±0.53	45.62±0.64
5	80	67.22±0.54	50.42±0.57	49.46±0.81
6	100	77.31±0.37	55.22±0.36	59.18±0.37
IC 50		47.75±0.54	80.74±0.45	76.07±0.28

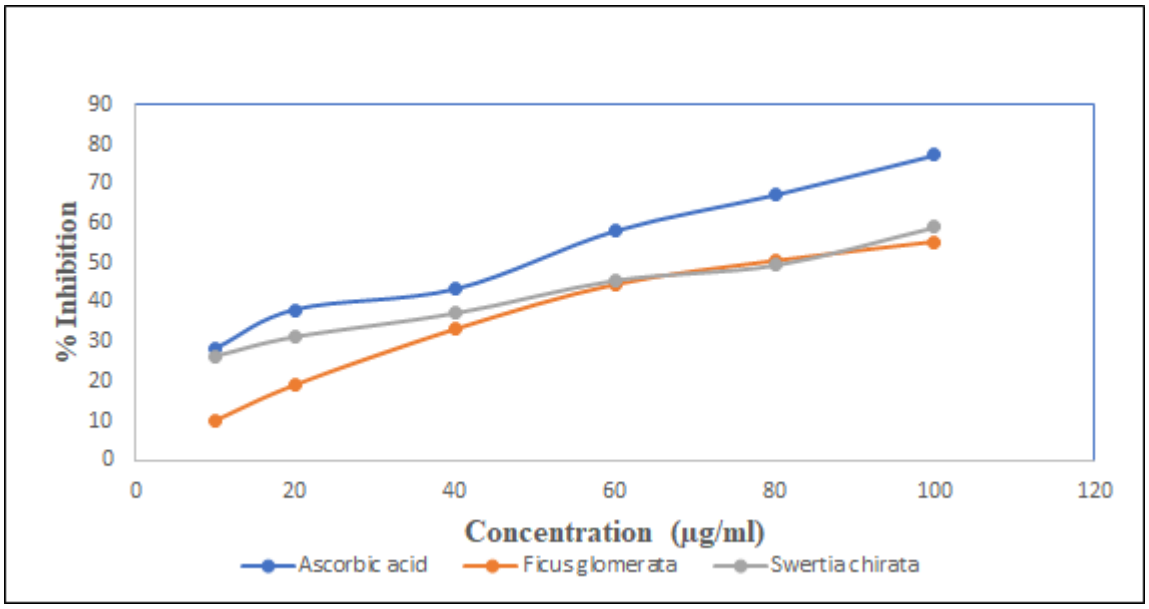

Figure 2: % Inhibition of ascorbic acid,*Ficus glomerata* and*Swertia chirata* hydroalcoholic extract using DPPH method

Conclusion:

Antioxidant properties obtained from natural extracts can be a promising alternative towards the use of synthetic oxidants and could help in avoiding the side effects that occurs due to use of synthetic antioxidants. The data obtained from this research prove to support the view that hydroalcoholic extract obtained from Ficus glomerata showed effective free radical scavenging activity. Besides antioxidant properties found in extracts of *Ficus glomerata* and *Swertia chirata*, a correlation can be observed between total phenolic content and flavonoid content with that of antioxidant activity. Further, the result obtained this research supports the use of hydroalcoholic extract of *Ficus glomerata* and *Swertia chirata*as a preventive agent in pathogenesis of some metabolic disorders. Proceeding

further with more extensive research on chemical composition and understanding of mechanism of action could help in development of drug for its therapeutic application.

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