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Original Research Article

Qualitative and quantitative analysis of phytochemicals of barleriaprionitis

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Abstract

Phytochemical analysis of plant products took a distinct place in organic chemistry as well as plant biochemistry in recent years. One of the challenges of phytochemistry is to carry out all the above operations on vanishingly small amounts of material. Frequently, the solution of a biological problem in, say, plant growth regulation, in the biochemistry of plant-animal interactions, or in understanding the origin of fossil plants depends on identifying a range of complex chemical structures which may only be available for study in microgram amounts. The present study investigates the qualitative and quantitative analysis of the major phytochemicals from leaves of medicinally important plant *B.prionitis.* Three solvent extraction system i.e. ethanol, ether and chloroform were used for this study. In ethanolic extract alkaloid, saponin, phenolic, carbohydrate, amino acid glycosides and flavonoids were found. In ether extract. Alkaloids and in Chloroform flavonoids were detected. In quantitative analysis the highest percentage of alkaloids were found. The preliminarystudy of phytochemials in *B.prionitis*confirms the presence of chemical constituents which can be used as diuretics in urinary problems.

Key Words: Diuretics, Phytochemical, Alkaloids, flavonoids, saponin, phenolic, carbohydrate, amino acid glycosides, etc.

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INTRODUCTION

Phytochemical analysis of plant products took a distinct place in organic chemistry as well as plant biochemistry in recent years. It is concerned not only the variety of organic substances accumulated by plants but also helps to determine chemical structure, distribution and biological function of plant substances. Thus, advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. One of the challenges of phytochemistry is to carry out all the above operations on vanishingly small amounts of material. Frequently, the solution of a biological problem in, say, plant growth regulation, in the biochemistry of plant-animal interactions, or in understanding the origin of fossil plants depends on identifying a range of complex chemical structures which may only be available for study in microgram amounts. *Barleriaprionitis* (Sanskrit: *Kuranta*; Marathi: *Vjradanti*) also known as the Porcupine Flower, is a species of plant of family *Acanthaceae*, native to India, Sri Lanka and Eastern Southern and Central Africa. The members of this family are mostly found in tropical to subtropical forests especially in the damp and marshy places. Phytochemicals defined in the strictest sense, as chemicals produced by plants. However, the termis generally used to describe chemicals from plants that may enhance health status of organisms, but are not essential nutrients (Srivastava *et al*. 2011). Because plant based foods are complex mixtures of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contain those Phytochemicals (Manjula *et al*. 2009). The most important of these bioactive constituents of plants are alkaloids, tannins,

flavonoids and phenolic compounds (Hill, 1952). Phytochemicals have been identified from plants (Taizand Zeiger, 2006). The phytochemical constituents of the medicinal plants were recorded by a number of workers (Joshi, 2000, Syed and Usha, 2005and Ramasubbu and Chandra Prabha, 2009). In India thousands of plant especially the angiosperms that all being exploited by the natives in tribal in variety of ways. The most important utilization of the seplantsist heir application in medicines (Camciuc *et al*., 1998andFelteretal.,2007). Ingeneral plants contain flavonoids that can either occurasagly conesoras O-or C-glycosides. Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities (Gurib-Fakim, 2006). Plant and their parts and the pattern of administration vary from person to person. Hence, in the present study it was aimed to screen the phytoconstituents of *B.Prionitis*for its optimum use.

MATERIALSANDMETHODS

In the present study the plant B. Prionitis was collected in earlymorning from around, Arogya Kendra, an area in Sant Hirdaram Nagar, Bhopal, India.

The experimental plant: Fresh matured plant leaves were obtained from the plant. The leaves were air dried under shade and hand crushed to obtain a 138.1 g. The plant material was shadowd ried for one week, the nitpowdered with the help of mixer grinder and used for preparation of plant extract.

Extraction of plant Material: Extraction was carried out in 500ml Ethanol at 60^{o}C for 8 hours using the soxhlet extractor. After extractionthe extracts were dried at room temperature untile xtractsolidifies.

Preliminary Phytochemical Analysis: Different organic solvent extracts of B. Prionitiswere used to screen the following phytochemicals likes terol, reducing sugar, alkaloids, phenoliccompounds, flavanoids, tannins, saponins, aminoacids, glycosidesand terpenoids. Phytochemical qualitative tests were carried out in extracts following the standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The various extracts of B. Prionitis obtained with different organicsolvents were subjected to same phytochemicaltests to screen the following phyto chemicals.

Qualitative Screening: Qualitative analysis was done to identify the presence of the following phyto constituents; alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids using standard procedures. These methods are given by Trease and Evans

Tests for Alkaloids:

a. **Dragendroff's Test:** About 0.2 g of the extracts was warmed with 2% H_2S0_4 for two minutes. It was filtered and few drops of Dragendroff's reagent was added. Orange red precipitate indicates the presence of alkaloids.
b. **Mayer's test:** To a few ml of filtrate, a few drops of Mayer's reagent was added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids. Each of the crude extracts was treated with dilute HCl andthe tests were performed with the filtrate.
c. **Wagner's Test**: Addition of Wagner's reagent to thefiltrate results in formation of reddish brownprecipitate indicating the presence of alkaloids.
d. **Hager's Test**: Filtrates were treated with Hager'sreagent. Formation of yellow colored precipitateindicates positive test.

Tests for Flavonoids

a. **Alkaline reagent test:** Extract was treated with 10 % NaOH solution; formation of intense yellow colour indicates presence of Flavonoid.
b. **NH_4OH test:** 3 ml of extract was treated with 10 % NH_4OH solution, development of yellow fluorescence indicates a positive test.
c. **Mg-turning test:** Extract was treated with Mg turning and add conc. HCl to this solution then add 5ml of 95 % ethanol, formation of crimson red colour indicates Flavonoid.
d. **Zn test:** 2 ml extract was treated with Zn dust and conc. HCl development of red colour indicates presence of Flavonoid.

Test for Phenolic compounds: The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Test for glycoside: Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genuine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Test for steroid: 20mg of the extract was treated with 2.5 ml of acetic anhydride and 2.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids and green bluish colour for steroids.

Test for tannins: To 0.5 ml of extract solution 1ml of water and 1- 2 drops of ferric chloride solution were

added. Blue color was observed for Gallic tannins and green black for catecholic tannins.

Test for amino acids

a. **Ninhydrintest:** To 1ml of sample, add 5drops of Ninhydrin Reagent. Heated in a boiling water bath for 2 min. a purple color indicates the presence of amino acids.
b. **Xanthoproteic test** To 3ml of the sample, add 1ml of concentrated nitric acid and heated for 3min. Then cooled and added 0.5 ml of NaOH. Reddish orange color indicates the presence of aromatic amino acids.
c. **Folin's test:**To 1ml of sample, add 1ml of Folin's phenol reagent followed by the addition of 1N sodium carbonate. The blue color indicates presence of tyrosine and tryptophan.
d. **Million's test:**To 1ml of sample, add 1ml of **Million's** reagent and heated for 3 minutes. Then 1% sodium nitrate is added. Red color indicates the presence of Tyrosine.

Quantitative estimation of Phytochemical plant

Determination of alkaloids: The method of Harbone (1973) was used for the estimation of alkaloids: 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. Then it was calculated with this formula:

Percentage of total alkaloids (%)= Weight of residue×100/Weight of sample taken

Total flavonoids content: The total flavonoids content was estimated using the procedure described by Zhishen *et al*. A total of 200 µL of plant extracts were diluted to 1 ml with distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 µL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 ml of sodium hydroxide (4%) solution was added and made up to 5 ml with distilled water. The mixture was shaken well and left for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink color showed the presence of flavonoid content. The total flavonoid content was expressed as equivalent mg RE/g extract on a dry weight basis using the standard curve.

Total phenolic content:- It was determined using following two methods

a) The total phenolic content was estimated using Folin-Ciocalteau reagent by the method of Sidduraju and Becker. About 20 µg of leaf extract were taken separately and it was made up to 1 ml with distilled water. Then 500 µl of diluted Folins-phenol reagent (1:1 ratio with water) and 2.5 ml of sodium carbonate Na_2CO_3 (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of color. After incubation, the absorbance was measured at 725 nm. A calibration curve of Gallic acid was constructed and linearity was obtained in the range of 10-50 µg/ml. The total phenolics content in the plant extracts were expressed as mg of Gallic acid equivalent (mg GAE/g extract) by using the standard curve.

b) The fat free sample was boiled with 50ml of ether for the extraction of the phenolic components for 15min. 5ml of the extract was pipetted out into a 50ml flask then 10ml of dist. water was added. 2ml of NH_4OH solution and 5ml of Conc. amyl alcohol were also added the samples were made up to mark and left to react for 30 min. For color develop this was read as 505 nm.

Tannin estimation: In this method 500 mg of the crude extract was shakenwith 50 ml distilled water for an hour and filtered. Thefiltrate was then subjected to reaction with 2 ml of (0.M) $FeCl_3$ in 0.1 N HCl and (0.008 M) potassiumferrocyanide after which the absorbance was read within10 minutes at 720 nm. The concentration of tannins wasexpressed as tannic acid equivalents in milligram pergram (TAE mg/g) of crude extract.

Saponin estimation: According to this method, the 20 g of dried leaf powderwas mixed with 20% aqueous ethanol and continuouslyheated over a water bath for 4 hours. 20 ml diethyl ether was added to the extracts and shaken. The ethereal layerwas discarded and the purification process was repeated.60ml n-butanol was added and then treated with 10 mlsodium chloride (5% w/v) and heated. The residue remaining rafter evaporation was weighed and expressed as percentage of leaf powder.

Estimation of Steroids: The Liebermann-Bur chard reaction method was used to detect sterols and terpenoids that give dark pink to green colour, due to the hydroxyl group reacting with aceticanhydride and H_2SO_4. Varying concentrations of cholesterol (10-1000 µg/ml) was used for standard calibration curve, which was read spectro-photometricallyat 640 nm. The concentration of steroids was expressed in milligrams/gram of the crude extract.

RESULTS AND DISCUSSIONS

The present study carried out on the *B. Prionitis*revealed the presence of medicinaly active constituents. The phytochemicalactive compounds of leaves of *B.Prionitis*were qualitatively analyzed separately using different organic solvents and the results are presented in Table 1. In these screening process esalkaloids, tannins, saponins, flavonoids and glycosides, phenols show different types of results indifferent solvents. Phytochemical screening of the various extracts of *B. Prionitis*leaves were used to study the presence of alkaloids, flavonoids, steroids, saponins, tannins and phytosterols and also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity. The importance of alkaloids, saponins and tannins invarious antibiotics used in Antioxidants, antibacterialantimicrobial, anthelemintic, antifertility, antidiabetic, anti-inflammatory, anti-arthritic, cytoprotective, hepatoprotective, diuretic, antidirrhoeal, enzymeinhibitory without any toxic effects. The alkaloid content of the leaves in ethanol and Pet. Ether extract was found to be (1.1±0.03 and 0.8±0.04) respectively. The flavonoid content of leaves in ethanol and chloroformextract of the plant was found to be (1.0±0.02and0.6±0.09) respectively, which is more than found inspecies like *A. indica* 0.62±0.10 and 0.52±0.20 in *C.* The saponin content of the plant in its leaves portion was found to be 2.05±0.1in ethanol extract

Table 12:

Sr. No	Phytochemical screening	Pet ether extract	Chloroform extract	Ethanol extract
	Alkaloids			
1	1. Mayer's reagent	+	-	+
	2. Hager's reagent	+	-	-
	3. Wagner's reagent	+	-	+
	4. Dragendorff's reagent	+	-	+
	Phenolic compounds and Tanins			
2	1. $FeCl_3$	-	-	+
	2. Lead acetate test	-	-	+
	3. Bromine water test	-	-	+
3	**Saponins**			
	1. Frothing test	-	-	+
	Carbohydrates			
4	1. Molisch test	-	-	+
	2. Fehling's solution	-	-	+
	3. Benedict's test	-	-	+
	Protein and Amino acids			
5	1. Millon's test	-	-	-
	2. Biuret test	-	-	+
	3. Ninhydrin test	-	-	+
	Glycosides test			
6	1. Borntrager's test	-	-	+
	2. Legal's test	-	-	+
	Flavonoids test			
7	1. Alkaline reagent test	-	+	+
	2. Shinoda test	-	+	+
	Phytosterols test			
8	1. Liebermann's test	-	-	+
	2. Libermann	-	-	+

CONCLUSION

The plant screened for phytochemical constituentsseemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

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