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PRELIMINARY PHYTOCHEMICAL SCREENING OF VARIOUS EXTRACT OF *PIPER BRACHYSTACHYUM* VAHL.

SASIKALA P*
DR. MEERABAI RS**

*M.Phil, Scholar, Dept. of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India
**Professor & Head, Dept. of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India

ABSTRACT

Medicinal plants have shown tremendous potential for the development of the new drug molecules for various serious. The plant *Piper brachystachyum* was screened for secondary metabolites. The objective of the study was to investigate the presence of various phytochemicals from ethanolic, methanolic, chloroform, petroleum ether, acetone and hexane extracts of *Piper brachystachyum*. These different extracts from leaf and stem were found to contain Alkaloids, Glycosides, Carbohydrates, Flavonoids, Quinones, Betacyanin, Coumarin, Protein, Resins, Phenols Saponins, Fixed oils and fats, Anthraquinones glycosides, Naphthaquinones and Phlobatannins.

KEYWORDS: Phytochemical, *Piper brachystachyum*

INTRODUCTION

India has both traditional and modern systems of medicines and these two systems of medicine use plants, minerals, metals and animals as source of drugs, where plants being the major source. It is estimated that roughly 1500 plant species in Ayurveda and 1200 plant species in Siddha have been used for drug preparation (Jain, 1987). In Indian folk medicine use of about 7500 plant species are recorded as medicinal plants (Anonymous, 1996).

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient culture without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs (Lown, 1993).

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defence mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, Proteins and Common sugars are constituents of Secondary compounds (Abdul Wadood *et al*., 2013).

Piper, the pepper plants or pepper vines are an economically and ecologically important genus in the family Piperaceae .*Piper brachystachyum* is a perennial woody climber with many sub-branches. Plants trail both on trees and rocks with the help of sticky roots on nodes. Leaves are elliptic-ovate, sometimes lanceolate, caudate and acuminate. Fruits globose one seeded berries, found in spikes. The common names of *Piper brachystachyum* is Wild pepper, Chab, Kattumulaku, Kattukurumulaku and Chavya, Chavika, Vanamaricha, Aranyamaricha.

Materials and Methods

Preparation of plant extract

5g of powdered plant materials were each separately dispersed in 50ml of each water, ethanol, methanol, chloroform, petroleum ether, acetone and hexane. The solution was left to stand at room temperature for 24 hrs and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

Preliminary phytochemical analysis of different crude extracts

Extracts were tested for the presence of active principles such as Alkaloids, Glycosides, Carbohydrates, Flavonoids, Quinones, Betacyanin , Coumarin, Protein, Resins, Phenols Saponins, Fixed oils and fats, Anthraquinones glycosides, Naphthaquinones and Phlobatannins.

Test for Alkaloids (Kokate *et al*., 2005)

Mayer's Reagent: 1ml of extract was added to 2ml of Mayer's reagent and development of cream precipitate indicate the presence of alkaloids.

Wagner's Reagent: 1ml of extract was added to 2ml of Wagner's reagent and development of reddish brown precipitate indicate the presence of alkaloids.

Tannic acid test: Alkaloids give buff colour precipitate with tannic acid test.

Test for glycosides (Shilika and Vijayalaxmi, 2012)

Sulphuric acid test: To 1ml extract few drops of conc. Sulphuric acid was added and mixed well. The contents were allowed to stand for few minutes appearance of reddish brown precipitate indicate the presence of glycosides.

Test for Carbohydrates (Kokate *et al*., 2005)

Fehling's test: Test solution was mixed with few drops of Fehling's reagent and boiled in water bath, observed for the formation of blue colour

Test for Flavonoids

Alkaline Reagent: Extracts were treated with few drops of Sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicate the presence of Flavonoids (Vasandha *et al*., 2011).

Lead acetate solution test: Test solution when treated with few drops of lead acetate solution would result in the formation of yellow precipitate (Vasandha *et al*., 2011).

Zinc hydrochloride test: To the test solution mixture of zinc dust and conc.HCl acid was added. It gives red colour after few minutes (Vasandha *et al*., 2011).

Flavonols: To the extract a pinch of Boric acid and few drops of Acetic acid were added. Bright yellow colour with green fluorescence indicate flavonols (SwetaPrakash, 2011).

Rao&Sheshadri test: To the extract, few drops of con. Nitric acid was added. Brilliant blue colour indicate confirmed the presence of phlorglucinol derived Flavanones (SwetaPrakash, 2011).

Test for Quinones (Anjali &Sheetal, 2013)

Dilute NaOH was added to 1ml of crude extract. Blue green or red coloration indicate the presence of quinones.

Test for Betacyanin (Sofoware, 1931)

To 2ml of plant extract, 1ml of 2N Sodium hydroxide was added and heated for 5min at 100^0C. Formation of yellow colour indicated the presence of betacyanin.

Test for Coumarin (Sofowara, 1931)

To 1ml of extract, 1ml of 10% Sodium hydroxide was added. Formation of yellow colour indicated the presence of coumarin.

Test for Protein (Vasandha *et al*., 2011).

Biuret test: The extracts were treated with 1ml of 10% Sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate was added. Formation of purplish violet colour indicate the presence of proteins.

Xanthoprotein: 3ml of test solution was taken in a test tube. To this 1ml of conc. Sulphuric acid was added along the sides of the test tube. Yellow precipitate has to be observed.

Trichloroacetic acid: To the solution when trichloroacetic acid is added, precipitate is formed.

Test for Resins (Vasandha *et al*., 2011).

Acetone- water test: Extracts were treated with acetone and small amount of water was added and shaken. Appearance of turbidity indicate the presence of resins.

Test for phenols (Vasandha *et al*., 2011).

Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicate the presence of phenols.

Test for Saponins (kokate *et al*., 2005)

Foam test: To 2ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for fixed oils and fats (Vasandha *et al*., 2011)

Stain test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicate the presence of fixed oils.

Anthraquinones glycosides (Kokate *et al*., 2005)

Hydroxy-anthraquninones: The sample when treated with potassium hydroxide solution red colour is produced.

Naphthaquinones (Kokate *et al*., 2005)

Junglone test: Sample has to be treated with 2ml of chloroform extract and 2ml of ethyl ether with dilute ammonia solution. Pink colour indicate naphthoquinones.

Phlobatannins (Anjali, 2013)

The crude extract of the plant sample was boiled with 2% aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

RESULTS

Table-1 Preliminary Phytochemical screening of leaf of *Piper brachystachyum* Vahl.

S.No	Tests	Reagents	Ethanol	Methanol	Chloroform	Petrolem Ether	Acetone	Hexane
1	Alkaloids	Mayers	+	+	+	+	+	-
		Wagners	+	+	+	+	-	+
		Tannic acid test	+	+	+	-	-	-
2	Flavonoids	Alkaline	-	-	-	-	-	-
		Lead acetate	+	+	-	-	+	-
		Rao&Sheshadri	-	+	-	+	-	-
		Flavanols	-	+	-	-	-	-
		Zinc hydrochloric test	-	+	-	-	+	-
3	Glycosides	Sulfuric acid test	-	-	+	+	+	+
4	Carbohydrates	Fehlings	+	+	+	-	+	-
5	Proteins	Xanthoprotein	+	+	--	-	+	-
		Biruet test	+	+	-	-	-	-
		Trichloro acetic acid	+	+	-	-	-	-
6	Phenols	Ferric chloride test	+	+	-	-	-	+
7	Saponins	Foam test	+	+	-	+	-	+
8	Resins	Acetone water test	+	-	-	-	+	-
9	Fixed oils		+	-	-	-	-	-
10	Coumarin		+	+	-	-	-	-
11	Quinones		-	-	-	-	-	-
12	Anthraqunione Glycosides	HydroxyAnthraquniones	+	+	-	-	-	-
13	Betacyanin		+	+	-	-	-	-
14	Phlobatannins	Precepitate test	+	+	-	-	+	-
15	Naphthaquinoe	Junglone test	-	-	-	-	-	-

RESULTS

Natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases. The current study was oriented towards the screening of *Piper brachystachyum* for secondary metabolites. Preliminary Phytochemical analysis reavealed the presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Quinones ,Betacyanin, Coumarin, Protein, Resins, Phenols Saponins, Fixed oils and fats, Anthraquinones glycosides, Naphthaquinones

and Phlobatannins. From this analysis, ethanol and methanol extract of leaf was found to be have more constituents than the other extract.

Discussion

Preliminary phytochemical screening test may be useful in detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these facilitate their quantative and qualitative separation of pharmacologically active compounds (Vadisha *et al*., 2012).

The secondary metabolites (phytochemicals) and other chemical constitutents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardio depressant properties. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia. The presence of saponins in methanol and methanolic extracts and glycosides in acetone and chloroform extracts might play a role in the cardioprotective potential of *Piper brachystachyum* (Table 1).

Since the yield of bioactive metabolites in a plant extract also varies considerably with the method/solvent of extraction it is plausible that the ethanolic and methanolic extracts were generally more potent than the other extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvents (ethanol and methanol) than other extracts. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound (Satheesh kumar *et al*., 2012).

Alkaloids are used as anaesthetic agents and are found in medicinal plants (Abdul *et al*., 2013). Alkaloids are often toxic to man and many have dramatic physiological activities; hence their wide use in medicine. They are usually colourless, often optically active substances, most are crystalline but a few (e.g. nicotine) are liquid at room temperature (Gayatri and Sahu, 2011).

Glycosides are the organic compounds from plants or animal sources which on enzymatic or acid hydrolysis give one or more sugar moieties along with nonsugar moiety. Tannins are the natural substances which are present in solution in the form of cell sap and also in distinct vacuoles.

Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals (Kahkonen

et al., 1999). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Proestos *et al*., 2006).

The presence of all the above in the leaf extract of P. *brachystachyum* possibly indicates its medicinal value and these bioactive compounds it separated using proper techniques could be used as medicines for various ailments.

Conclusion

The presence of phytoconstituents make the plant useful for treating different ailments and have potential of providing useful drugs of human use. In the present study, we have found that most of the biologically active phytochemicals were present in the ethanol, methanol, chloroform, petroleum ether, acetone and hexane. Since the ethanolic and methanolic and chloroform extract of the plant contains more constituents it can be considered as a potential plant for further investigation.

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