

SCREENING AND EVALUATION OF SECONDARY METABOLITES PRESENT IN *PIPER CUBEBA*

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ABSTRACT

Objective: Bioactive compounds like phytochemicals are extracted mainly from plants, as they serve as traditional herbal medicine. The secondary metabolite such as alkaloids, flavonoids, and phenolic compounds serve as natural antioxidants and has been widely used due to its therapeutic applications. The objective of this study was to do preliminary phytochemical screening of the spice *Piper cubeba*.

Methods: Totally, three solvents were used to prepare the extract. Of all the three extracts used, methanolic extract showed higher release of secondary metabolites when compared to ethyl acetate and hexane extract. Further quantitative analysis was carried out with the methanolic extract.

Results: The presence of alkaloids, flavonoids, phenolic compounds, and tannins was observed. A significant result such as 1040 µg of Gallic acid equivalent/g of total concentration of flavonoid and 1280 µg of quercetin equivalent/g of total phenolic content was obtained.

Keywords: Secondary metabolite, Alkaloid, Flavonoid, Phenolic content.

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INTRODUCTION

Plants are regarded as the richest resource for many potential drugs. Although usage of plants as medicine has been known for millennia, its therapeutic value is gaining significance in recent years [1-3]. Natural antioxidants such as spices and herbs help to prevent the disease and cure various ailments [4-6].

The secondary metabolites produced by plants exhibit various structural diversity, which is found to be biologically active. These include a wide set of products such as alkaloids, steroids, tannins, phenols, glycosides, and flavonoids [3,7,8]. One such therapeutically significant medicinal plant *Piper cubeba* belongs to the Piperaceae family; it has more than 700 species distributed in the tropical and subtropical regions of the world. It has many therapeutic uses and can be used as diuretic, hepatoprotective, antipyretic, and antioxidant [9]. Bronchitis and other associated ailments can be treated by this plant. Furthermore, it is used to treat paralysis and rheumatism. This present study evaluates and analyses the existence of huge secondary metabolites in the seeds of *P. cubeba*.

METHODS

Collection of plant materials

P. cubeba seeds were collected from local market and shade dried at room temperature (RT) for a week. Dried seeds were blended to a fine powder and stored in an airtight container at RT.

Preparation of plant extract

The powdered plant material was extracted with methanol, ethyl acetate, and hexane in the ratio of 1:10 and kept in shaking condition for 24 h. The extract was filtered using Whatman No. 1 filter paper. The process was reciprocated using the same plant material thrice by changing the solvent. The combined filtrate was subjected to condensation. The extracts were placed in the distillation unit to separate the solvent [10]. The separated residues were redissolved in different solvents to get a yield 10 mg/ml solutions for future work.

Phytochemical screening

Qualitative analysis

Detection of alkaloids

A few ml of dilute hydrochloric (HCL) was added in 50 mg of extract and filtered. The extracted filtrates were used for Mayer's test to identify the presence of alkaloids.

Mayer's test

Mayer's reagent was added to the few ml of extracted filtrate along the sides of the test tube. A white creamy precipitate indicated the presence of alkaloids.

Mayer's reagent

About 5 g of potassium chloride and 1.358 g of mercuric chloride was dissolved in 10 ml and 60 ml of water. Both the solutions were mixed and made up to final volume of 100 ml with water.

Detection of phenolic compound

Ferric chloride test

About 50 mg of the extract was dissolved in 5 ml of distilled water. To the mixture, 4-5 drops of 5% neutral ferric chloride solution were added. The presence of phenolic compounds was identified by the formation of a dark green color.

Detection of glycosides

About 50 mg of the extract was mixed with concentrated HCL acid and hydrolyzed for 2 h in a water bath, and the filtered hydrolyses were used for following test.

Borntrager's test

About 2 ml of the filtrate was added to 3 ml of chloroform and shake well. Chloroform layer should be separated and 10% of ammonia solution was added. Formation of pink color indicates the presence of glycosides.

Detection of flavonoids

A few ml of extract was added with 10 ml of ethyl acetate and heated in a steam bath for 3 min. 4 ml of the filtrate was mixed with 1 ml of dilute

ammonia solution and shaken well to form a yellow color appearance for the presence of flavonoids.

Detection of tannins

In a test tube, take 2 ml of the extract and mix with 2 ml of distilled water, with that few drops of 0.1% FeCl₃ solution were added. Green precipitation was formed and indicated the presence of tannins.

Detection of reducing sugars

About 100 mg of extract was stirred in 50 ml of water and filtered. The filtrate was used for the following test.

Fehling's test

About 1 ml of Fehling's solution I and II was mixed with 1 ml of filtrate and kept in a water bath. Red color formation indicates the presence of sugar.

Fehling's solution

Fehling's solution I: About 34.66 g of copper sulfate was dissolved in distilled water and made up to 500 ml with distilled water.

Fehling's solution II: About 173 g of potassium sodium tartrate and 50 g of sodium hydroxide was dissolved in water and made up to 500 ml.

Detection of saponins

Foam test

About 50 mg of the extract was diluted and made up to 20 ml with distilled water. It was shaken continuously for 15 min. A foam layer was formed about 2 cm and it indicates the presence of saponins.

Detection of proteins

About 100 mg of the extract was dissolved in 10 ml of distilled water and filtered. The filtered extract was used for biuret test.

Biuret test

A drop of 2% copper sulfate solution was added to 2 ml of filtrate and 1 ml of ethanol (95%) was added with excess of potassium hydroxide pellets. Pink color formation indicates the presence of protein.

Quantitative analysis

Determination of total phenolic contents

Spectrophotometric method was used to determine the total phenolic concentration. Methanol solution of the extract in the concentration of 1 mg/ml was used in the analysis. A mixture was made using 0.5 ml methanol solution of extract and 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water followed by the addition of 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% of NaHCO₃. Then, the samples were incubated at 45°C for 45 min. The absorbance was determined using spectrophotometer at 765 nm [11]. The experiment was repeated thrice and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid and the calibration line was constructed. Depending on the absorbance value, the total phenolic content concentration was determined and was expressed in terms of Gallic acid equivalent (GAE) [12].

Determination of flavonoid concentrations

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl₃ solution dissolved in methanol. The samples were incubated for an hour at RT. The absorbance was determined using spectrophotometer at $\lambda_{max} = 415$ nm. The mean value of absorbance was obtained from the triplicate samples. The same procedure was repeated for the standard solution of quercetin and the calibration line was constructed. Based on the measured absorbance, the total concentration

of flavonoids was obtained from the measured absorbance and is expressed in terms of quercetin equivalent (QE) [12].

RESULTS AND DISCUSSION

Preliminary phytochemical constituents of *P. cubeba* leaves revealed the presence of secondary metabolites such as tannins, phenolic compounds, flavonoids, and alkaloids while glycosides, saponins, proteins, and reducing sugars were absent. The results are incorporated in Table 1.

On comparison with the methanol extracts, the plant extract of ethyl acetate and hexane shows minimum amount of secondary metabolites.

Alkaloids are mainly used for pain killer drug development, and it is largest group of phytochemicals in the plant [13]. It is reported that piperine is used in the treatment of anti-inflammatory, antimalarial, and antileukemia [4] and plays a major role in herbal cough syrups. Mostly, it helps to increase the absorption of selenium, vitamins, and carotene and also increase the body's natural thermogenic activity [14]. Methanolic extract of *Piper nigrum* has been administered to significantly improve memory performance and has exhibited antioxidant potential [15]. Since the methanolic extracts have shown good results, the quantitative analysis was carried out with that. In the quantitative analysis of

Table 1: Phytochemical screening of *Piper cubeba* using different extracts

S. No.	Compound	Methanol	Ethyl acetate	Hexane
1.	Alkaloids	+	+	-
2.	Phenolic compounds	+++	+	-
3.	Glycosides	-	-	-
4.	Flavonoids	+++	+	+
5.	Tannins	+	-	-
6.	Reducing sugars	-	-	-
7.	Saponins	-	-	-
8.	Proteins	-	-	-

+++ denotes highly present, + denotes mildly present and -denotes absence

Table 2: Phenol and flavonoid content of *Piper cubeba* in methanolic extracts

S. No.	Phytochemical present	Amount
1.	Total phenolic content	1280 (μ g of GAE/g of extract)
2.	Total flavonoid content	1040 (μ g of QE/g of extract)

GAE: Gallic acid equivalent, QE: Quercetin equivalent

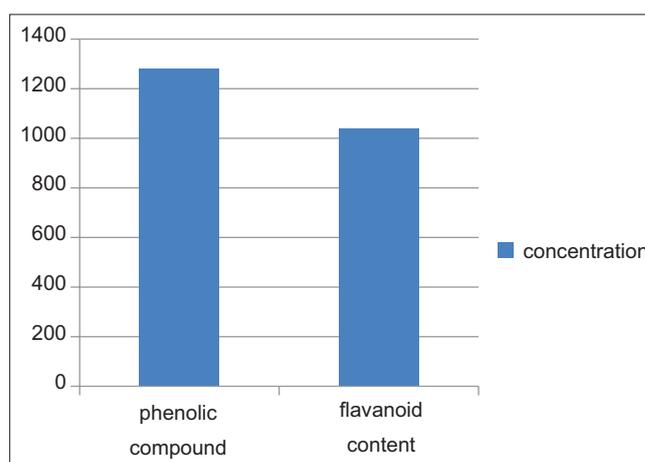


Fig. 1: Comparative study on phenolic and flavonoid content present in *Piper cubeba*

P. cubeba, the total concentration of flavonoid was estimated to be 1040 µg of GAE/g of extract and the total concentration of flavonoids was found to be 1280 µg of QE/g of extract (Table 2).

The secondary metabolites such as phenolic compounds have high amount of antioxidant activity [16,17]. The results obtained in this study showed a significant level of phenolic compounds. Flavonoids are another class of secondary plant metabolites, also known as Vitamin P [18]. These metabolites are rich to produce yellow pigments and other coloration in plants. Flavonoids are absorbed by humans, and they seem to anti-inflammatory, antiallergic, and anticancer activities [19].

From Fig. 1, it is evident that the phenolic contents are quite higher when compared with the flavonoid compounds.

CONCLUSION

The phytochemical screening indicates the presence of significant amount of alkaloids, phenolic compounds, tannins, and flavonoids in the crude methanolic extract. Many such compounds are known to possess high antioxidant activity. The higher amount of polyphenolic compound is evident to show higher antioxidant activity. Based on the results of current study, *P. cubeba* is a great source of secondary metabolites. Further analysis regarding biological activities of the plant will be conducted soon.

AUTHORS' CONTRIBUTIONS

Both the authors had contributed equally in performing the assays and writing the manuscript.

COMPETING INTEREST

The authors declared that they have no conflicts of interest.

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