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Original Article

PHYTOCHEMICAL SCREENING OF INFLORESCENCE OF *PIPER BETLE*

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ABSTRACT

Objective: The objective of the current study is to determine the phytoconstituents present in the Inflorescence of *Piper betle* (IPB).

Methods: Phytochemical screening was performed to analyse the phytoconstituents present in IPB and the same was identified by TLC. The phytoconstituents were also estimated quantitatively.

Results: Phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins and polyphenols. TLC results indicated and confirmed the presence of alkaloids, polyphenols, flavonoids and tannins. On quantification IPB was found to contain 4.2±1.661 mg/ml and 1.523±0.156 mg/ml of flavonoids and tannins respectively.

Conclusion: The present study suggests that Inflorescence of *Piper betle* can be a good source of secondary metabolites.

Keywords: Inflorescence, Phytochemicals, *Piper betle*, TLC, Flavonoids, Polyphenols, Tannins

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INTRODUCTION

Piper betle is an indigenous plant to India belonging to the family Piperaceae. The leaf commonly known as Paan is consumed in various parts of India [1]. The plant is very much popular in India than in any other country in the world. It is used as a special item offered to guests in the form of betel morsel in order to show respect. For such traditional use of the betel leaf in the Indian society, the leaf really stands alone without any parallel even today. *Piper betle* is a perennial dioecious creeper having semi woody stem and climb by means of short adventitious roots. The betel is a spice whose leaves are found to have medicinal properties and can cure many common illness [2].

Fresh leaves were found to contain protein (3-3.5%), carbohydrates (0.5-6.1%), fibre (2.3%), minerals (2.3-3.3%) and fat (0.4-1.0%). The leaf was also found to contain Nicotinic acid, Vitamin A, Vitamin C, Thiamine and Riboflavin. The leaf is rich in enzyme likes diastase and catalase as well [3].

The leaf also contains significant amounts of flavonoids and polyphenols [4]. The betel leaf has a peculiar aroma due to the presence of essential oils of phenols and terpenes. The terepenoids in betel leaf include 1,8-cineole, cadinene, camphene, caryophyllene, limonene and pinene [5]. *Piper betle* leaf contains good amount of phytoconstituents and secondary metabolites, some of these will also be present in other plant parts. The aim of this study is to screen the Inflorescence of *Piper betle* for some of these phytoconstituents. Inflorescence *Piper betle* contains eugenol and hydroxychavicol and is often added to betel quid to improve aromatic flavour. IPB aqueous extract was found to be a potent hydrogen peroxide scavenger and was found to supress the lucigenin enhanced hydrogen peroxide chemiluminescence. IPB aqueous extract was also a potent scavenger of the superoxide radical [6].

Piper betle leaf was found to contain various pharmacological activities like antioxidant activity, anti-fungal activity, anti-leishmanial activity, anti-ulcerogenic activity, antiplatelet activity, immunomodulatory activity, anti-filarial activity, anti-inflammatory activity [7], gastroprotective activity, anti-diabetic activity and antifertility activity [8].

The goal of this study was to identify the various phytoconstituents present in IPB.

MATERIALS AND METHODS

Chemicals

Methanol, Catechol (SD Fine chem), Quercetin (Sigma-Aldrich), Tannic acid (Nice chemicals), sulphuric acid, hydrochloric acid, chloroform, ferric chloride, lead acetate, toluene, ethyl acetate, glacia acetic acid, formic acid, vanillin, aluminium chloride, sodium hydroxide, ethanol, acetic anhydride, Folin-Ciocalteu reagent, Folin-Denis reagent was procured from Sigma. TLC silica gel plate was of 60 F_{254} which was procured from Merck.

Plant source

Inflorescence of *Piper betle* was collected from a home grown garden in T C Palya, Bengaluru, Karnataka, India.

Extraction

The plant extract was prepared from fresh IPB after washing them under tap water to remove dust particles. The fresh IPB [4.5g (w/v)] was extracted with methanol by homogenising in a motor and pestle followed by incubation for 1h at room temperature on a magnetic stirrer with continuous stirring. The mixture was then centrifuged at 10,000rpm for 5 min, RT. The supernatant was filtered and used for further experimental analysis.

Phytochemical analysis

The fresh methanol extract was subjected to various phytochemical screening for the detection of the presence of various phytoconstituents.

Test for alkaloids

To 1 ml of the extract, add a few drops of concentrated hydrochloric acid followed by few drops of Dragendroff's reagent. Appearance of reddish brown colour indicates the presence of Alkaloids [9].

Test for flavonoids

Few drops of concentrated sulphuric acid was added to 1 ml of the extract. Occurrence of a stable yellowish orange indicates the presence of flavonoids [10].

Test for steroids

Salkowski's test

To 1 ml of the extract add equal volumes of chloroform followed by 2 ml of concentrated sulphuric acid. Formation of red precipitate indicates the presence of steroids [10].

Libermann test

To 1 ml of the extract add equal volumes of chloroform followed by 2 ml of concentrated sulphuric acid and acetic anhydride. Formation of a green precipitate indicates the presence of steroids [10].

Test for saponins

Shake vigorously 1 ml of the extract and 5 ml of distilled water in a test tube. Lather formation indicates the presence of saponins [10].

Test for terpenoids

To 5 ml of the extract, add 2 ml of chloroform followed by 3 ml of concentrated sulphuric acid so as to form a layer. Formation of a reddish brown precipitate at the interface indicates the presence of terpenoids [11].

Test for tannins

Ferric chloride test

To 1 ml of the extract add equal volumes of freshly prepared 10% ferric chloride. Occurrence of greenish black colour indicates the presence of tannins [10].

Lead Acetate test

To 1 ml of the extract add 2 ml of 10% Lead Acetate. Formation of a white precipitate indicates the presence of tannins [10].

Test for phenols

To 1 ml of the extract add 2 ml of 10% Lead Acetate. Formation of a white precipitate indicates the presence of phenols [10].

Thin layer chromatography

TLC of alkaloids

The extract was spotted on TLC Silica gel 60 F_{254} plates used as a stationary phase using capillary tubes. Chloroform/Methanol (9:1) was used as mobile phase, 1% Nicotine in Methanol (w/v) was used as a standard and Dragendroff's reagent was used as a colour stain to develop the chromatogram. Appearance of orange spots indicates the presence of alkaloids [12].

TLC of polyphenols

The extract was spotted on TLC Silica gel 60 F_{254} plates used as a stationary phase using capillary tubes. Ethyl acetate/Methanol/Formic acid (16:4:1) was used as mobile phase, 1% Catechol in Methanol (w/v) was used as a standard and 2% ferric chloride was used as a colour stain to develop the chromatogram. The plate was then visualized under UV light (λ 366 and 254 nm). Appearance of brown to black spot indicates the presence of polyphenols [12].

TLC of flavonoids

The extract was spotted on TLC Silica gel 60 F_{254} plates used as a stationary phase using capillary tubes. Chloroform/Methanol (9:1) was used as mobile phase, 1% Quercetin in Methanol (w/v) was used as a standard and 2% ferric chloride was used as a colour stain to develop the chromatogram. The plate was then visualized under UV light (λ 366 and 254 nm). Appearance of brown to black spot indicates the presence of flavonoids [12].

TLC of tannins

The extract was spotted on TLC Silica gel 60 F_{254} plates used as a stationary phase using capillary tubes. Toluene/Ethyl acetate/Glacial Acetic acid/Formic acid (20:45:20:5) was used as mobile phase, 1% Tannic acid in Formic acid (w/v) was used as a standard and 0.5% vanillin in 4% sulphuric acid was used as a colour stain to develop the chromatogram. Appearance of purple to brown spots indicates the presence of tannins [12].

Quantitative determination of phytoconstituents

Total phenol content

0.4 to 2.0 ml aliquots of standard Catechol (5µg/ml) was pipetted out in to labelled test tubes and the volume was made up to 3.0 ml using distilled water. 3.0 ml of distilled water was taken as blank. 0.5 ml of Folin-Ciocaltue reagent was added to all tubes followed by incubation for 3 min, RT. 2.0 ml of 20% sodium carbonate was then added to all the tubes and incubated for 1 minute, RT. The absorbance of the coloured solutions were read at 650 nm in a colorimeter [13].

Total flavonoid content

0.2 to 1.0 ml of aliquots of standard Quercetin (1000µg/ml) prepared in ethanol was pipetted out in to labelled test tubes and the volume was made up to 1.0 ml using ethanol. 1.0 ml of ethanol was taken as blank. 4.0 ml of distilled water was added to all the tubes followed by the addition of 0.3 ml of 5% sodium nitrate and the tubes were incubated for 5 min, RT. 0.3 ml of 10% aluminium chloride was added to all the tubes and incubated for 1 minute, RT. 2.0 ml of 1M sodium hydroxide was added to all the tubes and the volume made up with 9.0 ml distilled water. The absorbance of the coloured solutions were read at 510 nm in a colorimeter [13].

Total tannins

0.2 to 1.0 ml of standard Tannic acid (50µg/ml) was pipetted out in to labelled test tubes and the volumes made up to 1.0 ml with distilled water. 1.0 ml of distilled water was taken as blank. 0.5 ml of Folin-Denis reagent was added to all the tubes followed by the addition of 1.0 ml of 10% sodium carbonate and the contents in the tubes were mixed well and the absorbance was read at 700 nm in a colorimeter [13].

RESULTS AND DISCUSSION

In this study the phytoconstituents in IPB were determined. This plant is a native species in India and is easily available and also possess a wide range of phytoconstituents for its enormous application in ethnobotanical and pharmacological activities.

Phytochemical analysis

Methanolic extract of IPB was subjected to phytochemical and pharmacological activities. The phytochemical screening was performed which showed the presence of various constituents like alkaloids, flavonoids, saponins, tannins and polyphenols (table 1). Eugenol, Chavibetol, Chavibetol acetate, Camphene, α-Pinene, β-Pinene, α-Limonene, Safrole and 1,8-Cineole are some of the secondary metabolites found to be present in leaf of *Piper betle* [14].

Table 1: Phytochemical analysis of IPB

Phytochemical screening	15% IPB extract
Test for alkaloids	++
Test for flavonoids	++
Salkowski's test	+
Libermann test	-
Test for saponins	++
Test for terpenoids	+
Ferric chloride test	-
Lead acetate test	++
Test for polyphenols	++

Analysis by TLC

TLC profiling confirmed the presence of phytoconstituents in the sample extract. The sample was found to be rich in polyphenols and flavonoids along with the presence of alkaloids and tannins (fig. 1). TLC investigation indicates important information about the polarity of the chemical constituents in a way that compounds displaying high Rf value in the less polar solvent system possess lesser polarity and the compounds with less Rf value possess higher polarity [14].

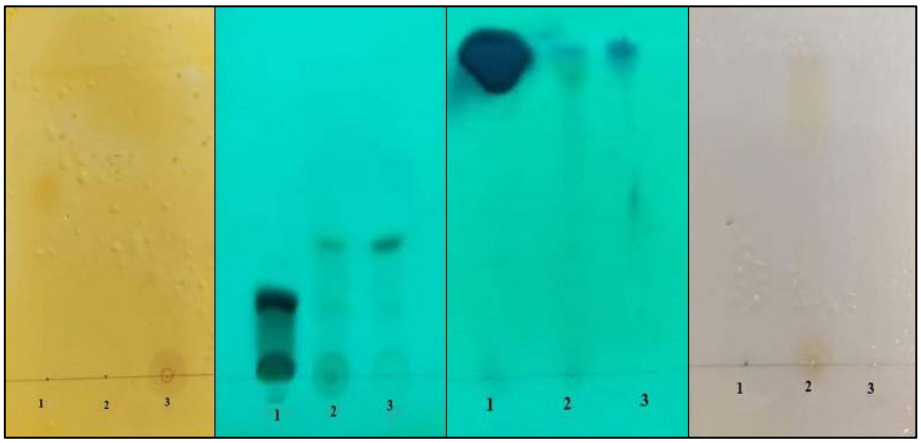

Fig. 1: TLC Profile of alkaloids, flavonoids, polyphenols and tannins respectively, lane 1–standard, lane 2 and lane 3 crude extract of IPB

Quantification of phytoconstituents

Total phenols, total flavonoids and tannins were quantitatively determined and their values expressed as mg/ml catechol equivalence, quercetin equivalence and tannic acid equivalence respectively (table 2, fig. 2). The samples were found to be quantitatively rich in Flavonoids, 4.2 mg/ml and 4.133 mg/ml respectively and Tannins 1.523 mg/ml and 2.066 mg/ml respectively.

The results of the present study indicate that IPB contains good amounts of phytoconstituents and can be used as a rich source for the extraction and purification of secondary metabolites with pharmacological activities. The inflorescence can be used as a potential natural therapeutic against many illness.

Table 2: Quantification of phytoconstituents

Phytoconstituent	Crude extract of IPB
Total Phenols	0.2566±0.035 mg/ml equivalence of catechol
Total Flavonoids	4.2±1.661 mg/ml equivalence of quercetin
Tannins	1.523±0.156 mg/ml equivalence of tannic acid

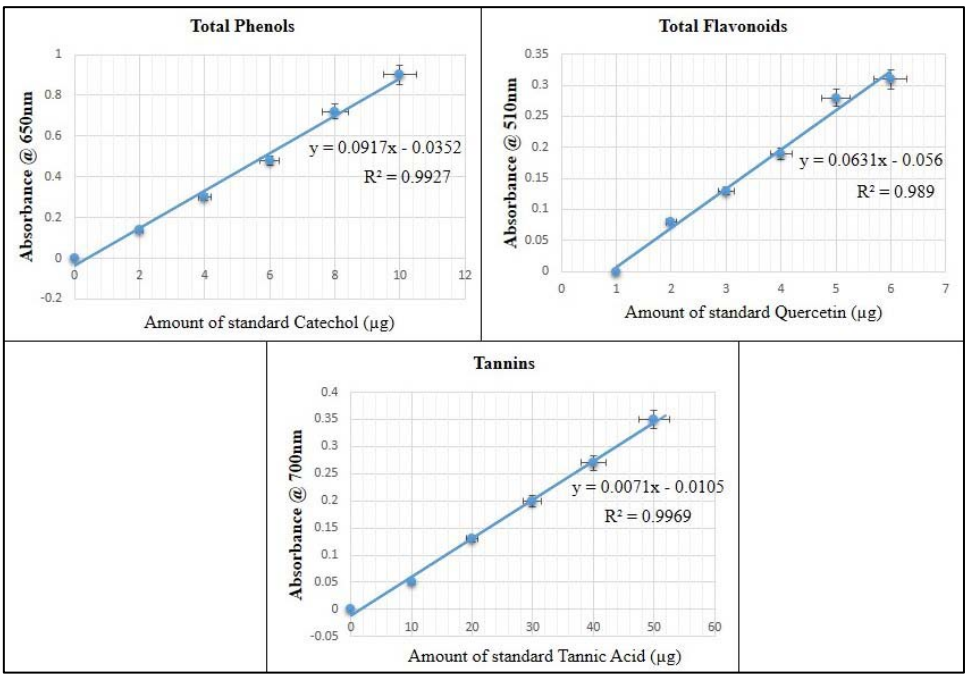

Fig. 2: Standard plots of total phenols, total flavonoids and tannins respectively for the quantification of phytoconstituents

CONCLUSION

The current study was focussed at the determination the various phytoconstituents in Inflorescence of *Piper betle* and quantification of the abundant phytoconstituents phenols, flavonoids and tannins. IPB was found to contain alkaloids, flavonoids, saponins, tannins and polyphenols. TLC further confirmed the presence of alkaloids, polyphenols, flavonoids and tannins. IPB was determined to contain rich amounts of flavonoids and tannins after quantification. Inflorescence *Piper betle* has a rich amount of phytoconstituents which can be beneficial for mankind for their physiological and pharmacological properties. Along with the betel leaf, IPB can also give positive results for the various pharmacological activities.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

None

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