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±.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

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 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 like 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 terpenoids 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 of 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 suppress 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, Gcatechol (SD Fine chem), Quercetin (Sigma-Aldrich), Tannase (Nice chemicals), sulphuric acid, hydrochloric acid, chloroform, ferric chloride, lead acetate, toluene, ethyl acetate, glacial 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 F254 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 Dragendorff'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].

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

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

DOI: http://dx.doi.org/10.22159/ijcpr.2020v12i6.40299. Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr
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 F254 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 Dragendorff’s reagent was used as a colour stain to develop the chromatogram. Appearance of orange spots indicates the presence of polyphenols [12].

TLC of polyphenols
The extract was spotted on TLC Silica gel 60 F254 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 F254 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-Ciocalteu 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 760 nm in a colorimeter [13].

Total flavonoid content
0.2 to 1.0 ml 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 0.1 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 possesses 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-Pinen, β-Pinen, α-Limonene, Saffrole and 1,8-Cineole are some of the secondary metabolites found to be present in leaf of Piper betle [14].

| 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    | ++              |

Table 1: Phytochemical analysis of IPB
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    | 0.256±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 focused 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.

ACKNOWLEDGMENT

The authors wish to acknowledge the Department of Chemistry (PG Biochemistry) and the management of Mount Carmel College, Autonomous, Bengaluru for offering their facilities and support.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

None

REFERENCES

1. VPB Rekha, Manideep Kollipara, BRSS Srinivasa Gupta, Y Bharath, Krishna Kanth Pulicherla. A review on *Piper betle* L.: nature’s promising medicinal reservoir. Am J Ethnomed 2014;1:276-89.

2. Sengupta R, Banik JK. A review on betel leaf (pan). Int J Pharm Sci Res 2013;4:4519-24.

3. Guha P. Betel leaf: the neglected green gold of India. J Human Ecol 2006;19:87-93.

4. Devjani Chakraborty, Bartha Shah. Antimicrobial, antioxidative and antihemolytic activity of *Piper betel* leaf extracts. Int J Pharm Pharm Sci 2011;3:192-9.

5. Sakat Mazumdar, Aditi Roychowdhury, Soumitra Banerjee. An overview of betel leaf (*Piper betle* L.): a review. Annals Food Sci Technol 2016;17:367-76.

6. Daniel Lei, Chin-Po Chann, Ying Yan Wang, Tong Mei Wang, Bor Ru Lin, Chun Hsun, et al. Antioxidative and antiplatelet effects of aqueous inflorescence *piper betle* extract. J Food Chem 2003;51:2083-8.

7. Kushagra Nagori, Mukesh Kumar Singh, Amit Alexander, Tekeshwar Kumar, Dhansay Dewangan. *Piper betle* L: a review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART-MS (Direct analysis in real time mass spectrometry). J Pharm Res 2011;4:2991-7.

8. D Pradhan, KA Suri, DK Pradhan, PBiswasroy. Golden heart of the nature: *Piper betle* L. JPharmacogn Pythochem 2013;1:147-67.

9. Amina Khatun, Mahmudur Rahman, Shamima Jahan. Preliminary phytochemical, cytotoxic, thrombolytic and antioxidant activities of methanol extract of *Murraya exotica* linn. leaves. Orient Pharm Exp Med 2014;4:223-9.

10. Shilika Shetty, KK Vijayalaxmi. Phytochemical investigation of extract/solvent fractions of *piper nigrum* linn. seeds and *Piper betle* linn. leaves. Int J Pharma Biosci 2012;3:34-44.

11. Abhayomi Sofowora. Recent trends in research into African medicinal plants. J Ethnopharmacogn 1993;38:197-208.

12. Junairiah Nimatuazhroh, Nabilah Istighfari Zuraidasanaaz, Liliis Sulkyoirini. Isolation and identification of secondary metabolites of black betel (*Piper betle* L. var Nigra). J Kimia Riset 2019;3:13-8.

13. Sarabjot Kaur, Poonam Monda. Study of total phenolic and flavonoid content, antioxidant activity and antimicrobial properties of medicinal plants. J Microbiol Exp 2014;1:23-8.

14. Samidha Kamtekar, Prushali Keer, Vijaya Patil. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharma Sci 2014;9:61-5.

15. Chaynika Sarma, Prasad Dasane, Sawinder Kaur, Jyoti Singh, Jaginder Singh, Yogesh Ghat, et al. Antioxidant and antimicrobial potential of selected varieties of *Piper betle* L. (Betel leaf). Annals. Of the Brazilian Academy of Sciences; 2018.

16. Awoola?ck MD, Taner P, Morita H. Isolation and structural characterization of flavonoids, flavonoids-from biosynthesis to human health. Goncalo C Justino Intech Open; 2017. DOI:10.5772/67881.