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PHYTOCHEMICAL INVESTIGATION OF EXTRACT/ SOLVENT FRACTIONS OF *PIPER NIGRUM* LINN. SEEDS AND *PIPER BETLE* LINN. LEAVES

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ABSTRACT

Phytochemical analysis of extract/solvent fractions of two medicinal plants; *Piper nigrum* Linn. (seeds) and *Piper betle* Linn. (leaves) was conducted to detect the presence of various phytochemical constituents. These two plants are extensively used in Ayurvedic system of medicine. Each analysis was carried out in triplicate. The qualitative analysis of crude extract and solvent fractions of *Piper nigrum* Linn. and *Piper betle* Linn. revealed the presence of various phytochemicals of pharmacological significance such as, alkaloids, steroids, tannins, phenols, flavones etc.

KEYWORDS

Phytochemical screening, *Piper nigrum* Linn., *Piper betle* Linn., Alkaloids, Phenol, Steroids, Flavones.

INTRODUCTION

Plants have been the pioneers of energy source to all living things on earth enabling the survival of life forms till date[1]. Also the plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants[2]. There have long been impressive correlations between the frequency and amount of fruits/ vegetables consumed and longevity and/or the delay in the onset of chronic diseases such as cancer and heart disease[3]. Plants in their different forms have also been the source of effective medication from ancient times. The field of ayurveda to a large extent is based on plants for curing various ailments. In plants the phytochemicals primarily identified and isolated for their therapeutic values are the secondary metabolites such as alkaloid, steroids, tannins, phenols, glycosides, flavones, quinones, pigments etc. that are produced as part of their normal metabolism[4].

Black pepper is used as spice as well as medicine by itself or as a part of some herbal remedies in combination with other well known herbs and spices. Betel leaves are used as a stimulant, antiseptic agent and as breath freshener. The leaf is used in several common household remedies. It has diuretic, analgesic and cooling properties. The present evaluation is aimed at identifying the presence of these therapeutically important secondary metabolites in leaves of *Piper betle* Linn. and seeds of *Piper nigrum* Linn[5,6].

MATERIALS AND METHODS

(i) Collection of plant material

The seeds of *Piper nigrum* Linn. were obtained from authentic dealers from Mangalore, Karnataka. The leaves of *Piper betle* Linn. were procured from a plantation in Konaje village, near Mangalore University, Mangalore taluk, Karnataka. The identity of the plant materials were confirmed by consulting the taxonomist in the department of Applied Botany, Mangalore University. The voucher specimen is deposited in the department of Applied Zoology.

(ii) Preparation of extract

The dried seeds of *Piper nigrum* Linn. were ground to fine powder and extracted with distilled ethanol by using Soxhlet method at 45°C. The extract obtained was concentrated in a rotary vacuum evaporator (Heidolph, Germany) at 45°C and then fractionated with different solvents viz., petroleum ether, diethyl ether, chloroform and methanol using a separating funnel. The leaves of *Piper betle* Linn. were washed and ground to a fine paste with distilled water and filtered through muslin cloth. The aqueous extract was concentrated with rotary vacuum evaporator at 45°C. The concentrated extract was fractionated with water-methanol (1:1) and distilled methanol using a separating funnel.

(iii) Phytochemical evaluation

The qualitative phytochemical analysis of crude extracts/solvent fractions of *Piper nigrum* Linn. and *Piper betle* Linn. was done using standard chemical tests (Harborne, 1973; Farnsworth, 1977; Kokate, 1985). The tests were conducted to study presence of different phytochemicals. The extract/ solvent fractions were hydrolyzed with dilute hydrochloric acid (1N) before analysis. The tests were carried out in triplicates by using uniform concentration of the sample.

Test for alkaloids

***i) Dragendroff's test*:** 1ml of the extract/ solvent fractions was taken in a test tube and few drops of Dragendroff's reagent were added. Orange precipitate developed indicated the presence of alkaloids.

ii*) Wagner's test:* 1 ml of extract/solvent fraction was added to 2 ml of Wagner's reagent. Development of reddish-brown precipitate revealed the presence of alkaloids.

iii) Mayer's test: 1ml of extract/solvent fraction was added to 2ml of Mayer's reagent and the development of pale whitish precipitate indicated the presence of alkaloids.

Test for steroids

i) Salkowski's test: 1ml of extract/solvent fractions mixed with equal amount of chloroform was treated with 2ml of concentrated sulfuric acid. Appearance of red precipitate indicated the presence of steroids.

ii) Libermann-Burchard's test*:* 1ml of test sample was dissolved in equal quantity of chloroform. To this mixture 2ml of conc. sulfuric acid and 2ml of acetic anhydride were added. The development of green coloured precipitate indicated the presence of steroids.

Test for glycosids

i) Sulfuric acid test: To 1ml extract/solvent fractions few drops of conc. sulfuric acid was added and mixed well. The contents were allowed to stand for few minutes; appearance of reddish precipitate indicated the presence of glycosides.

***ii) Molisch's test*:** To 1 ml of extract/solvent fraction 2 ml of Molisch's reagent was added and mixed well. To this mixture 2ml of conc. sulfuric acid was added along the side of the test tube and allowed to stand for few minutes. Appearance of reddish-violet ring at the junction of two liquids indicated the presence of glycosides.

Test for carbohydrates

i) Benedict's Test: To 5ml of Benedict's reagent, 1ml extract/solvent fraction was added and boiled for two minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

ii) Molisch's test: To 1ml of extract/solvent fraction 2 ml of Molisch's reagent was added and mixed well. To the mixture 2ml of conc. sulfuric acid was added along the side of the test tube and allowed to stand for few minutes. Appearance of reddish-violet ring at the junction of two liquids indicated the presence of carbohydrates.

Test for amino acids

i) Ninhydrin test: 2-3 drops of Ninhydrin reagent was added to 2ml of sample, development of purple color indicated the presence of proteins.

Test for saponin:

i) Aqueous test: To 1 ml of the test substance, 5 ml of water was added and the tube was shaken vigorously. Lather formation indicated the presence of saponins.

Test for flavones

***i) Aqueous test*:** Appearance of yellow color on treating 1ml of test substance with equal quantity of aqueous NaOH indicated the presence of flavones.

***ii) Sulfuric acid test*:** On addition of few drops of conc. sulfuric acid to test samples stable yellowish orange color indicated the presence of flavones.

Test for tannins:

***i) Ferric chloride test*:** To 1ml of test samples, equal amount of ferric chloride was added. The presence of tannins was indicated by the formation of greenish black color.

Test for phenols:

i) Lead acetate test: On addition of 2ml of 10% lead acetate solution to test samples white precipitate developed confirming the presence of phenols.

Test for oils and fats:

i. ***Spottest*:** Small quantity of test substance was pressed with Whatman filter paper. Appearance of oil stains indicated the presence of oils and fats.

ii. ***Saponification test:*** To 1ml of sample few drops of 0.1N KOH and few drops of phenophthalein indicator were added and heated in water bath for 1-2 hours. Formation of soapy solution indicated the presence of oils and fats.

Table 1

Results of the phytochemical analysis of Piper nigrum Linn. seeds

Phytochemicals	Test	Ethanol extract	Petroleum ether fraction	Diethyl ether fraction	Chloroform fraction	Methanol fraction
Alkaloids	Dragendroff's test	+	+	+	+	-
	Wagner's test	+	+	+	+	-
	Mayer's test	+	+	+	+	-
Steroids	Salkowski's test	+	+	+	+	-
	Libermann-Burchard's test	+	+	+	+	-
Glycosides	Sulfuric acid test	+	+	+	+	+
	Molisch's test	+	+	+	+	+
Carbohydrates	Benedict's test	+	+	+	+	+
	Molisch's test	+	+	+	+	+
Amino acids	Ninhydrin test	+	+	+	+	+
Saponins	Aqueous test	+	+	+	+	+
Flavones	Aqueous test	+	+	+	+	+
	Sulfuric acid test	+	+	+	+	+
Tannins	Ferric chloride test	+	-	+	+	+
Phenols	Lead acetate test	+	+	+	+	+
Oils and fats	Spot test	+	-	+	+	+
	Saponification test	+	-	+	+	+

+ Present; - Absent

Table 2
Results of phytochemical analysis of Piper betle Linn. leaves

Phytochemicals	Test	Water extract	Water-methanol fraction	Methanol fraction
Alkaloids	Dragendroff's test	+	+	-
	Wagner's test	+	+	-
	Mayer's test	+	+	-
Steroids	Salkowski's test	+	+	+
	Libermann-Burchard's test	+	+	+
Glycosides	Sulfuric acid test	+	+	-
	Molisch's test	+	+	-
Carbohydrates	Benedict's test	+	+	-
	Molisch's test	+	+	-
Amino acids	Ninhydrin test	+	+	+
Saponins	Aqueous test	+	+	+
Flavones	Aqueous test	+	+	+
	Sulfuric acid test	+	+	+
Tannins	Ferric chloride test	+	+	+
Phenols	Lead acetate test	+	+	+
Oils and fats	Spot test	+	+	+
	Saponification test	+	+	+

+ Present; - Absent

RESULTS AND DISCUSSION

The observations made for the phytochemical analysis of the crude extracts and solvent fractions of *Piper nigrum* Linn. seeds and *Piper betle* Linn. leaves are summarized in Table 1 and 2 respectively. Ethanol extract, diethyl ether and chloroform fractions of *Piper nigrum* contained all the phytochemicals analyzed. In case of methanol fraction, alkaloids, glycosides and steroids were absent; whereas the petroleum ether fraction did not have tannins, oils and fats. Water extract and water- methanol fraction of *Piper betle* contained all the phytochemicals tested. In methanol fraction alkaloids, glycosides and carbohydrates were completely absent. From these results it is evident that both the selected plants which are well known for their medicinal properties contain almost all the prominent phytochemicals. Much investigation has been conducted in recent years on the role of phytochemical compounds found in *Piper nigrum* Linn and *Piper betle* Linn[10]. Probably the combination of these secondary metabolites in these two plants consumed as food/ medicine provide the synergistic effect for prevention and treatment of various diseases.

CONCLUSION

The present analysis of the two plants was done as part of the study of their antioxidant,

antitumor and radioprotective properties. The results of the present study showed that both the plants contain various phytochemicals which could possess significant individualistic or synergistic effects against the free radicals and tumor growth.

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