



Assessment of Preliminary Phytochemical Screening and Anti-oxidant Potential of Different extracts of *Piper betle* L. leaves.

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ABSTRACT

In the present study, the phytochemical and antioxidant properties of pet. Ether, aqueous, ethyl acetate and ethanol extracts of *Piper betle* leaves were compared and investigated to find out the number of different phytoconstituents present in the plant which makes it remarkable for its use by traditional practitioners. Phytochemical screening of all the extracts (pet. Ether, aqueous, ethyl acetate and ethanol) showed the presence of alkaloids, steroids, phenolic compounds, tannins, saponins, carbohydrates, proteins, amino acids and organic acids. Further study showed potent *in vitro* antioxidant activity followed by Fenton's method. The ethnolic extract exhibited maximum activity followed by Ethyl acetate extract, aqueous extract and Pet. Ether extract simultaneously. DMSO was taken as control.

Keywords: *Piper betle*, Ethanolic and Ethyl acetate Extracts, Phytochemical constituents, Antioxidant activity.

INTRODUCTION

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethnopharmacological surveys conducted among herbal practitioners of traditional Arab medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins¹. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer². *Piper betle* Linn., (family Piperaceae) commonly known as the betel vine is an important medicinal and recreational plant in Southeast Asia. The most probable place of origin of betel vine is Malaysia but today the plants are also cultivated in India, Sri Lanka, Bangladesh, Burma and Nepal^{3,4}. Betel leaves are the most important plant part and are of medicinal, religious and ceremonial value in Southeast Asia. In India it is customary to serve betel leaf on various social, cultural and religious occasions and is also offered to guests as a mark of respect (referred to as tumbol)⁵. Based on the color, size, taste and aroma there are many varieties of betel

leaf and some of the most popular Indian varieties are the Magadhi, Venmony, Mysore, Salem, Calcutta, Banarasi, Kauri, Ghanagete and Bagerhati^{5,6}. The present study was undertaken to evaluate the phytochemical screening and *In-vitro* anti-oxidant activity of various leaf extracts of *Piper betle* by using Fenton's method.

MATERIALS AND METHODS

All the materials and reagents used for the study were from CDH, Ranchem and Hi Media Ltd., India.

Preliminary Phytochemical Screening

Standard screening tests of various leaf extracts of *Piper betle* were carried to find out various principal constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponin, tannins using standard procedures.⁷⁻⁸

Tests for Carbohydrates

A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates and further addition of Fehling's reagent if showed the brick red color confirmed the presence of reducing sugar.

Detection of Glycosides

Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides. Ammonia layer acquires pink color, showing the presence of glycosides.

Test for Proteins

The 2 ml of filtrate was treated with 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet color indicates the presence of proteins.

Test for Alkaloids

A 100mg of an extract was dissolved in dilute hydrochloric acid. Solution was clarified by filtration. Filtrate was tested with Dragendorff's and Mayer's reagents. The treated solution was observed for any precipitation.

Confirmatory Test: Five grams of the extract was treated with 40% Calcium hydroxide solution until the extract was distinctly alkaline to litmus paper, and then extracted twice with 10 ml portions of chloroform. Chloroform extracts were combined and concentrated in vacuo to about 5ml. Chloroform extract was then spotted on thin layer plates. Solvent system (n-hexane-ethyl acetate, 4:1) was used to develop chromatogram and detected by spraying the chromatograms with freshly prepared Dragendorff's spray reagent. An orange or dark colored spots against a pale yellow background was confirmatory evidence for the presence of alkaloids.

Tests for steroidal compounds

a) Salkowski's Test - 0.5g extracts were dissolved in 2ml chloroform in a test tube. Concentrated sulfuric acid was added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of steroid ring (i.e., the aglycone portion of the glycoside).

b) Lieberman's Test – 0.5 g extracts were dissolved in 2ml of acetic anhydride and cooled in an ice-bath. Concentrated sulfuric acid was then carefully added. A colour change from purple to blue- green indicated the presence of a steroid nucleus, i.e., aglycone portion of the cardiac glycosides.

Tests for Flavonoids

a) Tests for free flavonoids- 5mm of ethyl acetate was added to a solution of 0.5g of the extract in water. The mixture was shaken, allowed to settle, and inspected for the production of yellow colour in the organic layer, which is taken as positive for free flavonoids.

b) Lead acetate test- To a solution of 0.5 g extract in water, about 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

c) Reaction with Sodium hydroxide- Dilute sodium hydroxide solution was added to a solution of 0.5g of the extract in water. The mixture was inspected for the production of yellow colour which considered as positive test for flavonoids.

Tests for Saponin

Froth Test: 0.5g extracts were dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over 30 minutes period of time. If a "honey comb" froth above the surface of liquid persists after 30 minutes the sample is suspected to contain saponin.

Test for Tannins

a) Ferric chloride Test- A portion of the extracts were dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

b) Formaldehyde Test- To a solution of about 0.5g extract in 5ml water, three drops of formaldehyde and six drops of dilute hydrochloric acid were added. The resulting mixture was heated to boiling for 1min and cooled. The precipitate formed (if any) was washed with hot water, warm alcohol, and warm 5% potassium hydroxide successively. A bulky precipitate, which leaves a coloured residue after washing, indicated the presence of phlobatannins.

c) Test for Phlobatanins- Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

d) Modified iron complex Test- To a solution of 0.5g of the plant extract in 5mm of water a drop of 33% acetic acid and 1g sodium potassium tartarate was added. The mixture was warmed and filtered to remove any precipitate. A 0.25% solution of ferric ammonium citrate was added to the filtrate until no further intensification of colour is obtained and then boiled. Purple or blackish precipitates, which are insoluble dilute ammonia, denotes the presence of in hot water, alcohol, or dilute ammonia, denotes the presence of pyrogallol tannin.

Determination of *in vitro* antioxidant activity

Fenton's reaction⁹ was used for determination of *in vitro* antioxidant activity. The hydroxyl radical attached deoxyribose and initiated a series of reaction that eventually resulted in the formation of thiobarbituric acid reaction substance (TBARS). The measurement of TBARS thus gives an index of free radical scavenging activity. The reaction mixture consisted of a deoxyribose (3 mM, 100µl), ferric chloride (Fe³⁺⁺ 0.2 mM 50µl), EDTA (0.1mM 50 µl), ascorbic acid (0.1 mM 100 µl), stock solution of all the extracts (pet. Ether, aqueous, ethyl acetate and ethanol) at 10 mg/ml were prepared from which 100-1000 µl were added in reaction mixture, the final volume was made up to 1 ml by adding adequate quantity of phosphate buffer saline (pH, 7.4) and incubated for 1 hour at 37°C. The reaction was stopped by adding 0.5 ml of 5% TCA and 0.5 ml of 1% TBA the mixture was than incubated for 20 minutes in a boiling water bath. The



absorbance was measured at 532 nm. DMSO was used as control. The results are expressed in the form of percentage absorbance.

RESULTS AND DISCUSSION

Phytochemical screening of the extracts

The results confirmed the presence of alkaloids, glycosides, steroids, saponins and oils in both the extracts of the fruit of the plant. Some of the constituents were observed in one or the other extracts. These phytochemical constituents are good source of antimicrobial and antioxidant activity.¹⁰ The results of phytochemical screening are reported in Table 1.

In vitro antioxidant activity of the extracts

The extracts of *Piper betle* leaves had showed good antioxidant property in Fenton reaction model, the test drug(s) were compared to each other with DMSO as a control. The ethanolic extract showed potent antioxidant activity in comparison to ethyl acetate, aqueous and pet.ether extracts. The ethanolic extract exhibited maximum activity followed by Ethyl acetate extract, aqueous extract and Pet. Ether extract simultaneously. The results are reported in Table 2 and Figure 1. Thus the present investigation revealed that the ethanol and ethyl acetate have potent antioxidant activity in comparison to other extracts while taking DMSO as control.

Table 1: Presence of different primary and secondary metabolites in crude extracts of *Piper betle* leaves

Secondary Metabolite	Extract 2 (Pet. Ether)	Extract 3 (Aqueous)	Extract 4 (Eth. Acetate)	Extract 1 (Ethanol)
Carbohydrates	-	+	-	+
Flavonoids	+	+	+	+
Tannins	-	+	+	+
Saponin	-	+	-	+
Glycosides	+	+	+	+
Alkaloids	-	-	-	-
Proteins	-	-	-	+
Steroids	-	-	-	+

Table 2: Hydroxyl radical scavenging activity of different extracts of *Piper betle* leaf extracts. Fenton's Reaction (Hydroxyl Radical scavenging Activity)

Concentrations	Pet Ether extract	Aqueous extract	Ethyl Acetate extract	Ethanol extract
DMSO (Control)	87.97	87.97	87.97	87.97
5µg/ml	45.43	50.02	55.05	84.24
10µg/ml	42.87	46.34	46.88	90.11
25µg/ml	40.12	48.26	39.34	65.56
50µg/ml	37.10	47.55	31.89	34.89
100µg/ml	31.34	40.13	15.67	14.56
200µg/ml	28.68	37.22	01.11	04.12

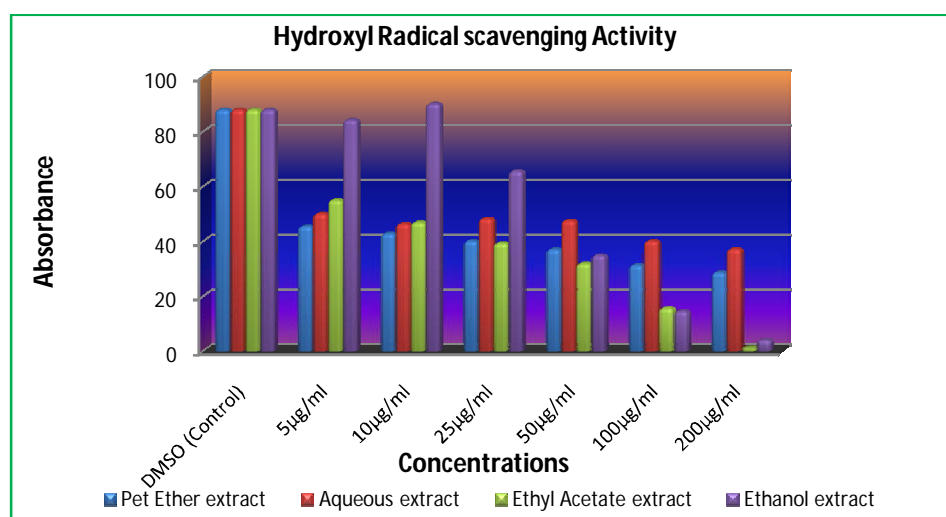


Figure 1: Hydroxyl radical scavenging activity of different extracts of *Piper betle* leaf extracts.

CONCLUSION

In the present investigation it is revealed that there are several phytochemical constituents present in the various extracts of *Piper betle* leaves. These phytochemical constituents are responsible for antioxidant activity of the plant. The results are in accordance with the results of *in vitro* antioxidant activity as revealed by Fenton's reaction. Further studies on isolation and characterization of the specific constituents are needed to validate our results. The study thus can be further utilized to formulate the natural antioxidant which can be used as a dietary supplement to fight against several diseases such as ageing, arteriosclerosis etc. which caused due to Reactive Oxygen Species (ROS).

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