

Comparative Study and Phytochemical Screening of Different Extracts of Piper betel L. Leaf

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ABSTRACT

The goal of this work was to compare the various extracts of Piper betel Linn leaves and to establish the phytochemical screening of all extracts (i.e., Aqueous, Methanolic and Ethanolic Extracts). Piper betel Linn leaves were taken and shade dried for 10 days. Dried leaves were size reduced and powdered before being passed through filter no. 40. The Decoction technique was used to create an aqueous extract. The Ethanolic and Methanolic extracts are prepared using the maceration process. Fresh leaves of Piper betel Linn were employed for the research of organoleptic characteristics. The presence of phytoconstituents was discovered to be greater in aqueous extracts than in ethanolic and Methanolic extracts. Apart from these, the presence of numerous phytochemical elements such as Alkaloids, Tannins, Saponins, Flavonoids, Saponin Glycosides, Cardiac Glycosides, Steroids, and so on was discovered in all three extracts. The percentage yield of aqueous extract was significantly higher than that of Methanolic and Ethanolic extracts. Piper betel Linn leaves may be identified and standardised using a rapid, efficient, and cost-effective method that involves the investigation of a number of physicochemical constants. The qualitative phytochemical investigation revealed that the aqueous extract of the leaf includes various secondary plant components with high therapeutic effectiveness. As a result, aqueous extracts of P. betel Linn. leaves can be used to identify useful secondary plant components for future medication development. This might be used for basic drug quality control and verification.

Keywords: Piper Betel, Phytoconstituents, Methanolic Extract, Ethanolic extract, Aqueous Extract

INTRODUCTION

Piper betel Linn (Paan) is an indigenous, evergreen creeper belonging to the family Piperaceae. While it is endemic to Malaysia, this plant is also widely distributed across Southeast Asia, including Bhutan, India, Bangladesh, Sri Lanka, Thailand, and Taiwan. *Piper betel* Linn is commonly known as Paan in India and widely used as Mouth freshener after meal.

From the old age, due to its edible properties, Betel leaves have a huge impact on the people's daily life. It also possesses various medicinal and therapeutic value. According to Ayurveda (one of the traditional systems of medicine), due to the pharmacological benefits, betel leaves are using by a huge number of populations in India and world [1,2].

According to Susrta Samhita and Bhabaprakash, Paan (also known as Tambool) is a aromatic, pungent, sharp and hot creeper. It has multiple benefits such as Laxative, Antimicrobial, Sedative, anti-inflammatory, Anti-diabetic, Anti-oxidant and Analgesic characteristics [3]. Paan is also used in treatment of Oral Ulcer. In India, Paan is chewing with other ingredient to treat mouth ulcers. National Botanical Research Institute, Lucknow has been classified *Piper betel* Linn. into five sub-categories, i.e., Bangla, Desawari, Kapor, Meetha and Sanchi [4].

Piper betel Linn. Leaves have been widely employed as a source of material for many traditional and modern medicine formulations because they contain a range of significant phytoconstituents. *Piper betel* Linn. leaves may be identified and standardised using

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a quick, efficient, and affordable process that involves the study of numerous physicochemical constants [5-6].

Taxonomical Classification of *Piper betel* Linn. leaf

Kingdom	<i>Plantae</i>
Order	<i>Piperales</i>
Family	<i>Piperaceae</i>
Subfamily	<i>Piperoideae</i>
Genus	<i>Piper</i>
Species	<i>Piper betel</i> L
Division	<i>Magnoliophyta</i>

Chemical Constitution of *Piper betel* Linn. leaf

The *Piper betel* Linn. leaf contains the following chemical components that are helpful for a number of medical ailments. The list of chemical substances discovered in leaves is compiled in Table 1.0. [1,2, 8,9].

Table 1.0: List of chemical constituent present in *Piper betel* Linn. leaf

Sr. no.	Chemical constituent
1.	β -sitosterol
2.	γ -sitosterol
3.	Hentriacontane
4.	Pentatriacontane
5.	n -triacontanol
6.	Stearic acid
7.	Chavicol

Pharmacological Value of Leaf extract

Various *Piper betel* leaf extracts have been studied for their potential medicinal and pharmacological benefits. In the alternating heptagons that are provided below, the list of numerous pharmacological benefits has been compiled.

1.	• Anti-cancer
2.	• Anti-ulcer
3.	• Anti-diabetic
4.	• Analgesic
5.	• Anti-oxidant
6.	• Anti-inflammatory
7.	• Anti-microbial

Fig. no. 1.: Pharmacological activities found in different extracts of *Piper betel* Linn. leaf [10-11]

Vernacular name of *Piper betel* Linn.

According to the traditional system of medicine, *piper betel* Linn. is known by a variety of common names. As several different languages are used in India, the word "piper betel" has several synonyms. All the data are collected in the provided table (Table 2.0).

Table 2.0: List of vernacular names of *Piper betel* Linn. leaf [12-13]

Sr. No.	Traditional system of Medicine or other Indian Languages	Vernacular names
1.	Hindi, Urdu	Paan
2.	Siddha	Vettilai Nagavalli or Kammaaruvetritai
3.	Unani	Paan or Tambool
4.	Ayurveda	Naagini/ Tambula/ Saptashiraa/ Bhujangalataa
5.	Bengali	Paan
6.	Gujarati	Paan

MATERIAL AND METHOD

Procurement of Plant material

Piper betel L. leaves were collected from the Local market of Dubagga, Lucknow. The leaf was authenticated by Dr Shashank Tiwari, Director (Academic and Research), Lucknow Model College of Pharmacy and Research, Lucknow.

The leaves were washed through tap water to remove dirt and attached impure particles and kept for air and shade dried for 10 days, followed by drying in Tray dryer for 10 minutes, grounded for size reduction by Grinder and subjected to size separation by Sieve no. 40 to get fine powder. Resulting Fine Powder was obtained, kept for further Studies by packing in zipper bags, followed by placing them in Desiccators.

Organoleptic Evaluation of *Piper betel* L. Leaves

During conducting an organoleptic examination, some sensory factors were noted, including odour, colour, taste, shape, apex, and leaf texture.

Preparation of Extracts

Piper betel L. leaves were cleaned, dried, and powdered into a coarse powder before being passed



through Sieve No. 40 to separate the sizes. As a consequence, Piper betel L. leaf powder was produced [11,14].

Preparation of Aqueous Extract of *Piper betel* L. Leaves

Piper betel L. fresh, washed, and dried leaves were made into an aqueous extract using the decoction technique. After grinding the leaves into a coarse powder, the coarse powder was separated into different sizes by being passed through Sieve No. 40. As a consequence, *Piper betel* L. leaf powder was produced. Aqueous extract of *Piper betel* L. leaves was made by combining 10 gm of finely crushed leaves with 100 ml of distilled water in a 500 ml beaker made of borosilicate glass. On a heating mantle, the mixture was boiled for 15 minutes at 80 degrees. Beaker was then taken out of the heating mantle and let to sit at room temperature. Whatman Filter Paper No. 1 was used to filter the mixture. The finished mixture was added to a petri dish and then Water was evaporated using a water bath at 80°C.

Preparation of Methanolic Extract of *Piper betel* L. Leaves

Fresh, washed, and dried leaves of *Piper betel* L. were macerated to create a methanolic extract. After grinding the leaves into a coarse powder, the coarse powder was separated into different sizes by being passed through Sieve No. 40. As a consequence, Piper betel L. leaf powder was produced. By combining 10 gm of finely powdered Piper betel L. leaves with 100 ml of methanol in a 250 ml volumetric flask made of borosilicate glass, a methanolic extract of the plant's leaves was created. stored this concoction in reserve for 96 hours. Whatman filter paper No. 1 is then used to filter the mixture after this step. The resulting mixture was put onto a petri dish, and then Methanol was evaporated using a water bath at 80°C.

Preparation of Ethanolic Extract of *Piper betel* L. Leaves

Fresh, washed, and dried leaves of *Piper betel* L. were macerated to create an ethanol extract. After grinding the leaves into a coarse powder, the coarse powder was separated into different sizes by being passed through Sieve No. 40. As a consequence, Piper betel L. leaf powder was produced. Piper betel L. leaf

ethanolic extract was made by combining 10 gm of finely powdered leaves with 100 ml of ethanol in a 250 ml volumetric flask made of borosilicate glass. stored this concoction in reserve for 96 hours. Whatman filter paper No. 1 is then used to filter the mixture after this step. The resulting mixture was put onto a petri dish, and then the ethanol was evaporated using an 80°C water bath [15-16].

Qualitative Phytochemical screening or analysis of *Piper betel* L. leaf extracts

The presence of several phytochemicals or phytoconstituents such alkaloids, glycosides, triterpenoids, flavonoids, phenolic compounds, steroids, cardiac glycosides, etc. is checked in all three extracts (i.e., aqueous, methanolic, and ethanolic extracts) using a variety of established techniques. Alkaloids, flavonoids, anthroquinone, saponins, carbohydrates, volatile oils, saponin glycosides, cardiac glycosides, tannins, and steroids were among the substances that were examined. [1, 16-17].

1. Mayer's test:

Solvent-free extracts were mixed with one mL of weak Hydrochloric acid before adding a few drops of Mayer's Reagent. The presence of alkaloids is confirmed by organic reddish brown precipitate or cream hue.

2. Wagner's test:

Solvent-free extracts were mixed with one mL of weak Hydrochloric acid before adding a few drops of Wagner's Reagent. The presence of alkaloids is confirmed by the appearance of brown or reddish brown precipitate.

3. Dragendroff's test:

Extracts were stirred with few drops of Dragendroff's Reagent. Reddish Brown colour or precipitate confirms the presence of the alkaloids.

4. Flavonoid test: (Alkaline Reagent Test)

Distilled water was used to dilute all extracts. The aqueous extracts were then treated with 1 mL of diluted Ammonium hydroxide solution, which produced a yellow fluorescence that vanished when



acid was added. The presence of flavonoids is confirmed by yellow fluorescence.

5. Cardiac Glycosides test:

Aqueous, Methanolic, Ethanolic Extract are separately diluted with 2 ml of distilled water. Then, solution of extracts was treated with 3.5% Ferric chloride solution. Allow to stand for 1-2 minutes. Add few drops of Conc. H₂SO₄ by the wall of the test tubes. A Reddish- Brown Colour is formed at Junctions which turns blue after few minutes confirm the presences of Cardiac Glycosides.

6. Saponin glycosides test:

Fehling Solution “A” and “B” was added in 2.5 ml solution of extracts.

7. Saponin test:

0.5 gm of extracted was diluted with 5 ml of water. Shake well the test tube for few minutes. Froths generation or 1 Cm layer of foam confirms the presence of Saponin.

8. Tannins Test:

2ml of all extracts solutions was treated with 5% Ferric Chloride solution gradually. Blue colour was obtained. On addition of more Ferric chloride solution, blue colour turns into Olive green. It confirms the presence of Tannins.

9. Molisch Test:

All extracts are diluted with water. All solutions were treated with Molisch reagent. Conc. Sulphuric acid was also added along the side of the test tubes. A purple ring at the junction confirms the presence of Carbohydrates.

10. Test for Anthraquinones:

All solutions of the extracts was treated with 10 ml benzene, and 10% Ammonia Solution. Shake the Test tubes well to confirms the colour of Anthraquinones.

11. Test for Steroids:

According to the Harborne method (1973), Diluted samples of extracts (2 ml) was treated with chloroform (2ml). Allow to stand for 1-2 minutes followed by the addition of Conc. Sulphuric Acid along the wall of test tubes to form the lower layer.

12. Test for Volatile oils:

1 ml of each diluted samples of extracts were mixed with HCl. White precipitate formation confirms the presence of the Volatile oils [15, 19-20].

RESULT AND DISCUSSION

1. Preparation of Extracts

Preparation of Aqueous Extract of *Piper betel* L. Leaves

The concentrated extract (1.32 gm, solid, Deep Brown colour) was stored in a sample vial with desiccators for future use.

Preparation of Methanolic Extract of *Piper betel* L. Leaves

The concentrated extract (0.93 gm, Semisolid, Dark Green coloured) was preserved in desiccators after being stored in a sample vial.

Preparation of Ethanolic Extract of *Piper betel* L. Leaves

The concentrated extract (1.03 gm, Semisolid, Dark Green coloured) was preserved in desiccators after being stored in a sample vial.

2. Percentage Yield of various extracts of *Piper betel* Linn. Leaves

All three extracts of *Piper betel* Linn. Leaves have different percentage yield, colour and state. The result data from the investigation is as follows (listed in table 3.0).

Table 3.0: Percentage Yield of various extracts of *Piper betel* Linn. Leaves

Sr. no.	Extract	Yield (in gm)	% yield	Colour	State
1.	Aqueous	1.32 gm	13.2 %	Deep Brown	Solid
2.	Methanolic	0.93 gm	9.3 %	Dark green	Semisolid
3.	Ethanolic	1.03 gm	10.3 %	Dark green	Semisolid



3. **Morphological or Organoleptic properties of *Piper betel* Linn. leaves** Morphological and organoleptic properties of *Piper betel* Linn. leaves were investigated. The result of investigation is listed below in table 4.0

Table 4.0: Morphological or Organoleptic properties of *Piper betel* Linn. leaves

Sr. No.	Morphological characteristics	Observation data	
1.	Dimension of leaf	Length: 7-16 cm	Width: 4-11 cm
2.	Colour and Condition	Fresh leaf: Green-dark green	
3.	Lamina	Reticulate, simple	
4.	Shape	Heart shaped	
5.	Odour	Aromatic, Pleasant and characteristic	
6.	Texture	Glossy and Smooth	
7.	Venation	Reticulate	
8.	Apex	Pointed	
9.	Taste	Aromatic	

4. **Phytochemical screening OR Phytoconstituent screening** phytoconstituent in extracts of *Piper betel* leaves. The result of this investigation is listed below.

Table 5.0: Phytochemical screening of *Piper betel* leaves

Sr.no.	Test name	Aqueous extract	Methanolic extract	Ethanollic extract
1.	Mayer's test	+	-	-
2.	Wagner's test	+	+	+
3.	Dragendroff's Test	-	-	-
4.	Flavonoids test	+	-	+
5.	Tannin test	+	+	+
6.	Test for Cardiac Glycosides	+	+	+
7.	Test for Saponin Glycosides	+	+	+
8.	Saponin test	+	+	-
9.	Molisch test	+	+	-
10.	Test for Anthraquinone	-	-	-
11.	Steroid test	+	+	+
12.	Volatile oils	-	-	-

Whereas “+” indicates for the presence and “-” indicate the absence of that phytoconstituents.

IMAGES







CONCLUSION

The current study shows that phytochemical screening detected the presence of alkaloids, tannins, flavonoids, steroid, anthraquinones, carbohydrates, saponins, cardiac glycosides, and saponin glycosides, which may be responsible for a range of medicinal and therapeutic actions. There is a lot of promise for in vivo studies because to the presence of several phytoconstituents.

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ETHICAL APPROVAL

This study does not require any ethical approval.

CONSENT TO PARTICIPATE

Not Applicable.

CONSENT FOR PUBLICATION

Yes, all authors gave their consent for the publication.

AVAILABILITY OF DATA AND MATERIAL

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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