

# Bioactive compound analysis in *Piper betle* leaf

<sup>\*1</sup>Shweta Tyagi, <sup>2</sup>I.P. Pandey, <sup>1</sup>Ashish Kumar

<sup>1</sup>D.A.V. (PG)College, Muzaffarnagar, India

<sup>2</sup>Professor Emeritus, Dehradun, Uttarakhand, India

<sup>\*</sup>Email: [shewtatyagi@gmail.com](mailto:shewtatyagi@gmail.com)

DOI 10.51129/ujpah-2022-33-2(10)

Received – November 30, 2022

Revised – December 05, 2022

Accepted – December 07, 2022

Published – December 24, 2022

**Abstract-** Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases. *Piper betle* commonly known as paan is used as a herbal medicine from ancient time to till present days. The objective of the present study was to test phytochemicals as well as biochemical compound in leaves of *Piper betle*. The results showed that all the bioactive compound abundantly present i.e. tannin, saponins, flavonoids, terpenoids. Glycosides, proteins, carbohydrates and phenol in aqueous and other (methanol, acetone, diethyl ether, acetone) extracts. Whereas steroids are absent in all the extracts.

**Key words:** *Piper Betle*, Bioactive Compounds, Herbal Medicine.

## Introduction

*Paan*, the treat – a betel leaf stuffed with a variety of ingredients – can be found in everywhere like people's homes, at restaurants, shopping centres and markets. *Tambul*, *tamalapaku*, *nagavalli*, *nagarbel*, *vettile* known by different names in different Indian languages. *Piper betle* commonly known as betel vine belongs to the family Piperaceae. It is a popular

medicinal plant in Asia. India grows nearly 40 varieties out of the nearly 100 cultivated worldwide.

From the ancient time, analysis of biologically active natural yields from plants has attracted several natural product researchers. In the ayurvedic and Unani system of medicine, the betel plant is used as an anthelmintic, appetite stimulant, vermifuge, astringent, diarrhoea, aphrodisiac, breath freshener, carminative, cardiac tonic, dentifrice, in the prevention of diuretic emmenagogues, induction and increase of menstrual flow, laxative, strengthen gums, nerve tonic and also in the treatment of urinary disorders (Satyavati GV). Betel leaves may help in relieving headaches, fighting against cancer, healing wounds, may reduce gastric ulcers, diabetes, and allergies. (Karandeep Kaur et al, 2018) Betel leaves contain several phytochemicals and nutritional components like proteins, fats, minerals, fibre, carbohydrates, potassium, calcium, vitamin C and other nutrients. (Proshanta Guha, 2022), which is recognized as a biologically active compound. These compounds have several significant pharmacological properties, in which antimicrobial, antidiabetic, antiulcer, anti-inflammatory, antimutagenic, and antioxidant properties are crucial once. Research on these compounds of betel leaves

have established their application as future active and additive ingredients in the pharmaceutical.

## **Material and method**

### **Collection of plant material**

The leaves of *Piper betle* were collected from the local market near at D.A.V(PG) College, Muzaffarnagar, UP, India and identified by the HOD of the Botany department, Dr. Sanjeev Kumar. The plant leaves were washed with running tap water.

### **Preparation of plant leaves extracts**

The fresh leaves of the plant *Piper betle* were homogenised, and about 20 g of the sample from each variety was extracted separately with 200mL(10%) of aqueous (sterilized distilled water) and four different solvents: methanol, acetone, di ethyl ether and ethanol at room temperature. The extracts were filtered and filtrates were concentrated in a rotary evaporator at 45 °C.

### **Phytochemical and Biochemical screening of leaves of *Piper betle***

Preliminary qualitative screening of bioactive compounds present in the plant *Piper betle* was carried out with the following Chemical tests in the aqueous and four other (alcoholic and ether) extract using standards to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), (Omoya and Akharaiyi, 2012), (Jyothiprabha and Venkatachalam, 2016). (Harborne and Williams, 2000).

#### **Test of alkaloids (Dragendorff's Test)**

5 ml each of the extracts, 5 ml of aqueous hydrochloric acid was added on a steam bath at 60°C for 5 min. The extract was filtered. Take one ml of the filtrate, few drops of Dragendorff's reagent were added. Appearance of Blue-black turbidity was positive for alkaloids.

#### **Test of steroids (Libermann- Burchard Test)**

1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

#### **Test of tannins (Braymer's Test)**

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

#### **Test of terpenoids (Salkowski Test)**

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish -brown layer at the junction of two solutions confirms the presence of terpenoids.

#### **Test of flavonoids (Alkaline reagent Test)**

2 ml of extract was treated with few drops of 1N sodium hydroxide solution and observed the formation of intense yellow colour. This yellow colour becomes colorless on addition of dilute hydrochloric acid, indicating the presence of flavonoids.

#### **Test of saponins (Foam Test)**

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence of the presence of the saponins.

#### **Test of Phenols (Ferric chloride Test)**

2ml of plant extract, 2ml of distilled water followed by 10 % FeCl<sub>3</sub> solution was added. Bluish black colour indicates the presence of phenol.

### **Test of Carbohydrate**

**Molisch's test-** 5 mg extract was taken in test tube than the 1 ml of Molisch's reagent was added into it. Mixture was shaken properly. After that, 2ml of concentrated Sulfuric acid was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

**Benedict's test-**Benedict's reagent was taken for the analysis of carbohydrate. the 5 mg extract was mixed with few drops of benedict's reagent, then allowed to boiled, the reddish-brown precipitate is found with the presence of the (reducing sugar)carbohydrates.

### **Test for proteins**

**Ninhydrin test-** aqueous extract of *Piper betle* was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for 2 min on water bath, if violet colour appeared with the presence of amino acids and proteins in the aqueous extract.

**Biuret's test-** 5 mg extract was added with the few drops of biuret's reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins.

**Test of Glycosides (Keller-kilani test)-** Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl<sub>3</sub>. The mixture was then poured into another test tube containing 2ml of concentrated H<sub>2</sub>SO<sub>4</sub>. A brown ring at the interphase indicated the presence of cardiac glycosides.

### **Results and Discussion**

Results of the phytochemical screening of the plant are indicated in table. Presence of the phytochemicals is indicated by a + sign while a – sign indicate absence of the tested phytochemical. This work principally focuses

on phytochemical screening in the leaves of *Piper betle* . The results are summarized and mentioned below. The current study was carried out on the *Piper betle* unconcealed the presence of medicinally active constituents. The phytochemical screening in the leaves of *Piper betle* showed that the abundant presence of all bioactive compound i.e.tannin, saponins, alkaloids, flavonoids, terpenoids, phenol, glycosides, protein and carbohydrates in aqueous and other extracts. While steroids are absent in aqueous and other leaf extract. The latest analysis investigation has ascertained that the bioactive and inhibitor potentials of those plants are attributed to the presence of phenols, flavonoids, alkaloids, terpenoids, saponin, and tannins (Agbor *et al.*, 2011). Terpenoids are concerned with medication and antineoplastic functions thus employment of the plant to treat burns, skin diseases, and bug stings (Bown Deniet *al.*, 1995), Flavonoids are also present in all the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah N, 1995; Rio DA, 1997]. Tannin rich medicinal plants are used as healing agents in a number of diseases Doughari JH (2012). Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants (Noble RL 1990; Madziga HA, 2010).

**Table- Screening of bioactive compounds present in different extracts of leaves of *Piper betle*.**

S.No.	Bioactive compound	Test name	Extracts				
			Aqueous	Methanol	Acetone	Diethyl ether	Ethanol
1	Proteins	Ninhydrin test	+	+	+	+	+
		Biuret test	+	+	+	+	+
2	Carbohydrates	Molisch's Test	+	+	+	+	+
		Benedict Test	+	+	+	+	+
3	Alkaloids	Dragendorff test	+	+	+	+	+
4	Flavonoids	Alkaline reagent test	-	+	+	+	+
5	Steroids	Libermann Burchard Test	-	-	-	-	-
6	Tannin	Braymer's Test	-	+	+	+	+
7	Saponin	Foam Test	+	+	+	+	+
8	Phenols	Ferric Chloride Test	+	+	+	+	+
9	Terpenoids	Salkowski Test	+	+	+	+	+
10	Glycosides	Keller-kilani test	+	+	+	+	+

## Conclusion

Natural products play a very important role within the field of recent medication analysis and development due to its low toxicity, simple handiness, and low price. Our results suggest that *Piper betle* can serve as potential source of bioactive healthy compounds and their consumption could be useful in the prevention of diseases. Determination of the natural phytochemicals and antimicrobial compounds can facilitate to develop of new drug candidates for antimicrobial medical aid. From the above studies, It is concluded that as the plants *Piper betle* studied, found to rich in phytochemicals, are full of pharmacological and medicinal significance and is used for herbal drugs.

Further study is required to find their potentials in the mentioned biological properties such as antidiabetic, anti-tumor,

etc. It is concluded that as the plants studied, found to rich in phyto-chemicals, are full of pharmacological and medicinal significance. It is concluded that as the plants studied, found to rich in phytochemicals, are full of pharmacological and medicinal significance

## Disclaimer Statment

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

## References

- Agbor, G. A.; Moumbegna, P. and Oluwasola, E. O. *et al.*, "Antioxidant capacity of some plant foods and beverages consumed in the Eastern Region of Nigeria," *African Journal of Traditional, Complementary, and Alternative Medicines*, 2012;8(4):362–369.

- Bown, Deni. The Royal Horticultural Society. *Encyclopaedia of Herbs and their uses*, 1995.
- Doughari, J.H. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, 2012.
- Harborne, J.B. and Williams, Ch.A. Advances in flavonoid research sinc.1992. *Phytochem.*, 2000; 55:481-504.
- Jyothi Prabha, V. and Venkatachalam, P. Preliminary phytochemical screening of different solvent extracts of selected Indian spices. *Int. J. Curr. Microbiol. App. Sci.*, 2016; 5(2):116-122.
- Kaur, Karandeep; Rai, Rashmi; Trivedi, V.; Milind, J. and Umekar. review on betel leaf used in various ailments [Internet]. [cited 2022 Aug 18]. Available from: [https://www.researchgate.net/publication/343749007\\_review\\_on\\_betel\\_leaf\\_used\\_in\\_various\\_ailments](https://www.researchgate.net/publication/343749007_review_on_betel_leaf_used_in_various_ailments).
- Omoya, F.O. and Akharaiyi, F.C. Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. *Int. Res. J. Pharm.*, 2012; 2(5):127-132.
- Proshanta, Guha. Betel Leaf: The Neglected Green Gold of India Nutritional composition of fresh betel leaf | Download Table [Internet]. [cited 2022 Aug18]. Available from: [https://www.researchgate.net/figure/Nutritional-composition-of-fresh-betel-leaf\\_tbl1\\_228624984](https://www.researchgate.net/figure/Nutritional-composition-of-fresh-betel-leaf_tbl1_228624984).
- Rio, D.A.; Obdulilio, B.G.; Casfillo, J.; Marin, F.G. and Ortuno, A. Uses and properties of citrus flavonoids. *J. Agric. Food. Chem.*, 1997;45(12):4505-4515.
- Salah, N.; Miler, N.J.; Pagange, G.; Tijburg, L. and Bolwell, G.P. et al. Polyphenolic flavonoid sassaflavone scavenger of aqueous phase radicals as chain breaking antioxidant. *Arch. Bio. Chem. Broph.*, 1995; 322(2):339-346.
- Satyavati, G.V.; Raina, M.K. and Sharma, M. Medicinal Plants of India. *Indian Council of Medical Research*, 1987; [Google Scholar]
- Sofowara, A. Medicinal plants and Traditional medicine in Africa. *Spectrum Books Ltd., Ibadan, Nigeria*. 1993;289.
- Trease, G. E. and Evans, M.C. *Pharmacognosy*. Elsevier, 14th ed. 2005;53:431-51.