Research article

PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT OF PIPER BETEL LEAVES FOR ANTIBACTERIAL ACTIVITY

Gopalghare Dipali¹, **Palshikar Gautam^{*1}**

¹JSPM's Jayawantrao sawant College of Pharmacy & Research, Hadapsar, Pune.

Corresponding Author: Mr. Gautam Sadashiv Palshikar

ABSTRACT:

Plants can be extracted and used for research work and are known to produce and store many Biochemical products. Different types of Herbal extract are commercially available in market. Become a medical use the potency of these extracts should be compatible & as per the standards prescribed in Pharmacopeia. Our aim is to evaluate the marketed extract sample and to check its authenticity and purity as per the standard parameter. The present study deals with preliminary phytochemical analysis & antibactertial activity of leaf with aqueous extract.

Keywords- Chemical Goldmines, Pharmaceutical compound, Herbal extract, Crude extract, Standardization, Evaluation.

INTRODUCTION

The **betel** (*Piper betel*) is the leaf of a vine belonging to the Piperaceae family, which includes pepper and kava. It is valued both as a mild stimulant and for its medicinal properties. Betel leaf is mostly consumed in Asia and elsewhere in the world by some Asian emigrants, as betel quid or in paan, with or without tobacco, in an addictive psycho-stimulating and euphoria-inducing formulation with adverse health effects. Betel is notable for staining the teeth of regular users. In Kerala as well as Sri Lanka, a sheaf of betel leaves is traditionally offered as a mark of respect and auspicious beginnings. Occasions include, greeting elders at wedding ceremonies, New Year, offering payment to Ayurvedic physicians and astrologers where usually money and or areca nut are kept on top of the sheaf of leaves and offered to the elders for their blessings. The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin. The betel plant originated from South and South East Asia.

The betel leaf is cultivated mostly in South and Southeast Asia, from Pakistan to Papua New Guinea. Since it is a creeper, it needs a compatible tree or a long pole for support. Betel requires well-drained fertile soil. Waterlogged, saline and alkali soils are unsuitable for its cultivation.^[8]

In Bangladesh, farmers called *barui* prepare a garden called a *barouj* in which to grow betel. The *barouj* is fenced with bamboo sticks and coconut leaves. The soil is plowed into furrows of 10 to 15 metres' length, 75 centimetres in width and 75 centimetres' depth. Oil cakes, manure, and leaves are thoroughly incorporated with the topsoil of the

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furrows and wood ash. The creeper cuttings are planted at the beginning of the monsoon season.

Macroscopic Characters

Leaves are simple alternate stipulate petiolate The petiole 0.75 to 3.8cm, ovate oblong broadly ovate cordate or obliquelu elliptic entire glabrous coriaceous 10 to 18 cms long and 5 to 10 cms broad acuminate oblique and rounded base primary or srbprinary nerves usually 7 (or 5to9); Secondary nerves reach to very near the apex; tertiaries numerous.

Microscopic Characters

Fig.2 Microscopy

TS of leaf through midrib shows In adaxial epidermis multiple epidermis with a single layer of rectangular epidermal cells with thick cuticle followed by two layers of larger hypodermal cells, Apostomatic . In abaxial epidermis, rectangular cells or 10mm contacts of silica bodies occur in a stellate mass with irregular outline (Fig:2)

Small rounded cells with hypodermal cells followed by small arc of sclerenchyma(adaxial part)large barrel shaped, epidermis, followed by2(or)3 layers of collenchyma cells(abaxial part) The palisade layer are well distingnuished they are double layered short wide compact cells and mesophyll cells are 3or4 layered and small lobed . Thick walled irregular secretory cells are seen with dense contents of probable an essential oil.

The leaf is dorsiventral mesomorphic even smooth and both surface with farrly rominent midrib The midrib portion of the leaf contains thin walled compact parenchymal cells in ground tissue. The vascular bundles located at the centre of midrib portion single ovate collateral cells with destea of xylem elements and a thick arc of phloem was observed cyclocytic stomata are seen only in the lower surface secretory glandular trichomes abundant on the epidermal layer with short stalk with buried in epidermal layer. The body is spindle shaped and horizontal oriented it is attached to stalk at one end and it is called ad pearl glands

Powder Characteristics

The leaf powder ps greyish green in colour having aromatic odour and burning tate it shows following powder characteristics,

- 1. pearl glands in secretory glandular trichome
- 2. cyclocytic stomata
- 3. palisade and spongy parenchyma cells
- 4. epidermal cells with auticlinal wall fibres.

PRILIMINARY PHYTOCHEMICAL SCREENING O F PIPER BETEL Linn

Determination of moisture content

The powdered material (1 g) was placed in a moisture dish and dried to a constant weight in an oven at 100 °C-105 °C. The loss of weight in mg/g of air-dried material was calculated.

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Determination of total ash content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was it was white. The residual ash was allowed to cool in a desicator. The content of total ash in mg/g of ignited to a constant weight by gradually increasing the heat to 500 $^{\circ}$ C-600 $^{\circ}$ C until air dried material was calculated.

> Determination of acid insoluble ash content

Hydrochloride acid (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried and ignited to a constant weight. The residue was allowed to cool in a desicator and weighed. The content of the acid insoluble ash in mg/g of air dried material was calculated.

Determination of water soluble ash content

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight. The water soluble ash content was calculated.

> Determination of ethanol extractable matter

Accurately weighed powdered material (4 g) was placed in a glass stoppered conical flask. Ethanol (95%; 100 mL) was added to the flask and it was weighed to obtain the total weight including the flask. Then, the flask was shaken well and kept for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of 95% ethanol. The contents were filtered rapidly. After that, 25 mL of the filtrate was evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h, cooled in a desiccator, and weighed. The content of extractable matter in mg/g air dried material was calculated.

MATERIALS AND METHODS

> COLLECTION OF PLANT MATERIAL

The *Piper betel* plant Linn. Was collected from the PUNE district OF Maharashtra India, during year 2014. The leaves of plant of piper betel collected and kept for drying.

> PREPARATION OF DIFFERENT CONCENTRATION OF PLANT EXTRACT

Dried extracts that is aqueous extract of *Piper betel* were powdered with the help of mortar & pestle .Various concentrations are made. All the solution evaluated for antimicrobial activity. Leaves of plant *Piper betel* Linn .were collected & kept for drying in shadow.

After drying of leaves it crushed and powdered. Powdered crude material is used for extraction by using solvent .Prepared extract was kept for evaporation in room

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tempreture. After some day it dried completely. Dried extract is use for phytochemical screening using various chemicals.

EVALUATION OF ANTIBACTERIAL ACTIVITY:

Requirements: 1. cultures:

Saline suspension of *Staphylococcus aureus* (NCIM 2654), Bacillus subtilis (NCIM 2196), *P. aureofaciens*(*NCIM 2026*), *P.Vulgaris*(*NCIM 2027*).

2. Media :

Nutrient agar plate.

3. Solvent :

Water

4. Apparatus:

Test tube, conical flask, pipette, Beakers, Petri plates, Cork borer.

Hot air oven ,Incubator, water bath ,Auto clave etc.

PROCEDURE:

1. Prepare nutrient agar petri plates for growth of bacterial cultures.

2. Spread the test cultures on the plates by spreading plate method.

3. The test cultures used such as S. aureus, Bacillus substilis, P. Vulgaris, P. aureofaciens.

4.Prepare well in greeded petri plates by using cork borer that is sterilised by burning with absolute ethanol.

5. Plant extract (0.1ml) are added in the labelled wells and incubated.

6. Bacterial test culture are incubated at 32°C -37°C for 48 hours.

7. The sensitivity of test organism to each of extracts is indicated by clear zones of inhibition around the well.

8. Measure the diameter of clear zone of inhibition.

RESULT & DISCUSSION

Petri plate method was used to determine the inhibition zones of *Piper Betel* Linn .aqueous extract. The plant leaves showned significant antibacterial activity against bacterial strains.

The gram negative bacteria show more zone of inhibition than gram positive bacteria. Hence gram negative bacterial strains are more susceptible to aqueous extract of piper betel leaves than gram positive bacterial strains.

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Fig. 1 Betel Plant cultivation in Bangladesh

PIPER BETEL LINN. LEAVES

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Fig.2



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Microscopic feature of Powder material of leaf and petiole of CB and AK.



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Volume 4 Issue 4, 2014

7

Fig.3. BEFORE GROWTH OF BACTERIAL SPECEIS



Fig.4.AFTER FORMATION OF ZONE OF INHIBITION



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www.earthjournals.org Volume 4 Issue 4, 2014

Fig.5. CHEMICAL TEST:



A-.Molisch test B-Keller killiani test(for glycosides) C-Test for phenols & tannins D.-Test for alkaloids(Dragondroffs reagent test)

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Sr.no.	NAME OF TEST	OBSERVATION
1.	Test for carbohydrate	+
2.	Test for protein	+
3.	Test for alkaloids	+
4.	Test for glycosides	+
5.	Test for amino acid	+
6.	Test for flavonoids	_
7.	Test for tannins	_
8.	Test for starch	_
9.	Test for fixed oil	_
10.	Test for phenols	+
11.	Test for saponins	_

Table-1 PHYTOCHEMICAL EVALUATION: (+) show presence, (-) show absence

Table 2. **RESULTS:**

Sr. no.	Name of bacterial strain used	Diameter of zone of in concentration.(in mm)			ubition in	following
		1%	2%	3%	4%	5%
1.	S. aureus	11	14	10	22	19
2.	B. subtilis	-	-	-	-	10
3.	P. aureofaciens	20	18	15	15	9
4.	P. vulgaris	22	11	9	10	11

Table-3 PHYSICOCHEMICAL PARAMETER OF PIPER BETEL LEAVES:

Sr.	Parameter	Inference in(%w/w)	
no.			
1	Total ash value	15.0 -17.3	
2	Acid insoluble ash	10.5-12.5	
3	Water soluble ash	9.5-11.2	
4	Moisture content	2.2-2.5	

CONCLUSION:

From the result we concluded that higher concentration of crude drug extracts shows more zone of inhibition towards gram positive bacterial strain where lower concentration of crude drug extracts show more zone of inhibition towards gram negative bacterial strain.

That is gram negative bacteria are more susceptible to the aqueous extract of piper betel than gram positive bacteria.

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