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STUDIES ON QUALITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Piper nigrum*

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Abstract:

The Piper betle L. plant materials such as [leaf and seed] were collected and allowed to shade dry to remove moisture content. The dried samples were used for further studies. The powdered plant materials were filled separately in the thimble and extracted successively using a soxhlet extractor with distilled water, acetone, chloroform, DMSO, ethanol and aqueous. All the extracts were subjected to systematic phytochemical screening for the presence of phytochemical constituents. Carbohydrates, Amino Acids, Proteins, Chloride, Alkaloids, Tannins, Phlobatannins, Steroids, Phenolic compounds, saponins are traced. Antimicrobial activity of the plant extracts were tested by agar well diffusion method against four bacterial pathogens [two Gram positive and two Gram negative strains] such as E. coli, K. pneumoniae, B. cereus and Staphylococcus aureus. In this assay, the leaf and seed of Piper betle extracts showed inhibition activities on Gram negative bacterium such as E. coli and K. pneumoniae. The aqueous extract showed inhibitory activity only on Gram negative bacterium B. cereus and S. aureus. . This present study the qualitative phytochemical analysis and antibacterial activity were analysed.

Key words: *Soxhlet extractor, Piper betle L. and Antibacterial activity*

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INTRODUCTION:

Nowadays, the use of antibiotics to control diseases are producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs. Herbal medicines are in great demand in both developed and developing countries as a source of wide biological and medicinal activities, high safety margins and lesser costs[1]. Herbal molecules are safe and will overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs were known to prevent cancer. Some herbs have antibacterial and antifungal properties that are useful for clinical use. Some of the *in vitro* studies have been conducted, in which herbal extracts were given to clinical drug resistant strains and different serotype strains of infection [2].

Piper betle L. leaves had been used in traditional medicine as carminative, stimulant, antiseptic, antifungal and antibacterial agent. The volatile oil known as betle oil is the chief constituent of the leaves. *Piper betle* L. can be a great benefit in treating diseases caused by bacteria and fungi [3]Fresh juice of betle leaves is also used in many ayurvedic preparations. Betle leaves have long been studied for their diverse pharmalogical actions. Traditional healers from different remote communities in India claim that their medicine obtained from these betle leaves is cheaper and more effective than modern medicine[4]. Patients belonging to these communities have a reduced risk of acquiring infectious diseases from resistant pathogens than the people from urban areas who may be treated with regular antibiotics[5]. A novel approach to the prevention of antibiotic resistance of pathogenic species is the use of new compounds that are not based on existing synthetic antimicrobial agents[6].

MATERIALS AND METHODS:

Selection of Plant material

In this present study, the plant *Piper betle* L. leaves and seeds were collected in Kadyal, Kanyakumari District, Tamilnadu. An adult, fresh leaves were picked out from the plant and also the matured seed were collected from the plants and transported to the laboratory for work.

The collected leaves were subjected to surface cleaning by rinsing the samples with sterile water, in order to remove dust particles present on the plant materials. The samples such as leaf and seeds were allowed to shade dry to remove moisture content. The dried samples were used for further studies.

Preparation of plant extracts

The leaves were cut into small pieces and seeds were made powdered using electric mixer grinder. All the samples were subjected to soxhlet extraction using five solvents such as Acetone, Chloroform, Dimethyl sulfoxide, Ethanol and Distilled water. Each 5grams of plant material was filled separately in the thimble and extracted successively with 60ml of solvents using a soxhlet extractor for three hours. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

Qualitative analysis phytochemical constituents

All the plant extracts were subjected to systematic phytochemical screening for the presence of chemical constituents like Carbohydrates, Amino Acids, Proteins, Chloride, Alkaloids, Tannins, Phlobatannins, Steroids, Phenolic compounds and Saponins [7].

Anti-microbial activity assay

Antimicrobial activities of five extracts of six plant materials were determined by agar well diffusion method [8] Four bacterial pathogenic strains such as Two Gram Positive strains [*Bacillus cereus* and *Staphyloccocus aureus*] and Two Gram negative strains [*Escherichia coli* and *Klebsiella pneumonia*] were used in this investigation.

RESULTS AND DISCUSSION:

The leaf of *Piper betle* L. was showed none of positive result in acetone, chloroform and ethanol extract. Dimethyl Sulfoxide [DMSO] extract was showed positive result for tannin. Aqueous extract showed positive results for chloride, alakaloid, flavonoid and steroid.

The fruit of *Piper betle* L. was showed none of positive result in acetone and chloroform extract. Dimethyl Sulfoxide [DMSO] extract was showed positive result for alkaloids and tannin. Ethanol extract was showed positive result only for phenol. Aqueous extract showed positive for chloride, alkaloid, flavonoid, tannins, phenols and steroid.

Also, most of phytochemical were absent in chemical solvent and the aqueous extract showed positive result for most of the phytochemicals. This was reported by Sindhu *et al.* [2013], in their study also acetone, ethanol and petroleum ether extracts of the plant shows least amount of secondary metabolites. Alkaloids which are one of the largest groups of phytochemicals in the plant which helped in the development of powerful pain killer medications [9] It is well documented that flavonoids are the

polyphenolic compounds which showed potential effects on human health and possess antiviral, antiinflammatory, antitumour, antihaemolytic and antioxidative activity [10].

Table 1. Phytochemical Constituents of *Piper betle* L. Leaf

Sl. No.	Phytochemicals	Acetone	Chloroform	DMSO	Ethanol	Aqueous
1	Carbohydrate	-	-	-	-	-
2	Amino acid	-	-	-	-	-
3	Protein	-	-	-	-	-
4	Chloride	-	-	-	-	+
5	Alkaloids	-	-	-	-	+
6	Flavonoids	-	-	-	-	+
7	Tannins	-	-	+	-	-
8	Phlobatannins	-	-	-	-	-
9	Phenos	-	-	-	-	-
10	Steroids	-	-	-	-	+
11	Saponin	-	-	-	-	-

Table 2. Phytochemical Constituents of *Piper betle* L. Fruit

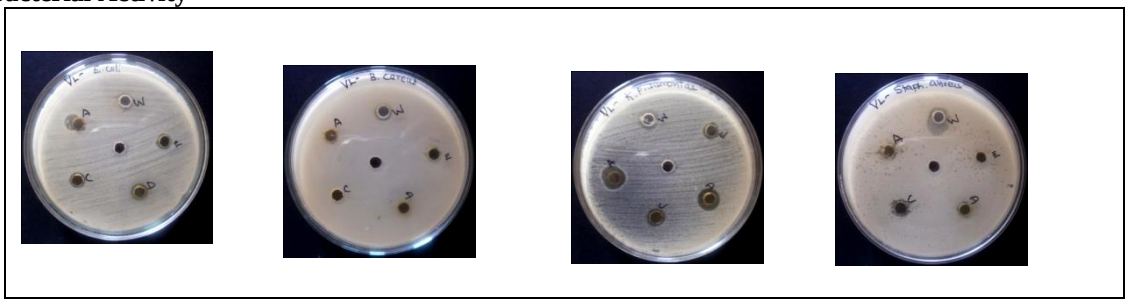
Sl. No.	Phytochemicals	Acetone	Chloroform	DMSO	Ethanol	Aqueous
1	Carbohydrate	-	-	-	-	-
2	Amino acid	-	-	-	-	-
3	Protein	-	-	-	-	-
4	Chloride	-	-	-	-	+
5	Alkaloids	-	-	+	-	+
6	Flavonoids	-	-	-	-	+
7	Tannins	-	-	+	-	+
8	Phlobatannins	-	-	-	-	-
9	Phenols	-	-	-	+	+
10	Steroids	-	-	-	-	+
11	Saponin	-	-	-	-	-

The leaf of *Piper betle* L. acetone extract was showed inhibitory activity on *Escherichia coli* [10mm], *Klebsiella pneumonia* [15mm], *Staphylococcus aureus* [14mm]. Chloroform extract was showed inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [10mm], *Staphylococcus aureus* [10mm]. DMSO extract was showed inhibitory activity on *Escherichia coli* [8mm],

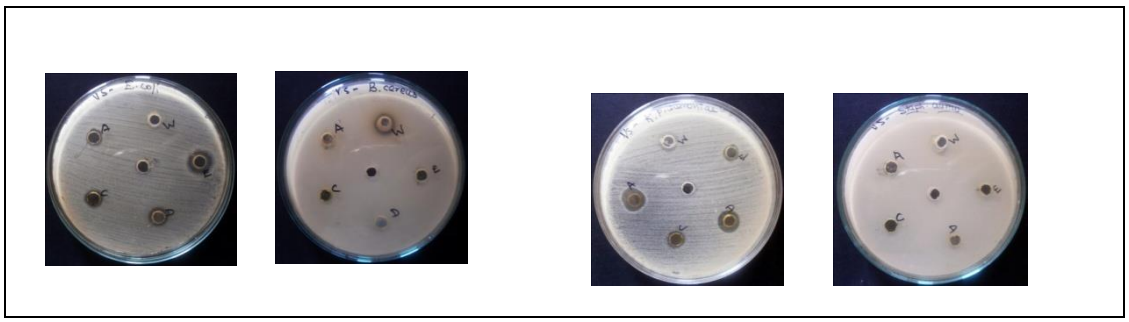
Klebsiella pneumonia [13mm]. Ethanol extract was showed inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [10mm] and aqueous extract was showed the inhibitory activity only on *Klebsiella pneumonia* [11mm] [Plate 1, Table1 & Figure 6]. The fruit of *Piper betle* L. acetone extract was showed the inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [12mm]. Chloroform extract was showed the inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [9mm]. DMSO extract was showed the inhibitory activity on *Escherichia coli* [9mm], *Klebsiella pneumonia* [12mm]; ethanol extract showed inhibitory activity on *Escherichia coli* [11mm], *Klebsiella pneumonia* [9mm] and aqueous extract showed the inhibitory activity only on *Klebsiella pneumonia* [10mm], *Bacillus cereus* [11mm].

The leaf and seed of *Piper betle* L. extracts was showed the inhibitory activity on *Escherichia coli*, *Klebsielia pneumonia* and *Staphylococcos aureus;* andonly the aqueous extract was also showed inhibitory activity against *Bacillus cereus. Piper betle* L. showed the significant inhibition against pyocyanin formation by *Pseudomonas aeruginosa* PA01 [11]. It can be found in large quantities in the sputum of cystic fibrosis patients and it disturbs ion transports, ciliary beatings and mucus secretion in the respiratory epithelial cells [12] Also, the crude *Piper betle* L. leaves extract may have influenced the adhesion between the cell surface of the bacteria and the host surface via ionic interaction which could be responsible for the adherence effect demonstrated[13] and also it has an influence on the cell surface area of the bacterial cells *Proteus vulgaris* and *Klebsiella* were inhibited to a great extent by the betle leaf extract, followed by *Pseudomonas* and *Staphylococcus aureus.*

Antibacterial Activity



Antibacterial Activity of *Piper betle* L. Leaf



Antibacterial Activity of *Piper betle* L. Fruit

CONCLUSION:

The present study concluded that, the preliminary screening of phytochemical constituents results demonstrated the presence of various bioactive metabolites. The antibacterial activity results showed the inhibitory activity of *Piper longum.* The results of the study also supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of pain relief, rheumatism, chills, flu, colds, muscular aches and fever. Further pharmacological evaluations, possible isolation of the therapeutic antibacterial from this plant are the future challenges. The future prospects of the present research work include isolation and purification of the therapeutic antimicrobials from the active extract and further pharmacological evaluation of the extracts and

clinical trials. So, further scientific assessment of these medicines for phytochemical biological and clinical studies as however greatly needed.

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