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

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Abstract:

The Piper nigrum 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 nigrum 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 nigrum L. and Antibacterial activity*

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INTRODUCTION:

The wide spread use of herbal remedies and health care preparations obtained from commonly used traditional herbs and medicinal plants have been raised due to the occurrence of natural products with medicinal properties. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by the microorganisms have also increased[1].Ethno medical practices are preferred largely because the medicinal plants are less expensive, readily available and reliable, and they are considered to have fewer side effect the modern medicines. A few reports on ethno medicinal uses of plants by the Kani tribals were available [2-5] in the adjoining areas of Kanyakumari district. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [6]. Bacterial resistance to antibiotics increases mortality likelihood of hospitalization and length of stay in the hospital. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies. Bacterial resistance to antimicrobial drugs is a worldwide problem that has emerged even among the common poultry pathogens [7].

The Piperaceae is also known as the pepper family, is a large family of flowering plants. The group contains roughly 3,610 currently accepted species in ten genera. The vast majority of peppers can be found within the two main genera: *Piper* L. [2000 species] and *Peperomia* Kuiz & Pav. [1600 species]. Members of the Piperaceae may be small trees, shrubs or herbs. The distribution of this group is the best described as pan tropical and sub-tropical of the world. There are 30 species of the genus in India and 700 species in the world. The most well-known species is *Piper nigrum* L. which yields most peppercorns that are used as species, including black pepper, although its relatives in the family include many other spices [8].

Thippali consists of dried fruits of *Piper longum* L. [Piperaceae] a slender, aromatic, creeping and perennial herb [9].It is commonly used to treat stomach ache, bronchitis, cough and tumour. It is also applied externally to sooth and relieve muscular pains, rheumatism, paralysis and inflamed skin.

Pippali contains an alkaloid piperine as chief constituent [10]. It is applied locally for muscular pain, inflammation and internally used as a carminative in conditions such as loss of appetite and sleeplessness [11]. In the Western part of India aqueous extract of the roots of *Piper longum* L. is used as food material [12]. In addition to this, there is a major role for *Piper longum* L. is preventing the cancer development in the experimental glioma model [13]. The extract of the root of *Piper longum* L. and its major compound, piperine exert anti-oxidant activity and are protective in the myocardial ischemic condition [14]. The alcoholic extract of the fruits of the plant *Piper longum* L. and its component piperine showed significant immunomodulatory and antitumor activity [15]. Piper nonaline, a piperidine alkaloid derived from long pepper, possess a mosquito larvicidal activity [16]. Piperine was the first amide isolated from *Piper* species and was reported to display central nervous system depression, antipyretic, and anti-inflammatory activity [17]. The *Piper longum* L. dried fruit's oil showed significant anti-inflammatory activity on carrageen an-induced rat paw edema [18]. Isolates from *Piper longum* L. fruit extracts showed antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria .

MATERIALS AND METHODS:

Sample Collection

In this present study, *Piper longum* L. was selected. The plant materials such as leaves and seeds was collected. 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 Elant 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.

Anti-microbial Activity Assay
Antimicrobial activities of five extracts of six plant materials were determined by agar well diffusion method. 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 longum* L. was showed none of positive result in acetone extract. Chloroform extract was showed positive for alkaloid. Dimethyl Sulfoxide [DMSO] extract was showed positive for chloride. Ethanol extract showed positive none of result. Aqueous extract was showed positive result for chloride and steroids [Table 1].

The seed of *Piper longum* L. was showed none of positive result in acetone, chloroform and ethanol extract. Dimethyl Sulfoxide [DMSO] extract was showed positive result for carbohydrate and tannin. Aqueous extract was showed positive result for carbohydrate, chloride, alkaloid, flavonoid, steroids and saponin [Table 2].

In phytochemical screening, leaf of *Piper longum* L. showed positive result for chloride, alkaloid and steroids; seed of *Piper longum* L. showed positive result for carbohydrate, chloride, alkaloid, flavonoid, tannin, steroids and saponin. The phytochemical constituents are mainly responsible for the medicinal properties of the plants. In this study, *Piper longum* L. contained carbohydrate, chloride, alkaloids, flavanoids, tannin, phlobatannin, steroids and saponin; and *Piper betle* L. contained chloride, alkaloids, flavanoids, tannin, phenols and steroids.

Phytochemical analysis of ethanol extract of *Piper nigrum* L. showed the presence of tannins and alkaloids[20]. From the study of, *Piper longum* L. contained alkaloid, tannins, terpenoids, resins, steroids, phenols, cardiac glycosides, triterpinods in leaf, stem, root and fruit of petroleum ether, acetone and ethanol extracts and from the study of *Piper longum* L. showed the presence of alkaloids, flavonoids, tannins and saponins. The metabolites such as chloride, alkaloids, flavanoids, tannin and steroids were commonly present in all the three plants and phenol was only present in *Piper betle* L[21]. They were known to show medicinal property as well as exhibiting physiological activities.

Table. 1 Phytochemical Constituents of *Piper longum* 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	Phenols	-	-	-	-	-
10	Steroids	-	-	-	-	+
11	Saponin	-	-	-	-	-

Table 2. Phytochemical Constituents of *Piper longum* 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	Phenos	-	-	-	-	-
10	Steroids	-	-	-	-	+
11	Saponin	-	-	-	-	+

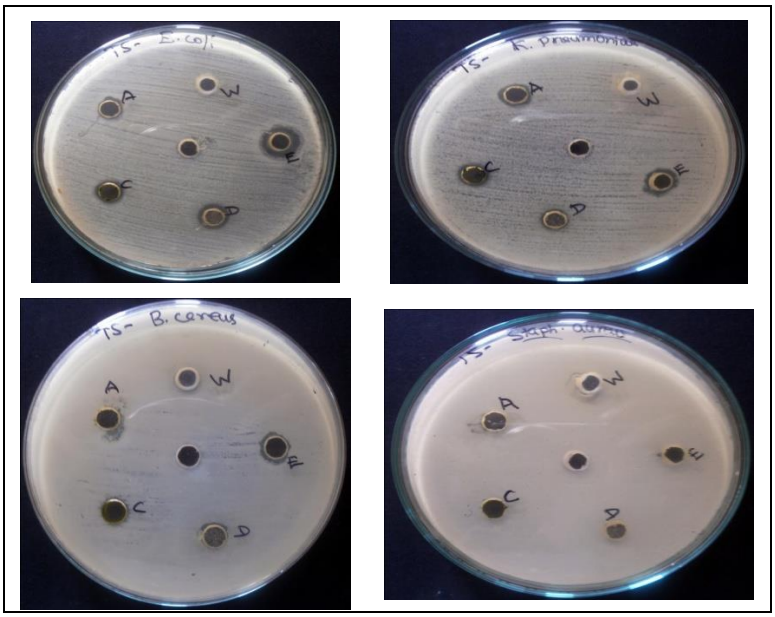

Plate : 1 Antibacterial Activity of *Piper longum* L. Fruit

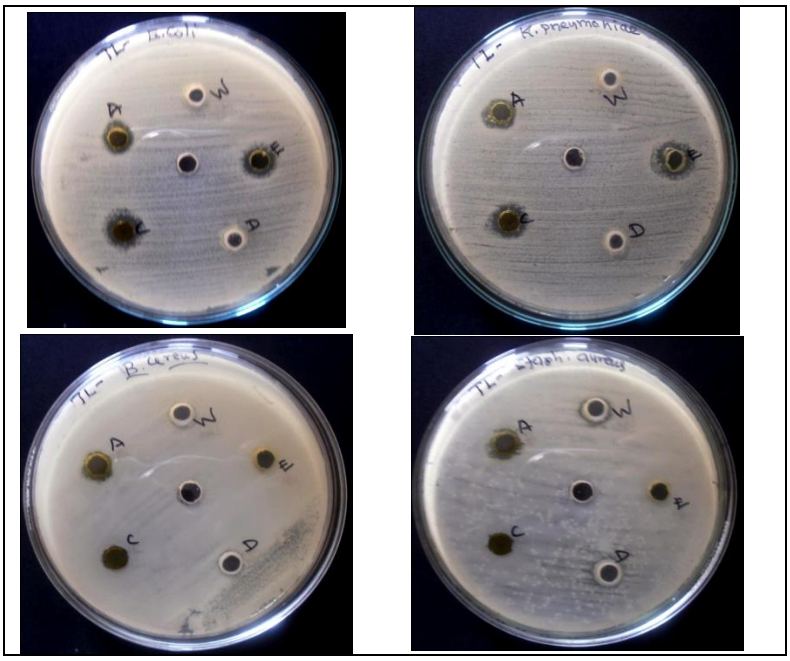

Plate : 2 Antibacterial Activity of *Piper longum* L. Leaf

The leaf of *Piper longum* L. acetone extract was showed inhibitory activity on *Escherichia coli* [10mm], *Klebsiella pneumonia* [11mm], *Bacillus cereus* [7mm], *Staphylococces aureus* [8mm]. Chloroform extract was showed the inhibitory activity on *Escherichia coli* [12mm], *Klebsiella pneumonia* [12mm] and *Bacillus cereus* [7mm]. DMSO extract was showed no inhibitory activity on all test organisms. Ethanol extract was showed the inhibitory activity on *Escherichia coli* [12mm], *Klebsiella pneumonia* [13mm] and aqueous extract showed inhibitory activity only on *Staphylococces aureus* [7mm] [Plate 1].

The seed of *Piper longum* L. acetone extract was showed inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [10mm]. Chloroform extract was showed the inhibitory activity on *Escherichia coli* [8mm], *Klebsiella pneumonia* [10mm]. DMSO extract was showed the inhibitory activity on *Escherichia coli* [10mm], *Klebsiella pneumonia* [8mm] and ethanol extract was showed inhibitory activity on *Escherichia coli* [13mm], *Klebsiella pneumonia* [10mm], *Bacillus cereus* [9mm]. Aqueous extract was showed no inhibitory activity [Plate 2].

The leaf and seed of *Piper longum* L[22]. extracts showed inhibitory activity on *Escherichia coli*, *Klebsielia pneumonia*, *Bacillus cereus*, *Staphylococcus aureus* and aqueous extract was showed inhibitory activity only on *Staphylococcus aureus. Piper longum* extracts showed a high antibacterial activity against gram-negative bacteria[23]. Similar reports have been observed by [24]. Also, the antimicrobial activity of ethyl acetate [hot extract] and hexane: water [1:1] extract of *Piper longum* L. were tested in three bacteria[25,26]. Among the three bacteria tested, hot ethyl acetate was extract showed the antibacterial activity against *Escherichia coli, Bacillus subtilis* and was found to be less active for *Staphylococcos aureus. Piper longum* L[27]. was reported as a strong antibacterial against *Bacillus cereus* and *Escherichia coli* [Williamsons, 2000].

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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