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Preliminary Phytochemical Screening and Antibacterial activity of *Piper longum* against different Pathogenic Bacterial Strains

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Abstract

The plant *Piper longum* Linn. was screened for secondary metabolites and anti microbial activity. Standardization of the solvent system and quantities required to get maximum separation of individual secondary metabolites was done. The methodology used showed that excellent separation of compounds was obtained. The development of such fingerprint for the fruits of Piper longum Linn is useful in differentiating the species from the adulterant and also acts as biomarker for this plant in the Pharmaceutical industry. The in vitro antibacterial activity was performed by Agar well diffusion method. The phytochemical extracts were examined for anti microbial activity against the pathogens like Escherichia *coli*, *Bacillus subtilis, Streptococcus pyogens and Staphylococcus aureus*

Keywords

Piper longum Linn, Antibacterial activity

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1. INTRODUCTION

Piper longum L. also known as 'long pepper' or 'pippali' belonging to the family Piperaceae and is one of the most extensively used medicinal plant in the ayurvedic system of medicine, particularly for diseases of respiratory tract. Four types of pippali namely 'pippali', 'vanapippali', 'saimhali' and 'gajapippali' are mentioned in ayurvedic literature Rajanighantu (Sivarajan and Balachandran, 1994) .

Pippali is used in over 320 classical compound medicinal formulations and in many modern herbal formulations (Singh *et al*., 2004). Several workers have investigated the species pharmacognostically, chemically and also pharmacologically in view of the

commercial, economical and medicinal importance of *Piper longum* (Neelam and Krishnaswamy, 2001). Since it is one among the 14 medicinal plants, which has high demand in indigenous drug industry, it is prioritized for cultivation and development by National Medicinal Plant Board.

Piper longum is indigenous to South Asian countries including India. It is used as a spice in ancient India.*Piper longum* is well adapted for cultivation as an intercrop in coconut, arecanut and rubber plantations of Kerala, but its cultivation is limited due to poor returns from the crop on account of high expenditure on harvesting due to staggered flowering and lack of high yielding varieties with high dry recovery. Being a semi domesticated crop, collection of diverse germplasm and selection appear to be the first and foremost method for developing high yielding varieties to suit different agro - climatic conditions and cropping systems.

Piper longum Linn. popularly known as pippali, belonging to the family Piperaceae, is an important medicinal plant, used in traditional medicine in Asia and Pacific Islands. The family Piperaceae comprises 12 genera and about 1400 species, mainly found in tropical region (Barroso, 1978). P. longum has immense potential in medicines, as it is reported as a good remedy for treating gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut-related pain and arthritic conditions (Singh, 1992). Almost all parts of it, namely roots, stems and fruits are medicinally important and used especially in the treatment of respiratory tract disorders like bronchitis, asthma, cough etc (Sivarajan and Balachandran, 1994). The principal pharmacological constituents are piperine and piplartine. The crude extract of P. longum contains 3-8 per cent of piperine (James, 1999). The plant is reported as endangered for Tamil Nadu and at lower risk for Kerala (Nair, 2000).

Piper longum is native of Indo-Malayan region. It was very early introduced to Europe and was highly regarded as a flavour ingredient by the Romans. P. longum, considered indigenous to the hotter parts of India was described by Krishnamurthy (1969). The distribution of long pepper in Calicut was recorded by Manilal and Sivarajan (1982). In India, it is widely distributed in low altitude evergreen forests, occurring in sub Himalayan hills, Assam, Khasi regions, Eastern Ghats and Western Ghats, and in low elevation of forest lands and lower hills of West Bengal. It was reported that from India, it has reached the rest of Asia and Mediterranean through the “spice route” and was used as a spice in all these regions (Ravindran and Balachandran, 2005).

Piper longum appears to be derived from two or three species that may include species from Malaysia and Indonesia. It was a product of either P. longum or P.peepuloides, while the java long pepper is from P. officinarum (Khushbu et al., 2011).The aim of the present study is to evaluate the preliminary phytochemical activity of medicinal plants namely *Piper longum* L against the Gram Positive Bacteria.

2. MATERIALS AND METHOD

Collection of Plant material

The seeds of *Piper longum* L. were collected in coorg District, Karnataka and authenticated by Dr.C.Murugan, Botanical Survey of India, TNAU Campus, Coimbatore with herbarium code number No.BSI/SRC/5/23/2019/3456. Dated 07-03-2019.

Preparation of extracts

The dried plant seed materials were manually ground to a fine powder. About 25 g of the each dried plant powder was soaked in 100 ml of Acetone and ethyl acetate (1:4) for 7 days with periodic soaking and then filtered using Whatman filter paper No. 1. The filtrate was then dried at 55 °C for 1 h using rotary vacuum evaporator (Buchi Type, India) and the yield percentage yield was calculated. The dried *P.Longum* Acetate (PLA) and ethyl acetate (PLEA) extracts were then aliquoted using 80 % di-methyl sulfoxide (DMSO) (Himedia, India) to prepare stock and working solution.

Preliminary Phytochemical analysis of *Piper longum* seed

Phytochemical screening was carried out for the analysis of secondary metabolite present in the plant extract. The phytochemical screening was carried out by the method of Paech and Tracey, (1955).

Detection of Phenols

Ferric chloride Test

1ml of each extract was diluted with water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green color indicated the presence of phenols.

Lead acetate Test

1 ml of each extract was diluted to 5ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. Formation of yellow precipitate indicated the presence of phenols.

Detection of Flavonoids

Alkaline reagent Test

To 1ml of each extract, few ml of 10% ammonium hydroxide was added. Yellow fluorescence showed the presence of flavonoids.

. Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

Detection of alkaloids

Wagner's Test (Iodine-Potassium-Iodide Solution)

1.0g of iodine and 2.0g of potassium iodide solution was diluted to 100ml. 1.0ml of each extract were acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

Meyer's Test (Potassium Mercuric Iodide)

1.36g of Mercuric chloride was dissolved in 60ml of distilled water and 5g of potassium iodide in 10ml of water. The two solutions were mixed and diluted to 100ml with distilled water. To 1ml of each extract few drops of reagent was added. Formation of white or pale precipitate indicated the presence of alkaloids.

Hager's Test

To 1ml of each extract, 1 or 2ml of Hager's reagent (saturated aqueous solution of picric acid) was added. Formation of prominent yellow precipitate indicated the presence of alkaloids.

Detection of tannins

Ferric chloride test

To 1.2ml of each extract few drops of alcoholic ferric chloride was added. Blue colour was formed indicating the presence of tannins.

Detection of glycosides

A small amount of each extract was dissolved in 1.0ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow color showed the presence of glycosides.

Detection of steroids

Libermann-Burchard's Test

To 1ml of each extract, 1ml of conc.sulphuric acid was added followed by the addition of 2.0ml of acetic anhydride solution. Formation of greenish color showed the presence of steroids.

Salkowski Test

To 1ml of each extract, 1.0ml of conc.sulphuric acid was added carefully along the sides of the test tube. Formation of red color showed the presence of steroids.

Detection of saponins

Froth test

In a tube containing about 5.0ml each extract, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3min. A honeycomb like froth was formed indicated the presence of saponins.

Detection of carbohydrates

Molisch's Test

To 2ml of each extract, two drops of alcoholic solution of α-naphthol was added, the mixture was shaken well and 1ml of conc.sulphuric acid was added slowly along sides of the test tube and allowed to stand. Formation of violet ring confirms the presence of carbohydrates.

Fehling's Test

34.66g of copper sulphate was dissolved in 500ml of water and 173g of potassium sodium tartarate and sodium hydroxide was dissolved in 500ml of water. The two solutions were mixed and made up to 1 litre.

To 1ml of each extract, 1ml of Fehling solution was added and heated on a boiling water bath for 2 minutes. Formation of red precipitate showed the presence of sugar.

Benedict's test

173g of sodium citrate and 100g of sodium carbonate were dissolved in 800ml of distilled water and boiled to make it clear. To this added 17.3g of copper sulphate and dissolved in 100ml water.

To 0.5ml of each extract, 0.5ml of reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Formation of characteristic colored precipitate showed the presence of sugar.

Detection of proteins

Million's Test

1g of mercury was dissolved in 9ml of fuming nitric acid. When the reaction was completed, equal volume of water was added. To 0.5ml of each extract, few drops of million's reagent were added. Formation of white precipitate showed the presence of proteins.

Ninhydrin Test

10mg of ninhydrin was dissolved in 200ml of acetone. To 2ml of each extract, few drops of ninhydrin solution were added. Formation of characteristic purple color showed the presence of amino acids.

Detection of Terpenoids

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentratedH_2SO_4 was added and heated for about 2 min. A greyish colour indicated the presence of terpenoids.

Test organisms

Test organisms Clinical isolates of bacteria were used for the bioassay studies. The isolates included Escherichia *coli*, *Bacillus subtilis, Streptococcus pyogens and Staphylococcus aureus*. The isolates were maintained on freshly prepared nutrient agar slants and kept in a refrigerator at 4ºC until required for use.

Agar well diffusion assay

The anti-bacterial activity of PAM and PAEA extracts performed by agar well diffusion method using Nutrient agar (NA) (Himedia, India) by following the methods specified in Clinical and Laboratory Standards Institute [19]. Briefly, 1 % overnight culture of test pathogen was swabbed uniformly over freshly prepared Nutrient Agar plates and allowed to set. Then, agar plugs was incorporated onto the wells and wells were cut onto the medium by using gel puncture. Penicillin -G (0.03 mg/ml) was used as positive control. 30 µl of plant extract (APL and EAPL), and DMSO was used as negative control.

Preliminary phytochemical screening of solvent extracts

The phytochemical constituents are mainly responsible for the medicinal properties of the plant. The data of phytochemical analysis of *P.longum* is presented in the table 1. It showed the presence of alkaloid, tannins, terpenoids, steroids, phenols, glycosides, triterpinods in acetone and ethyl acetate extracts of fruit. By comparing with the Acetone extracts and Ethyl acetate 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 (Kam and Liew,2002). Saponin found to be present in *P.longum* extracts and have supported the usefulness of this plant in managing inflammation.In thee present analysis acetone extract reveals the presence of all secondary metabolites other than carbohydrate in Ethyl Acetate extract Saponin,glycosides and Tannins were absent . In both extract carbohydrates were absent.

3.RESULTS

Table 1: Qualitative phytochemical analysis of *Piper Logum*.

Secondary Metabolites	Acetone	Ethyl Acetate
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	-
Glycoside	+	-
Tanins	+	-
Terpinoids	+	+
Steroids	+	+
Phenols	+	+
Proteins	+	+
Carbohydrates	-	-

Antibacterial Activity

Among the four different types of pathogens used in the study. The maximum zone of inhibition was observed against *Bacillus subtilis streptococcus pyogenes* (19 and 18 mm) in acetone extract (Table 2). The maximum zone of inhibition was obtained in acetone extract when compared to ethyl acetate extract. The antimicrobial activity of the tested extracts and fractions are comparable with the standard drugs. These activities may be due to strong occurrence of different chemical compounds such as flavonoids, tannins, alkaloids, steroids, phenols and saponins.

S.NO	Organisms	Acetone Extract	EthylAcetate Extract	Penicillin-G	DMSO
1	*Bacillus subtilis*	19	14	20	Nil
2	*Escherichia coli*	17	19	24	Nil
3	*Staphylococcus aureus*	16	13	23	Nil
4	*Streptococcus pyogenes*	18	15	20	Nil

Table 2: Antibacterial activity of Acetone and Ethyl Acetate *P.Longum* extracts against pathogenic bacteria

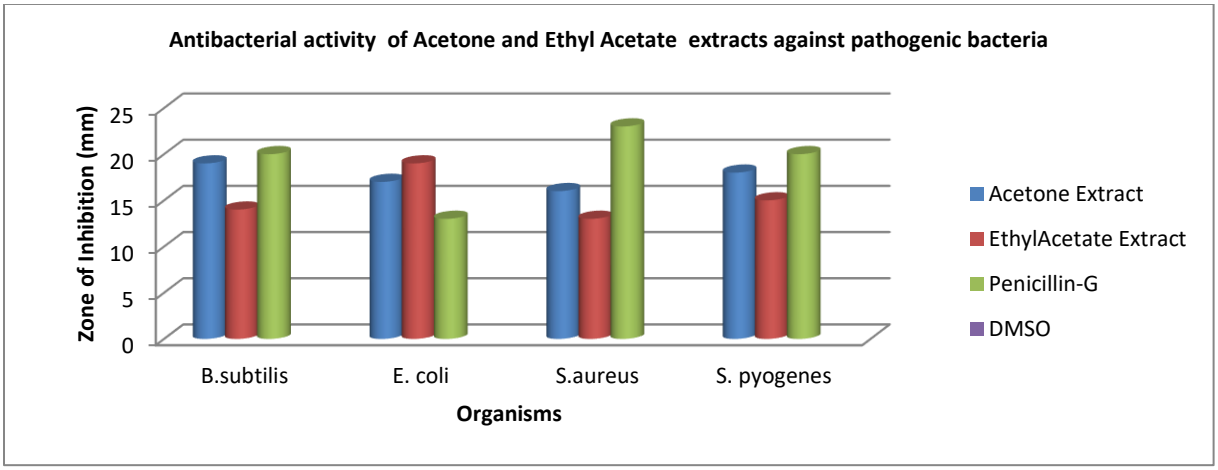

Fig 2 Antibacterial activity of Acetone and Ethyl Acetate extracts against pathogenic bacteria

4.DISCUSSION

Infectious diseases are the major cause of morbidity and mortality worldwide. The number of multidrug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters organ transplantation and ongoing epidermis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment. [2] In this present study, preliminary screening for antimicrobial activity showed, that the Acetone extract exhibited maximum inhibitory zone (19mm) against *Bacilius*. While the ethyl acetate showed least inhibitory activity. The antimicrobial assay by agar-well diffusion method revealed that acetone extract of medicinal plants exhibited broad spectrum activity against tested isolates as compared to eehyl acetate. Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. The present investigbation corroborates with studies of many authors.

5.CONCLUSION

It can be concluded that most fraction of medicinal plant *P.longum* showed potential antimicrobial activities against the tested bacterial strains. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. Results of our findings confirmed the use of P.longum as traditional medicine. However, these medicinal plant species may be subjected to detailed phytochemical and pharmacological studies in order to find out new drugs against pathogenic bacterial and fungal strains.

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