Mintage Journal of Pharmaceutical & Medical Sciences
RESEARCH ARTICLE

PRILIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *PIPER LONGUM* L. (PIPERACEAE)

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Received -01-02-13; Reviewed and accepted -16-02-13

ABSTRACT

Piper longum L. (Thippali) is a native of the Indo-Malaya region, belongs to family Piperaceae. It has been used as a therapeutic agent in the treatment of various pathological conditions. *P.longum* was reported to contain alkaloids, phenols, tannins, flavanoids, steroids, lignin & saponins in it. The objective of study is to evaluate the antimicrobial activity with their preliminary phytochemical study. The petroleum ether, acetone and ethanol extracts of all the parts (Leaf, fruit, stem and root) were tested for antimicrobial activity against certain bacterial strains of *E.coli, Steptococcus faecalis, Steptococcus pyogens* and *Salmonella paratyphi* A. by in vitro disc diffusion method and the results are recorded as the zone of inhibition.

Keywords: *Piper longum*, Piperaceae, alkaloids, saponins, antimicrobial activity.

INTRODUCTION

Plants, as the source of medicine, have been playing an important role in the health services around the globe (Thomson, 2010). About three quarters of the world's population relies on plant and their extracts for health care (Kunwar, 2008). A good number of our population particularly those living in rural areas depend largely on herbal remedies for the treatment of different types of diseases. It indicates the importance of the individual plants in the health care system. Use of herbal medicine in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importance (Diallo *et al*., 1999). The medicinal value of plants lies in some chemical substances that they contain. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds (Edeoga *et al*., 2005).

The genus *Piper* (L.) contains more than 700 species they grow in tropical and subtropical rain forest. Pipali (Thippali) consists of dried fruits of *Piper longum L. (Piperaceae)* a slender, aromatic, creeping and perennial herb, native of the hotter parts of the country and found wild as well as cultivated extensively in Assam, lower hills of Bengal, ever green forest of Western Ghats, along west coast of Southern States and also recorded from Car Nicobar Islands (Satyavathi, 1987). It is most commonly used to treat stomach ache, bronchitis, cough, tumours. It is also applied externally to sooth and relieves muscular pains, rheumatism, paralysis and inflamed skin. Pippali contain an alkaloid piperine as chief constituent (Kokate, 1980). *Piper longum* contain piperine, piper longamine, volatile oil, resin, gums and fatty oil. The fruits of *Piper longum* are useful in spleen disorders, bronchitis tuberculosis and jaundices (Kiritikar, 1987). The present study reveals relevant phytochemical and antimicrobial data of *Piper longum.*

MATERIALS AND METHODS

Collection of plant materials

Fresh plant parts (Leaf, stem and rhizome) were collected randomly from the foot hills of Attapady, Palakkad district, Kerala, India. Fresh plant material were washed with tap water, air dried and then homogenized to a fine powder and stored in air-tight bottles.

Preparation of plant extract

Sample (50 gm) of the shade-dried powder of *Piper longum* was extracted in a Soxlet extractor successively with 250 ml Petroleum ether, Acetone and Ethanol until colourless extract was obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure (Harborne, 1998). After complete solvent evaporation, each of these solvent extracts was weighed and subjected to phytochemical analysis and antibacterial activity assay.

Preliminary phytochemical screening

The freshly prepared crude Petroleum ether, Acetone and Ethanol extract of *Piper longum* was qualitatively tested for the presence of phytochemical constituents by standard methods (Trease, 1989). A known quantity of the extract was also subjected to the quantitative determination of phytochemicals such as the total proteins (Lowry *et al,*. 1951), total free amino acids (Moore and Stein 1948) and total carbohydrates (Hedge and Mofreiter 1962).

Test organisms

Clinical isolates of bacteria were used for the bioassay studies. The isolates included *Escherichia coli*, *Steptococcus faecalis*, *Steptococcus pyogens* and *Salmonella paratyphi A*. The isolates were obtained from PSG medical hospital, Coimbatore. The isolates were maintained on freshly-prepared nutrient agar (oxoid) slants and kept in a refrigerator at 4ºC until required for use.

Anti-bacterial Activity:

The antibacterial assay of aqueous and methanolic extracts was performed by Bauer *et al.* (1996). The Nutrient Agar media was poured into the petridishes and were streaked with the test organism. For the agar disc diffusion method, the disc (0.5 cm) was saturated with 100 mg/ml of the extract was then placed on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth. A reading of more than 6mm indicated growth inhibition. All the assays were done in triplicate and the results were given in mean ± SD. For each bacterial strain, Amphicillin positive controls were used.

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

Secondary Metabolites	Petroleum ether				Acetone				Ethanol			
	L	F	S	R	L	F	S	R	L	F	S	R
Alkaloid	-	+	+	-	+	+	+	+	+	+	+	+
Flavonoid	-	-	-	-	-	-	-	-	-	-	-	-
Saponin	+	-	+	-	+	-	+	-	+	-	+	+
Glycoside	-	-	-	-	+	-	-	-	+	-	-	-
Tanins	+	+	+	-	+	+	+	-	+	+	+	+
Terpinoids	-	+	-	-	+	+	-	+	+	+	-	+
Resins	-	-	-	-	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	+	-	-	-	+	+
Phenols	+	+	-	-	+	+	+	+	+	+	+	+

Cardiac Glycosides	-	+	+	-	-	+	+	+	+	+	+	+
Triterpinoids	-	-	-	-	+	-	+	+	+	+	+	+

L : Leaf, F : Fruit, S : Stem, R : Root

Table 2: Composition of primary metabolites in *Piper longum* L.

Sl.no	Plant Parts	Protein (mg/g fresh weight)	Amino acid (mg/g fresh weight)	Carbohydrate (mg/g fresh weight)
1	Leaf	11.47±0.87	8.7±0.81	2.5±0.21
2	Fruit	13.47±0.77	8.2±0.55	2.75±0.72
3	Stem	11.56±0.56	7.01±0.27	2.79±0.11
4	Root	14.72±0.22	7.3±0.21	2.6±0.27

RESULT AND DISCUSSION

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, resins, steroids, phenols, cardiac glycosides, triterpinods in petroleum ether, acetone and ethanol extracts of leaf, stem, root and fruit. By comparing with the ethanol extracts acetone 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 (Kam and Liew,2002). Saponin found to be present in *P.longum* extracts and have supported the usefulness of this plant in managing inflammation.

The biochemical analysis of primary metabolites such as carbohydrate, proteins and amino acid in the plant parts (leaf, stem, root and fruit) are illustrated in table 2. The amount of carbohydrate in fruit and stem is higher when compared to root and leaf. The stem and root having minimum amount of amino acid than leaf and fruit.

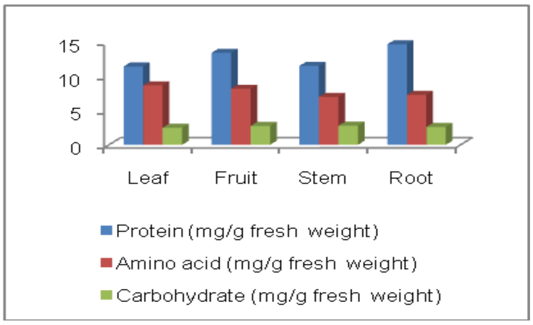

Figure 1: Composition of primary metabolites in *Piper longum* L.

Table 3: Antibacterial efficiency of *Piper longum.*

Solvent	*Steptococcus pyogens*				*Escherichia coli*				*Salmonella paratyphi A*				*Steptococcus faecalis*			
	L	F	R	S	L	F	R	S	L	F	R	S	L	F	R	S
Control	25	27	26	28	25	24	30	28	26	25	24	28	25	26	27	22
Petroleum ether	12	16	14	16	12	13	18	13	13	13	14	16	13	15	13	13
Acetone	13	18	16	18	14	14	20	17	15	15	15	17	14	17	14	12
Ethanol	14	20	18	20	15	16	22	20	17	16	16	19	17	18	15	14

L : Leaf, F : Fruit, S : Stem, R : Root

The antimicrobial activity of *P.longum* extracts has been evaluated *in vitro* against two gram positive bacterial stains such as *Steptococcus faecalis, Steptococcus pyogens* and two gram negative bacteria such as *E.coli* and *Salmonella paratyphi A* (Table 3). The gram negative bacteria *E.coli* showed maximum inhibition zone in ethanol extract of root (22mm) where *Salmonella paratyphi A* showed maximum inhibition zone in ethanol extract of stem (19mm). The gram positive bacteria *Steptococcus faecalis* and *Steptococcus pyogens* showed maximum inhibition zone in both the ethanol extracts of stem and fruit (18mm, 20mm resp.). *Piper longum* was reported as a strong antibacterial against *B. cereus* and *E. coli* (Elizabeth, 2000), which is traditionally used for chronic bronchitis, asthma (Kapoor, 1990).

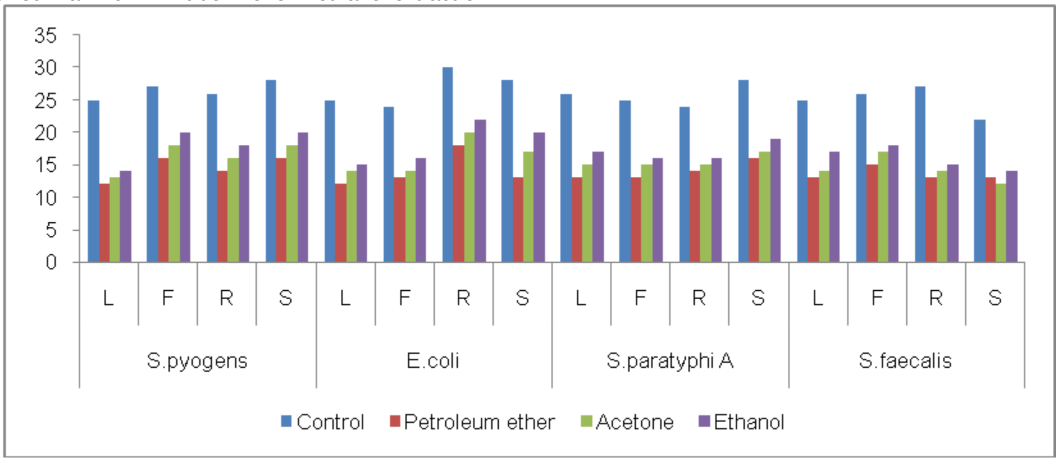

Figure 2: Antibacterial efficiency of *Piper longum.*

CONCLUSION

Since the plant, *Piper longum* which is also used for the treatment of various diseases and disorders, it is important to standardize it for use as a drug. Thippali was found to possess higher the rate of phytoconstituents and promising antibacterial activity. It is also confirmed that, this spicy product triggers natural immune system to fight against enteric bacterial infection. This study would provide the preliminary scientific evidence for ethno-botanical and traditional use of Thippali for prevention of enteric bacterial infections.

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