International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 3 | JUL-SEPT| 2017 | 123-128
Original Research Article – Biological Sciences| Open Access| UGC Approved | MCI Approved Journal

THERAPEUTIC POTENTIAL OF *PIPER LONGUM*

Sheeja T Tharakan[1]* and Manju Madhavan[2]
[1, 2] Vimala College (Autonomous), Thrissur, Kerala, India- 680 009

***Corresponding Author Email:** sheejatharakan@gmail.com

ABSTRACT

Aims: *Phytochemical screening, total phenolic quantification, antioxidant and anthelmintic activity of hot water extract extract of Piper longum L.* ***Methods:*** *Preliminary phytochemical analysis and total phenol quantification of hot water extract of Piper longum was done by standard procedures. The antioxidant activity was evaluated by DPPH radical scavenging activity. Anthelmintic activity was checked against Indian earthworm Pheretima posthuma.* ***Results and Discussion:*** *In the present study, phytochemistry of hot water extract of Piper longum, revealed the presence of phytochemicals like carbohydrate, amino acid, protein, alkaloid, steroids, phenols and tannins. The extract also showed DPPH radical scavenging activity. In anthelmintic studies, in 100 mg/ml of Piper longum, earthworms get paralyzed after 2 min and died after 14 min. The standard drug used was Albendazole. The antioxidant and anthelmintic effect of Piper longum may be due to presence of secondary metabolite like alkaloids or tannins.* ***Conclusion:*** *The result of the present study clearly indicates that the hot water extract of Piper longum can produce antioxidant and anthelmintic activity. However, much scope for additional exploration of Piper longum is necessary for assessing its potential in the treatment of various diseases.*

KEY WORDS

Piper longum, Antoxidant activity, DPPH, Anthelmintic activity, Pheretima posthuma

INTRODUCTION

In this world, there are no plants without any medicinal value, so they have been used for therapeutic purpose long before pre-historic period. The traditional medicinal practices like Unani, Ayurveda, and Siddha have been established their deep roots in herbal therapies from ancient days itself [1]. Herbal medicines prepared from various plant parts like leaves, stem, roots, etc. contain secondary metabolites and are used for treating a number of diseases. Spices and herbs are distinguished as the resource of natural antioxidants and thus play a significant function in the chemoprevention of diseases and aging [2]. The benefits resulting from the use of natural products rich in bioactive substances has promoted the rising interest of pharmaceuticals, food and cosmetic industries. Hence there is a need for systematic analysis of herbal drugs in respect to the present scenario.

Constant exposure to chemicals and pollutants may show the way to augment the number of free radicals in body to cause irreversible oxidative damages. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease [3]. Helminths are group of parasitic worms present inside the body whose infection may cause chronic illness and cause mortality to human being as well as live stocks [4]. Although synthetic drugs are used for treatment, they suffer from limitations such as side effects and toxicity. These drugs are also unaffordable and inaccessible to the people of the developing countries. The factors paved the way for herbal formulation as an alternative to anthelmintic drugs.

Piper longum L. (Family: Piperaceae) commonly known as long pepper (catkin) is widely used as a household remedy in treating respiratory disorders. Mainly its useful part is seeds. Ayurvedic system of medicine recommends *Piper longum* for treating cardiac disorders [5]. It is an ingredient in 'Trikatu churna' an ayurvedic medicine used to treat cough, fever, sneezing etc [6]. The main objective of the present study is to analyze phytochemistry, total phenol quantification, antioxidant and anthelmintic potential of hot water extract of *Piper longum* L. seeds.

MATERIALS AND METHODS

Preparation of crude plant extract

Piper longum L.(catkin) was purchased from the local market. The dried seeds were powdered using a mixer grinder. About 10 g of dried, ground plant materials were soaked separately in water (100 ml) for one week. The soaked material was stirred and heated to boiling point and stirred using sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The extracts were dissolved in distilled water to make a concentration of 1 mg/ml.

Phytochemical Analysis

The plant extract was diluted with distilled water for phytochemical analysis of primary and secondary metabolites using standard procedures [7, 8].

Total phenolic content

The concentration of phenolics in seed extract was determined using spectrophotometric method [9]. The diluted working solutions of the test extracts were prepared in water. The reaction mixture was prepared by taking 5 µl, 2.5 µl, 1µl of hot water extract and made up the volume to three ml of distilled water, added 0.5 ml of Folin-Ciocalteu's reagent and 2 ml 20% Na_2CO_3. Blank was concomitantly prepared**,** containing 3 ml water, 0.5ml Folin-Ciocalteu's reagent and 2ml of 20% of Na_2CO_3. The samples were thereafter incubated in a dark for 30min. The absorbance was determined using spectrophotometer at 650 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of catechol and the calibration line was constructed (Figure 1). Based on the measured absorbance, the concentration of phenolics was read (µg/µl) from the calibration line; then the content of phenolics in extracts was expressed in terms of catechol equivalent (µg of CE/µg of extract).

Antioxidant activity

The ability of the seed extracts to scavenge DPPH free radicals was assessed by the standard method [10]. The stock solution of extracts were prepared in water to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 5, 2.5 and 1µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of .002% DPPH. After 30 min incubation in darkness at room temperature (23°C), the absorbance was recorded at 517 nm Labtronics NT 290 Spectrophotometer. Control sample contained all the reagents except the extract. Percentage inhibition was calculated, whilst IC_{50} values were estimated from % inhibition versus concentration plot. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations [11].

The optical density was recorded and % inhibition was calculated using the formula given below Percent (%) inhibition of DPPH activity = {(A-B)/A} x100, where, A = optical density of the blank and B = optical density of the sample.

Anthelmintic activity

The anthelmintic assay was carried out as per the Ayaiyeoba et al method [12]. Adult earthworms (*Pheretima posthuma*), were used to evaluate anthelmintic activity *in vitro*. Earthworms of 3-5 cm in length and 0.1-0.2 cm in width (same type) were collected from Kerala Agricultural University, Mannuthy. Test samples of hot water extract was prepared at the concentrations, 25,50,100 mg/ml in distilled water and three worms i.e. *Pheretima posthuma*, of approximately equal size were used for all the experimental protocol were placed in each nine-cm petri dish containing 25 ml of above test solution of extracts. Albendazole (25 mg/ml and 50mg/ml) was used as reference standard as advocated earlier. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously.

RESULT AND DISCUSSION

Preliminary phytochemical analysis of hot water extract of *Piper longum*

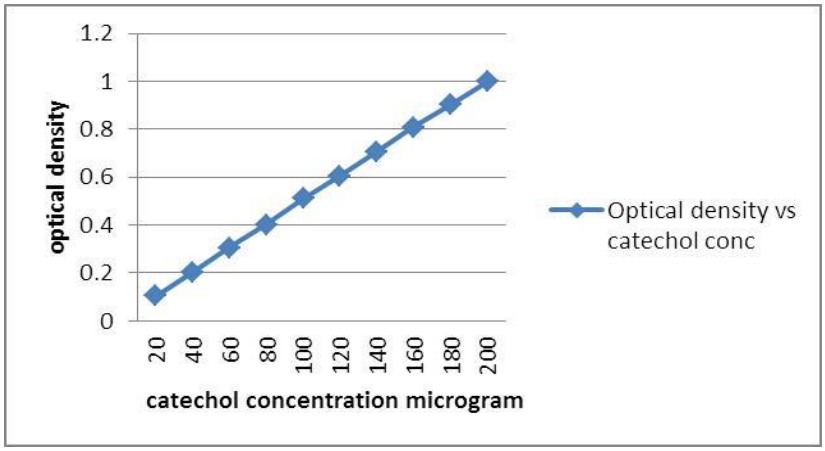
Plants have always been a loaded with a variety of phytochemical. They have also well-known for their therapeutic potential activities in the field of pharmacognosy. The medicinal property of a plant may be attributed to the presence of various secondary metabolites such as phenolics, terpenoids, or alkaloids. The phytochemical analysis of *Piper longum* showed the presence of carbohydrate, amino acid, protein, alkaloid, steroid, phenol and tannins (Table 1). According to Dhanalakshmi *et al.,* [13] aqueous extract of *Piper longum* contain phytochemicals like protein, alkaloids such as piperlongumine and piperine, volatile oils, saponins, carbohydrates and amygdalin.

Table 1. Preliminary phytochemical screening of hot water extract of *Piper longum*

Sl No	Plant Constituents	Test / Reagent	Observations	Presence / Absence
1	Carbohydrate	Molisch Test	Violet Ring	+++
		Iodine Test	Blue Colouration	_
		Benedict's Test	Appearance of green, yellow or red colouration	+++
2	Proteins	Biuret Test	Violet to pink colouration	++
3	Amino Acids	Ninhydrin Test	Blue to violet colouration	++
4	Alkaloids	Mayer's Test	White precipitate	++
5	Steroid	Salkowski reaction	Chloroform layer appears red and acid layer shows greenish yellow fluorescence	++
6	Saponins	Foam test	Persistent Foam	_
7	Phenols and Tannins	Folin Test	Blue Colouration	++
		Bromine water	Decolouration of Bromine water	++
		Acetic acid	Red Colouration	++

+ low, ++ Average, +++ High, '–' Nil

Figure 1: Catechol calibration curve



Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method. Catechin is one of the polyphenol compounds, so total phenolic content of hot water extract of spices were expressed as microgram (µg) catechin equivalents (CE) / µg of extract (Figure 1). *Piper longum* had the highest phenolic content 75µg CE/ 5µg of extract, 57µg CE/ 2.5µg of extract and 39µg CE/ 1µg of extract, using the standard curve of catechin (Table 2). Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, anthelmintic and anticancer [14]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers [15]. Alkaloids inhibit the growth of micro-organisms and can reduce the risk of fungal infection. Phenols are

detoxifying agents, antibacterial and antifungal agents, inhibitors of procarcinogen activation, inducers of drug binding of carcinogens and inhibitors of tumourogenesis [16].

Table 2: Total phenol quantification of hot water extract of *Piper longum*

Sl. No.	Plant Extract	Concentration (µg/ µl)	Phenol concentration (µg/µl CE)
1	*Piper longum*	5	75
		2.5	57
		1	39

DPPH free radical scavenging activity of hot water extract of *Piper longum*

DPPH assay is characteristically used for measurement of free radical scavenging potential of an antioxidant molecule. It is considered as one of the standard and easy analytical technique for the assessment of antioxidant properties of compounds. When compared to the control, Table 3 showed the decrease in absorbance of DPPH free radical due to the scavenging ability of the different concentrations of plant extract. It was observed that the remarkable scavenging activity of *Piper longum* for 1 µg, 2.5 µg and 5 µg was 10%, 47.6% and 63.8% respectively. IC50 value (µg/ml) of *Piper longum* was found to be 3.59µg/ml. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components. The bioactive compounds present in the plant extracts were able to discolour DPPH solution by their hydrogen donating ability [17]. From the results it appears that the extracts of Piper longum possess hydrogen donating capabilities and it will act as an antioxidant.

Table 3. DPPH free radical scavenging activity of hot water extract of *Piper longum*

Sl No	Plant Extract	Concentration (µg)	Optical density (OD)		Percentage of inhibition	IC50 (µg)
			Initial	Final		
1	Control		0.340			
		1	0.169	0.306	10	
2	*Piper longum*	2.5	0.207	0.178	47.6	3.59
		5	0.309	0.123	63.8	

Anthelmintic activity of hot water extract of *Piper longum*

The anthelmintic activity of different concentrations of hot water extract of *Piper longum* and standard drug Albendazole were studied by observing the time taken for paralysis and death of earthworm in different concentrations of aqueous extract of these spices. In 50 mg/ml of standard drug Albendazole, the earthworm gets paralysed and died after 2 min and 6 min respectively. In 25 mg/ml of Albendazole the time for paralysis and death of *Pheretima posthuma* was found to be 4 min and 18 min respectively. In 100 mg/ml of *Piper longum*, earthworms get paralysed after 2 min and died after 14 min, while in 50 mg/ml of *Piper longum*, the earthworms took about 39 min and 120 min for paralysis and death. In 25 mg/ml extract the earthworms get paralysed after 67 min and died after 168 min (Figure 3). When compared to standard drug Albendazole, it was found that the anthelmintic effect of hot water extract of *Piper longum* was found to be dose-dependent manner.

Albendazole binds to free ß-tubulin, inhibiting polymerisation and thus interfering with microtubule dependent glucose uptake by the worms [18]. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer [19]. The effect would be due to presence of alkaloids which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in local homeostasis which is essential for the

development of helminths. The possible mechanism of action of tannins may be interfere with energy generation by uncoupling oxidative phosphorylation, or may interfere with glycoprotein of cell surface, or can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [20].

Figure 2: DPPH free radical scavenging activity of hot water extract of *Piper longum*

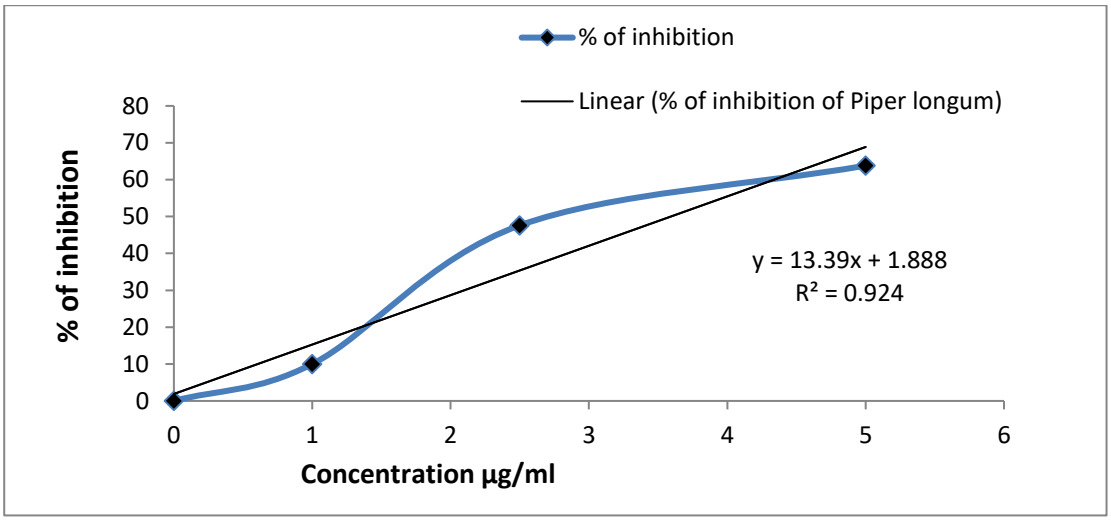
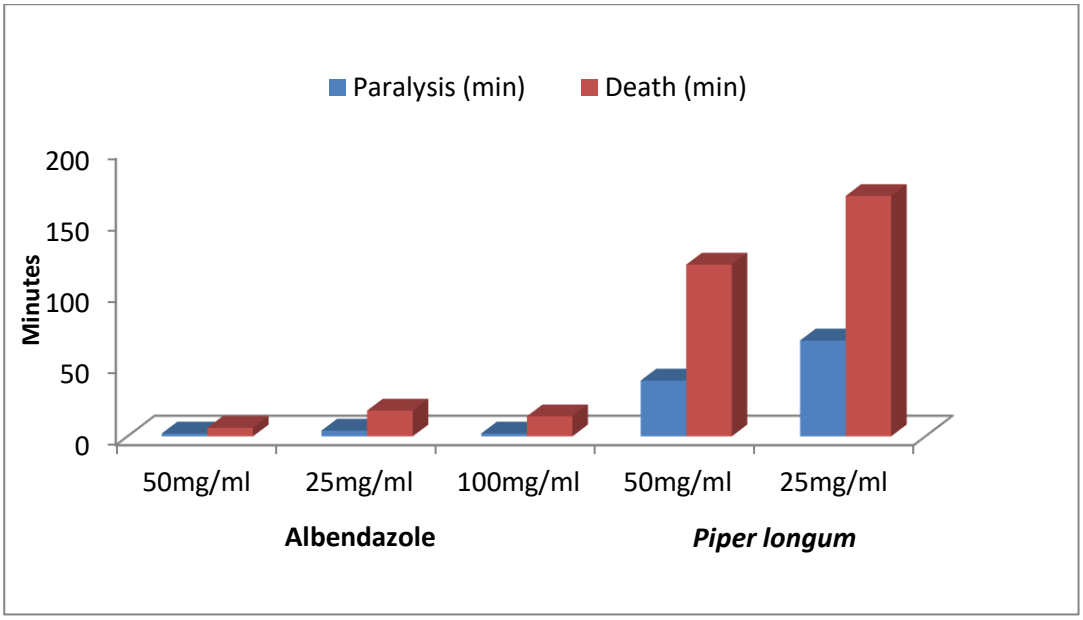


Figure 3: Anthelmintic activity of hot water extract of *Piper longum*



CONCLUSION

Phytochemical analysis showed the presence of primary and secondary metabolites. Hot water extract of *Piper longum* contain alkaloids, tannins and phenols. The result of the present study clearly indicates that the hot water extract of *Piper longum* can produce antioxidant and anthelmintic activity. Further investigations are needed for the isolation and identification of the active components and to elucidate its mechanisms of action, as well as their potential role in the biological activity, but they can be used as a flavouring agent in the food industry.

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***Corresponding Author:**
Sheeja T Tharakan*
Email: sheejatharakan@gmail.com