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RESEARCH ARTICLE

Phytochemical screening Antioxidant and Anti–Bacterial activity of *Piper longum*

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ABSTRACT

Fruit extract of *Piper longum* (Pipericeae) are used in India for reducing oxidative stress and also for its anti-bacterial activity. The objective of this study is to investigate the phytochemical constituents. Phytochemical screening of *Piper longum* fruit powder in different organic solvent revealed the presence of alkaloids, tannins, flavonoids. Antioxidant activities were evaluated by using *In-vitro* antioxidant assay models like phospho-molybdenum and reducing power assay. Anti-bacterial activity was evaluated using streptomycin as a standard against *E.coli* ATCC (LB M1655) to find MIC value of crude extract. The ethanolic extract showed maximum anti-bacterial activity. The percentage of antioxidant activity by phospho-molybdenum assay was in the order acetone>ethanol> water> petroleum ether. The results obtained in this study showed that the fruits of *Piper longum* have antioxidative and anti-bacterial properties which provide a basis for the traditional use of the plant. The maximum values of both antioxidative and anti-bacterial activity and was found to be 300 µg/mL (acetone extract) and 200 µg/mL (ethanol extract) respectively.

INTRODUCTION

Medicinal plants are used in herbalism. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Plants have been the source of medicines for thousands of years; species of the genus *Piper* are among the important medicinal plants used in various systems of medicine. *Piper* species are widely distributedin the tropical and subtropical regions of the world and are of high commercial and economic importance.

***Piper longum* fruit**

Traditional medicines hold a great promise as source of easily available effective therapy for many diseases like upper respiratory infections, inflammatory reactions etc. In this context these herbs were used as medicines by our ancient people, particularly in tropical developing counties, including India. These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating diseases. Oxygen is the life of all cells. Although it is so important, its partial reduced forms are dangerous to the cells. In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionization radiations. Free radicals are derived from molecular oxygen under reducing conditions. Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. Hydrogen peroxide (H_2O_2), superoxide anion radical (O_2) hydroxyl radical (OH^-), lipid peroxide radical (ROO^-), singlet oxygen ($1O_2$), nitric oxide (NO^-) and oxynitrite ($ONOO^-$). Out of which Hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$) is not free radicals. Excess amount of these free-radicals can lead to cell injury, which results in many diseases like cancer, diabetes, neurodegenerative disorders.

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) plays important roles in scavenging the free radicals and preventing cell injury (Bergendi *et al*., 1999). Molecules such as vitamin C and E inhibit lipid peroxidation in cell The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the development of "antioxidants". In the present study was carried out to evaluate the antioxidant activity of *piper longum* fruit extracted using different solvents and their aqueous extracts. Also this herb have been used also as antibacterial agent, hence the activity was also carried out against other organisms such as *Escherichia coli ATCC* (LB M1655) *strain.*

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MATERIALS AND METHODS

Plant collection

Fruits of *Piper longum* were collected from Krshsna and Sons Shop, Tamil Nadu. The plant was authenticated by Botanist, Bangalore University, Bangalore, Karnataka, India.

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade and are purchased from Lancaster Research Lab, Chennai, India and Himedia Lab, Mumbai, India.

Extraction of plant material

Extraction was carried out at room temperature under normal conditions. About 15g of shade dried powder of fruits of *Piper longum* was successively extracted with ethanol, acetone, de-ionized water, and petroleum ether. The extract obtained was filtered, concentrated by evaporating at 100^0C in a water bath and dried.

Phytochemical analysis

The extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner's and Meyer's tests), saponins (foam and froth tests), (Acetone-water test), $FeCl_3$ test, tannins (gelatin test), and flavonoids (Alkaline reagent and Lead acetate tests), the screening was done as per the standard method.

Test for alkaloids

- Dragendorff's test: 2mg of the test extract and 5ml of distilled water was added, 2M hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendrof's reagent was added. Formation of orange red precipitate indicated the presence of alkaloids.
- Hager's test: 2mg of the test extract was taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.
- Wagner's test: 2mg of extract was acidified with 1.5%v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
- Mayer's test: few drops of Mayer's reagent, 2mg of extract was added formation of white or pale yellow precipitation indicated the presence of alkaloids

Test for flavonoids

- Ferric chloride test: test solution with few drops of ferric chloride solution shows intense green color
- Zinc hydrochloride acid reduction test: test solution with zinc dust and few drops of hydrochloric acid shows magenta red color
- Lead acetate solution test: test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

Tests for saponins

- Foam test: to the extract solution, a drop of sodium bi carbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. The formation of honey comb like froth indicates the presence of saponins.

Test for tanins

- Ferric chloride test: 1-2ml of the extract, few drops of 5%w/v $FeCl_3$ solution was added. A green color indicated the presence of gallotanins; while brown color indicates the presence of pseudotanins.
- Gelatin test: test solution when treated with gelatin solution gives white precipitate

Antioxidant Activity

Determination of phospho-molybdenum assay

The antioxidant activity of all the extract was determined by the phospho-molybdenum Method as described by Prieto *et al* [8]. 0.3 ml of extract of different concentrations (100 to 500µg/ml) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95^0C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer against blank. Distilled water (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid.

Reducing Power Assay (Iron (III) to iron (II) reduction)

The Ferric reducing power method was applied with slight modifications of the method in which, 2.5 mL of extract solution of different concentrations (100 to 500 µg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassiumferricyanide (2.5 mL, 0.1%). This was incubated at 50^0 C for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added.2.5 mL of the reaction mixture was mixed with distilled water (2.5mL) and ferric chloride (0.5 mL, 0.1%). The solution absorbance was measured at 700 nM. Increasing absorbance of the reaction mixture indicates increasing reducing power. The same procedure was applied for ascorbic acid which acts as the standard. Increase in the absorbance indicates increase in reducing power.

Anti- bacterial activity

The different dilutions (100- 500µg/ml) of crude extract of *Piper longum* were tested for its anti-bacterial activity on *E.coli* ATCC (LB M 1655) strain. Evaluation was carried out by agar well diffusion method.

Procedure for Inoculum

A loopful of *E.coli* ATCC (LB M 1655) strain was taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test-tube were kept in shaker incubator at 37°C for 24 hours. 1ml of overnight culture was plated on nutrient agar by spread plate method. Thereafter, a sterile cork borer of 5.0 mm diameter was used to punch wells in the seeded nutrient agar. 100µl of different crude extracts were added and the plates were incubated at 37^0C and observed for zone of inhibiton after 24 hours.

RESULT AND DISCUSSION

In this study of *In Vitro* antioxidant activity, preliminary phytochemical screening of the different fractions of dried fruit extracts revealed the presence of alkaloids, tannins, flavonoids and the results are tabulated in Table 1.

Table 1. Screening of secondary metabolites

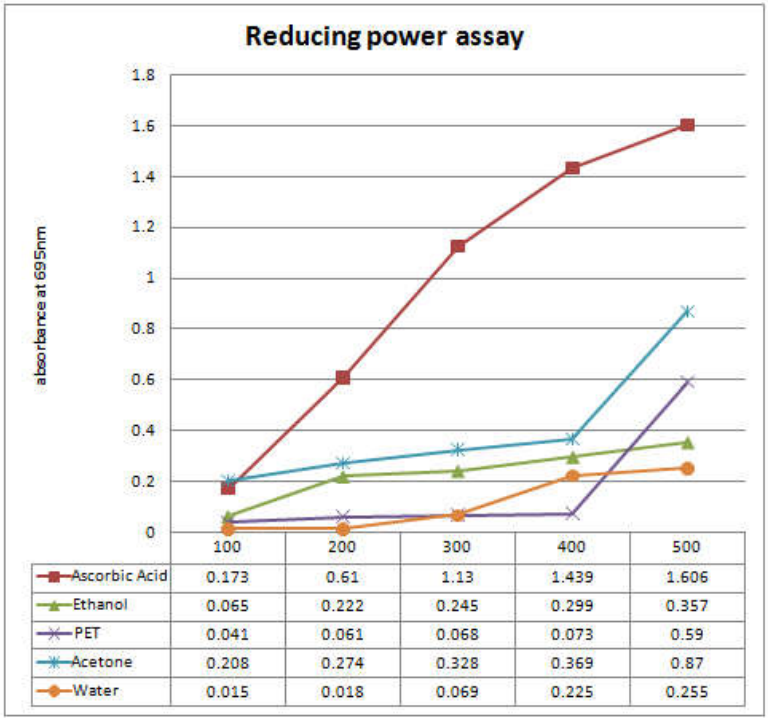
	Ethanol	Petroleum ether	Acetone	Water
Test for alkaloids				
Dragendorff's test	+	+	-	+
Hager's test	+	+	-	+
Mayer's test	+	+	-	+
Wagner's test	+	+	-	+
Test for flavonoids				
$FeCl_3$ test	-	-	+	-
Zinc test	-	-	+	-
Lead acetate test	-	-	+	-
Test for saponins				
Foam test	-	-	-	-
Test for tanins				
Gelatine test	+	-	+	-
Ferric chloride test	+	-	+	-

The results of the free radical scavenging potentials of different fractions tested by reducing power assay method and phospho-molybdenum assay are depicted in Fig 1 and 2. The phospho-molybdate method is quantitative, since the total antioxidant capacity is expressed as ascorbic acid equivalents. The percentage of antioxidant activity was in the order acetone> ethanol> pet.Ether> water. The IC_{50} value of acetone fraction was found to be 275 µg/mL, whereas the IC_{50} value of ethanol 350 µg/mL, In reducing power assay, acetone fraction seemed to have quite high reducing activity compared to ethanol, water and Pet ether. The reducing power assay of different extract is given in Fig 1.

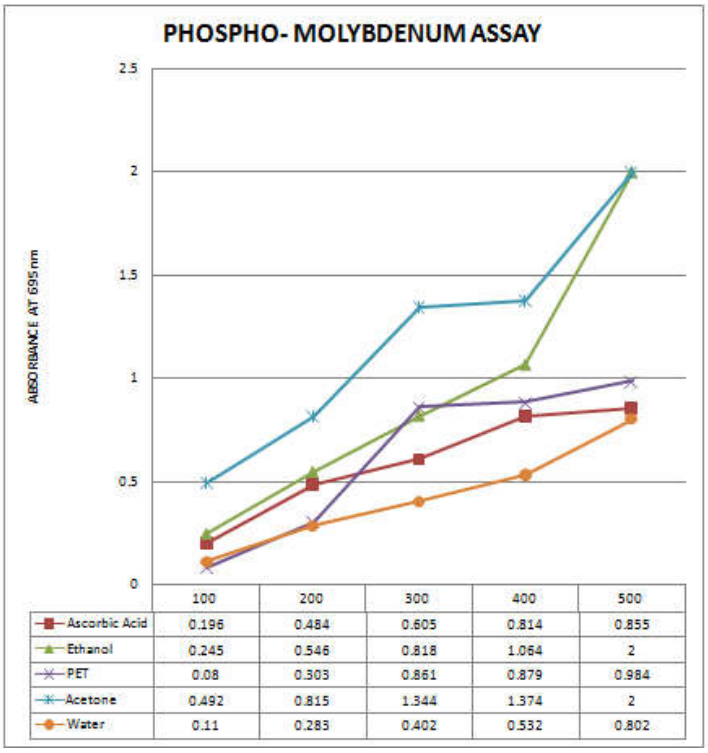
Table 2. Minimum Inhibition Concentrations (MIC)

CONCENTRATION µg/mL	ETHANOL	PET.ETHER	ACETONE
100	4mm	-	5mm
200	8mm	-	6mm
300	10mm	-	12mm
400	12mm	-	13mm
500	13mm	-	18mm

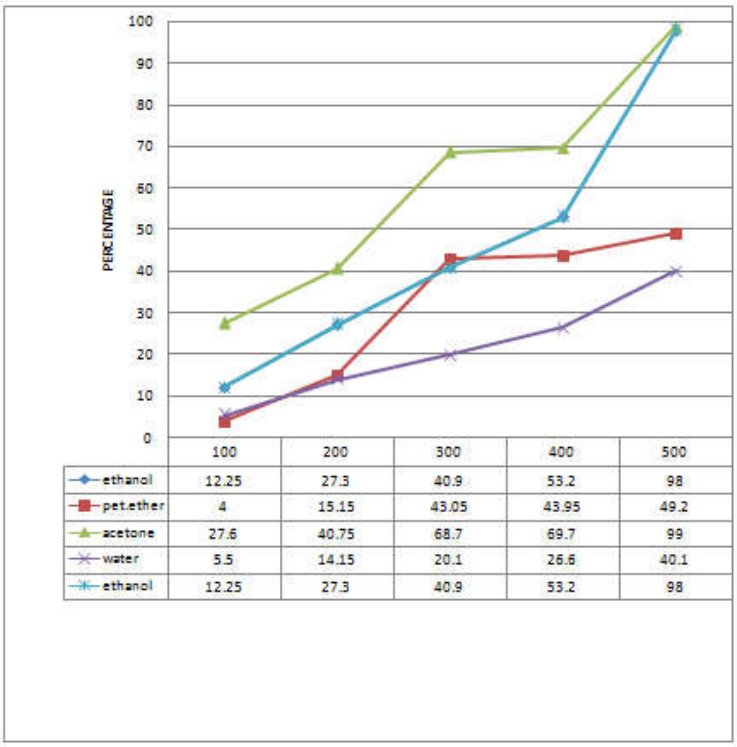
Minimum Inhibitory concentration of different estract against *E.coli* ATCC (LB M 1655) is given in Table 2.



Graph 1



Graph 2



	100	200	300	400	500
ethanol	12.25	27.3	40.9	53.2	98
pet.ether	4	15.15	43.05	43.95	49.2
acetone	27.6	40.75	68.7	69.7	99
water	5.5	14.15	20.1	26.6	40.1
ethanol	12.25	27.3	40.9	53.2	98

Graph 3

Acetone extract exhibited minimum inhibitory concentration ranging from 5 mm-18 mm ecept pet ether extract which did not inhibit the growth of tested organism used in the study at any concentration ranging from 100 µg/mL -5000 µg/mL.

Conclusion

Two methods were used to estimate the amount of antioxidant activity in the fruits of *Piper longum*. Among the two methods, antioxidant activity was more when performed with phospho-molybdate assay. Ethanol and acetone extracts seemed to have more antioxidant activity than any other extracts. This study has revealed the potential for antioxidant activity and also lent scientific justification to the traditional use of plant.

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