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Phytochemical and Antimicrobial activity of medicinal plant – *Piper longum*

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Abstract: *Plant based medicinal compounds is long established to be used as traditional treatment for innumerable human diseases from time immemorial in many parts of the world. Their use has been multiplied through various researchers and application due to a number of side effects by the use of synthetic drugs, antibiotics and due to the high cost.In the present study,the various extracts were prepared from Piper by maceration method. The preliminary phytochemical analysis were carried on various extracts by standard procedures. The antimicrobial activity is performed on chloroform extract of Piper longumbased on the results of phytochemical analysis. The results of antimicrobial activity performed on bacterial strains reveals that the medicinal plant is pharmaceutically important.*

Key words: *antibiotic, bacterial strains, phytochemical, antimicrobial.*

1. INTRODUCTION:

India is one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world(1). World Health Organization estimated that over 80% of the people in developing countries depend on traditional medicines for their primary health needs(2). Thephytochemicalsalso work as nutrients and fibres to activate the defense system against disease(3).Studies on the phytochemistry of plants have shown that aromatic and medicinal plants have a great source of diverse nutrient and non – nutrient molecules which act as antioxidant and antimicrobial agents(4-7).The resistance of microorganisms against the traditional antibiotics needs urgent attention for the development of the new drug molecules. It is well documented from ancient times that active principles from plant origin have been used as medicines for various diseases and microbial infections(8).

DESCRIPTION OF *PIPER LONGUM*:

Piper longum and plants belonging to the genus piper are common in Indian Ayurveda system of medicine(9). It has been used as medicine in rural and tribal areas in the world(10). The fruits and roots has numerous medicinal uses for diseases of respiratory tract, viz cough, bronchitis, asthma and analgesic when applied for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative(11).

Fig:1 Represents *Piper longum*

2. LITERATURE REVIEW:

Ounchokdee*et.al* performed the Antifungal activity of *Piper longum*fruits extract against plant pathogenic fungi namely *Colletotrichumcapsici*, *C. gloeosporioide* and *fusariumoxysporum f.* The ethanol crude extract exhibited potent activity against all tested fungi using disc diffusion method. The crude extract was then partially fractionated in order by column chromatography using six organic solvents. The results revealed that the fractions eluted with diethyl ether(DE) and ethyl acetate (EtOAc) exhibited potent antifungal activity against tested plant pathogens(12).

NitinB.L.*et.al* performed the phytochemical screening analysis of root and stem of *Piper longumlinn* using Thin Layer Chromatography profile to compare their chemical constituents. The profile suggested that there is similarity between root and stem cuttings but the percentage of chemical constituents was found more in stem(13).

The above reviews, reveals that the medicinal plant *piper longum* has pharmacognostic importance. Based on the above information the present study was performed on *piper longum* leaves and fruits.

3. MATERIALS AND METHODS:

Maceration:

The dried fruits and leaves of *Piper longum* is placed in a stoppered bottle with the chloroform and ethanol. It is allowed to stand at room temperature for a period of one week with frequent agitation until the soluble matter has dissolved. The mixture is then filtered and subjected to evaporation. The extracts were subjected to phytochemical screening analysis(14).

PRELIMINARY PHYTOCHEMICAL SCREENING ANALYSIS:

a) Determination of Alkaloids:

About 3 ml of concentrated extract was taken in a test tube and 1 ml of HCl was added to the mixture which is heated gently for 20 minutes and is then cooled and filtered. The filtrate was used for the following tests.

Wagner's test:

The filtrate was treated with Wagner's reagent . Formation of brown reddish precipitate indicates the presence of alkaloids(15).

b) Test for Saponins:

i) Froth test:

Exactly 0.5 g of the extract was dissolved in 2 ml distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for Saponins(16)**.**

ii) Foam test:

5 ml of the extracts were mixed with 20ml of distilled water and then agitated in a graduated cylinder for about 15 minutes. Formation of foam indicates the presence of Saponins.

c) Test for phenol:

Ferric chloride test:

Extracts were treated with 4 drops of alcoholic $FeCl_3$ solution. Formation of bluish black colour indicates the presence of phenols(17).

d) Test for Phytosterols:

LibermannBurchard's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

e) Test for Proteins

Xanthoprotein test

The extracts were treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins(18).

f) Test for Triterpenes
Salkowski test

The extracts were treated with chloroform solution and is then shaken with one or two drops of concentrated sulphuric acid and is then allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes(19).

g) Test for Glycosides
i) Glycoside test

0.5 mg of the extract was dissolved in 1 ml of water and then aqueous NaOH solution was added to the extract. Formation of yellow colour indicates the presence of glycosides(20).

ii) Concentrate H_2SO_4 test

To 5ml extract, add 2ml of glacial acetic acid, one drop 5% $FeCl_3$ and concentrated H_2SO_4. Appearance of brown ring indicates the presence of glycosides(21).

h) Test for Tannins
Lead acetate test

To the extracts few drops of 10% lead acetate solution were added. Formation of precipitate indicates the presence of tannins(22).

i) Test for steroids
Salkowski's test

5 ml of the extract was dissolved in 2 ml chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of steroids(23).

j) Test for coumarins:

10% NaOH (1ml) was added to 1 ml of the plant extracts. Formation of yellow colour indicates the presence of coumarins(24).

k)Test for Flavonoids
i) Pew's test

To two ml of the extract zinc powder was added in a test tube, followed by dropwise addition of concentrated HCl. Formation of purple red or cherry colour indicates the presence of flavonoids.

ii) NaOH test:

To two ml of the extract sodium hydroxide were added in a test tube. Formation of intense yellow colour that becomes colourless on addition of few drops of dilute HCl indicates the presence of flavonoids(25).

l) Test for carbohydrates
Molisch's test

To two or three ml of the aqueous extract two drops of alpha napthol solution in alcohol is added and shaken well. Then add concentrated sulphuric acid from the sides of the test tube. Violet ring formation indicates the presence of carbohydrates(26).

ANTIMICROBIAL ACTIVITY OF CHLOROFORM EXTRACTS OF *PIPER LONGUM*:

The antimicrobial activity of chloroform extract of piper longum leaves and fruits were analysed by agar well diffusion method(27).Muller Hinton Agar plates were prepared and inoculated with test organisms (Staphylococcus aureus, Enterococussps., Klebsiellapneumoniae, Pseudomonas aeruginosa) by spreading the bacterial inoculum on the surface of the media with the help of sterile swap. Wells (8mm in diameter) were punched in the agar by using cork borer. Extracts with concentration 50 ml were added. Positive control used is ciprofloxacin. The plates were incubated at 37 degree Celsius for 18 to 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and recorded in mm.

4. Analysis - Discussion:

Qualitative phytochemical screening analysis:

The phytochemical screening analysis performed on chloroform and ethanol extracts of *piper longum* leaves and fruits were discussed in the Table 1 and 1.1.

Table 1 Preliminary phytochemical screening Analysis of *Piper longum* leaves:

Name of the phytochemical	Name of the test	C	E
Alkaloids	Wagner's test	+	-
Saponins	Froth test	+	+
	Foam test	+	+
Phytosterols	Libermann- Burchard's test	+	+
Proteins	Xanthoprotein test	+	+
Triterpenes	Salkowski test	-	-
Glycosides	Glycoside test	+	-
	Concentrate sulphuric acid test	+	-
Tannins	Lead acetate test	+	+
Steroids	Salkowski's test	+	-
Coumarins	10%NaOH+1ml plant extract	-	-
Flavonoids	Pew's test	-	-
	NaOH test	-	-
Carbohydrates	Molisch's test	-	-

where, C - Chloroform extract, E - Ethanol extract + indicates present , - indicates absent
Table1 represent the phytochemicals present in chloroform and ethanol extracts of *piper longum*leaves. It is found that maximum number of phytochemicals were present in *piper longum* chloroformextract compared to ethanol extract.

Table 1.1 Preliminary phytochemical screening Analysis of *Piper longum*fruits

Name of the phytochemical	Name of the test	C	E
Alkaloids	Wagner's test	+	-
Saponins	Froth test	+	+
	Foam test	+	+
Phytosterols	Libermann- Burchard's test	+	-
Proteins	Xanthoprotein test	+	+
Triterpenes	Salkowski test	-	-
Glycosides	Glycoside test	+	+
	Concentrate sulphuric acid test	+	+
Tannins	Lead acetate test	+	+
Steroids	Salkowski's test	+	-
Coumarins	10% NaOH+1ml plant extract	-	+
Flavonoids	Pew's test	-	-
	NaOH test	-	-
Carbohydrates	Molisch's test	-	-

where, C - Chloroform extract E - Ethanol extract + indicates present - indicates absent
Table 1.1 represents the phytochemicals present in chloroform and ethanol extracts of *piper longum* fruits. It is found that maximum number of phytochemicals were present in chloroform extract of *piper longum* compared to ethanol extract.

Antimicrobial activity of chloroform extracts of *piper longum:*

The chloroform extract of *piper longum* leaves and fruits were subjected to antimicrobial studies in gram positive and gram negative bacteria's. The results were discussed in Table 2and Fig 1.1.

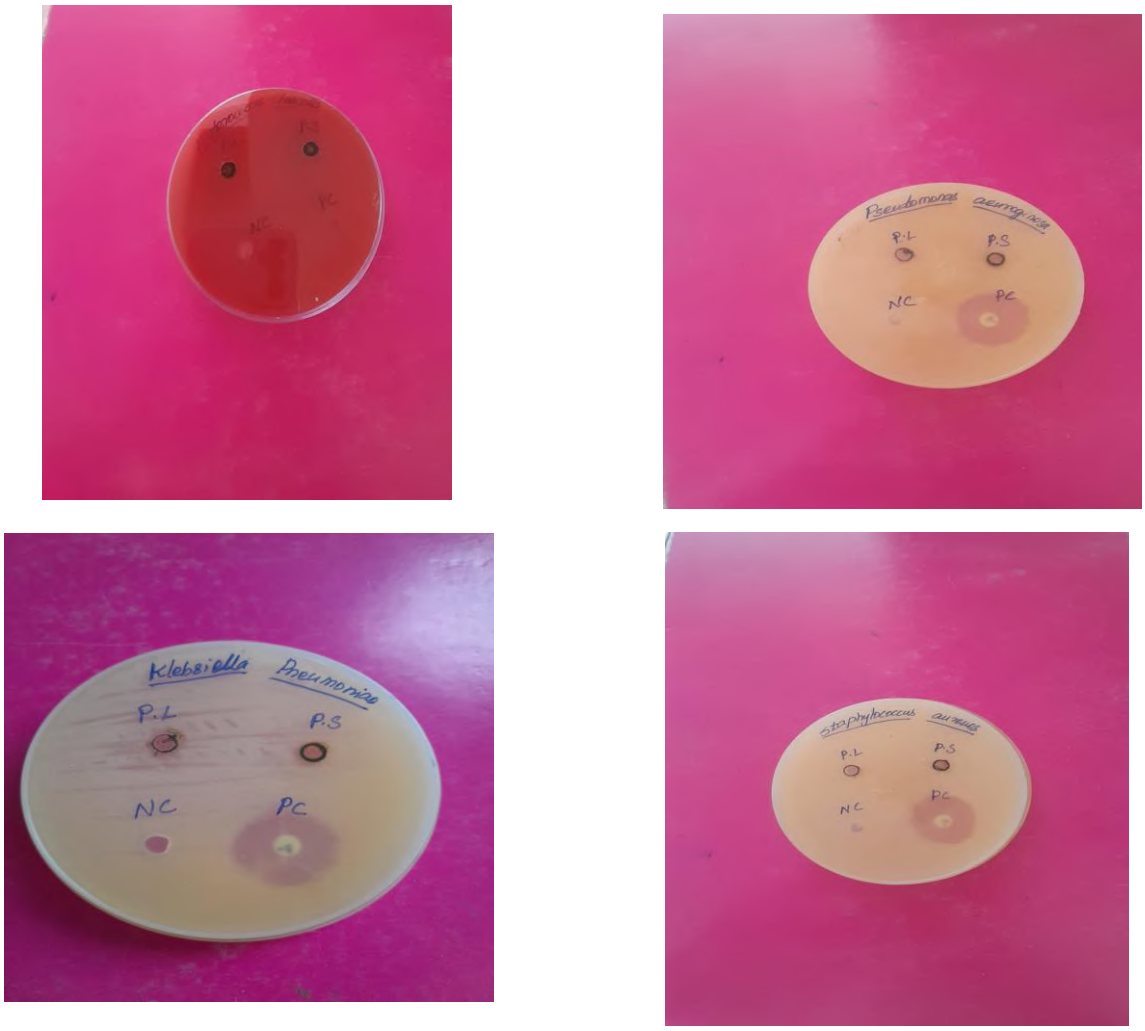

Fig 1.1 Represents the antimicrobial activity of chloroform extract of *piper longum* leaves and fruits

Table 2 Antimicrobial Activity of chloroform extract of *piper longum* fruits and leaves by Agar well diffusion method

Sl.No	Name of the bacterial strains	Zone size in diameter (mm)		
		Positive control (Ciprofloxacin)	Chloroform extract of *Piper longum* fruits	Chloroform extract of *Piper longum* leaves
1.	*Klebsiellapneumoniae*	26 mm	6 mm	6 mm
2.	*Staphylococcus aureus*	26 mm	6 mm	6 mm
3.	*Pseudomonas aeruginosa*	27 mm	6 mm	6 mm
4.	Enterococcus sps.	24 mm	6 mm	6 mm

The results showed that 6mm zone of inhibition is observed for all the bacteria tested against the positive and negative control. This shows that the plant has minimum antimicrobial activity against the tested bacterial strains.

5. FINDINGS:

Phytochemical screening analysis reveals that the maximum number of phytochemicals were present in *piper longum* chloroform leaves extract compared to ethanol exrtract. In case of *piper longum* fruits the maximum number of phytochemicals were present in *piper longum* chloroform fruits extract compared to ethanol extract.

Antimicrobial studies performed on chloroform extract of *piper longum* fruits and leaves showed that there is minimum antimicrobial resistance for the chloroform extracts of *piper longum* fruits and leaves.

6. CONCLUSION:

The phytochemical screening analysis reveals that the bioactive components were present more in chloroform extract of leaves and fruits than ethanol extracts.The antimicrobial activity performed on chloroform extract of piper longum fruits and leaves showed that the bioactive components responsible for antibacterial activities were present in inappropriate amounts.

7. RECOMMENDATION:

Further studies should be extended for characterization and to find the amount of each bioactive component present in the chloroform extracts of leaves and fruits of *piper longum*. So that the active components responsible for antimicrobial activity of chloroform extract of *piper longum* leaves and fruits can be evaluated.

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