Research Journal of Agriculture and Biological Sciences, 3(6): 852-857, 2007

Antimicrobial Screening of Different Extracts of *Piper longum* Linn.

[1]M. Abbas Ali, [2]Noor Mahbub Alam, [3]Mst. Sarmina Yeasmin, [3]Astaq Mohal Khan, [2]M. Abu Sayeed

[1]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh
[2]Department of Applied Chemistry and Chemical Technology, Rajshahi University, Rajshahi-6205, Bangladesh
[3]BCSIR Laboratories, Rajshahi-6206, Bangladesh.

Abstract: The antibacterial and antifungal activities of various solvent extracts of *Piper longum* were determined against a wide variety of pathogenic bacteria and fungi respectively. Crude extracts of *Piper longum* showed mild to moderate activities against most of the tested bacteria. On the other hand, the antifungal activities exhibited by all the crude extracts were not prominent. Petroleum ether extracts of *Piper longum* were found to be inactive against most of the tested organisms. Ethyl acetate extracts showed relatively better anti-microbial effect against most of the tested organisms. It has been expected that the present work on antimicrobial screening of the plant materials will lead to the scientists who continue work may have clinical success concerning the killer diseases.

Keywords: *Piper longum*, extract, antibacterial activity and antifungal activity

INTRODUCTION

Many microorganisms can cause several diseases and now, in this world of modern science, man can face any challenge against any disease. But in spite of the tremendous advancement of medical science and technology, diseases are the leading health problem particularly in the under privileged population in the remote rural areas in the developing countries. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

Piper longum Linn., sometimes called Indian Long Pepper, is a flowering vine in the family *Piperaceae*, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant, and has a similar, though generally hotter, taste. The root and fruit of *Piper longum* are used in palsy, gout and lumbago. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive[1]. They have a pungent pepper-like taste and produce salivation and numbness of the mouth. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma etc; as counter-irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative. Besides fruits, the roots and thicker parts of stem are cut and dried and used as an important drug in the Ayurvedic and Unani systems[2].

Due to the fact that the plant *Piper longum* is very useful, as found by above mentioned reports and the fact that little information cited in the literature[3-6] is available on the biological activities, there is a need to find out more about the potentiality of this plant as an antimicrobial agent. The present study is, therefore, designed to assess the potency of different solvent extracts of *Piper longum* on some selected microorganisms.

MATERIALS AND METHODS

The plant *Piper longum* was collected from botanical garden of the Rajshahi University, Bangladesh. Organisms used in present study were collected from the Department of Biochemistry and

Corresponding Author: M. Abu Sayeed, Department of Applied Chemistry and Chemical Technology, Rajshahi University, Rajshahi-6205, Bangladesh.
Tel.: +88-0721-750452, E-mail: radwiya44@yahoo.com

Molecular Biology and from the Institute of Environmental Science, Rajshahi University, Bangladesh. All solvents used for this study were redistilled and purified. Other chemicals, including culture media used were of analytical grade unless otherwise specified.

Plant Samples: The plant materials of *Piper longum* were washed with water, dried in sunlight and pulverized into fine powder by a grinding machine and then stored in airtight container. 50 g of each of powdered plant materials of root, stem and leaves was extracted separately at room temperature using petroleum ether (40-60^{0}C) as extracting solvent with gentle stirring for seven days (three times within this period). The resultant extracts were combined and combined extract was filtered and concentrated under a vacuum to obtain semi-solid mass. Extraction was carried out successively with ethyl acetate, chloroform and methanol from the residue left after extraction with petroleum ether applying the same procedure as mentioned above.

Antimicrobial Screening: *In vitro* antibacterial and antifungal screening were performed with petroleum ether, ethyl acetate, chloroform and methanol extracts of root, stem and leaves of *Piper longum* against 13 pathogenic bacteria (5 gram-positive and 8 gram-negative) and 6 fungi by the standard disc diffusion method[7-9]. Nutrient agar medium was used for determining antibacterial activity whereas potato dextrose agar medium (PDA) was selected for antifungal activity. Standard antibiotic discs of Kanamycin (30 µg/disc) and Clotzimazole (30 µg/disc) were also used for comparison in antibacterial and antifungal tests respectively.

The crude extracts were dissolved in sufficient amount of the respective solvents, so that each 10 µl of solutions contained 400 µg of the test materials for antibacterial activity and 300 µg for antifungal activity tests. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm using a transparent scale. The diameters of the zones of inhibition by the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc used.

RESULTS AND DISCUSSIONS

As can be seen from Table 1, ethyl acetate, chloroform and methanol extracts obtained from *Piper longum* root showed mild to moderate activity against most of the tested bacteria. But inhibitory effect of petroleum ether was observed against only the bacteria *Streptococcus aureus*. The results were compared with those of Kanamycin as a standard antibiotic. Of the four extracts, only ethyl acetate extract showed activity against gram- negative *Klebsiella species*. Ethyl acetate extract also displayed excellent activity against gram-positive *Sarcina lutea* (22 mm) and gram-negative *Shigella sonnei* (21 mm) whereas methanol extract showed strong activity against gram-negative *Shigella flexneriae* (17 mm). The organism *Shigella boydii* was resistant to all the extracts. But the activities, on overall consideration, of ethyl acetate extract were higher as compared to those of other extracts extracted from *Piper longum* root.

The three extracts (ethyl acetate, chloroform and methanol) from *Piper longum* stem displayed mild to moderate activity against most of the bacteria tested while the petroleum ether extract was found to be active against only gram-positive *Streptococcus aureus* and gram-negative *Shigella boydii* (Table 2). Ethyl acetate extract displayed strong activity against gram-negatives *Shigella boydii* (17 mm) and *Shigella flexneriae* (17 mm) whereas methanol extract was strongly active against gram-positive *Streptococcus β-haemolyticus* (18 mm). The bacteria *Shigella dysenteriae* was resistant against all the crude extracts of *Piper longum* stem. Moreover, all of the extracts were appeared to be active against the organisms *Streptococcus aureus* and *Shigella boydii*.

With regard to antibacterial activity of different extracts of leaves of *Piper longum*, all the crude extracts (except petroleum ether), we reported herein (Table 3), appeared to have mild to moderate activity against most of the bacterial strains, but petroleum ether extract showed activity against only gram-positive *Streptococcus aureus*. Moreover, all the extracts were inactive against gram- negative *Shigella dysenteriae*. Results also indicated that all the three extracts (ethyl acetate, chloroform and methanol) were found to be strongly active against gram-positive *Streptococcus aureus* (15-17 mm) and gram-negative *Shigella boydii* (17-20 mm) and the activities, on overall consideration, of methanol extract were not so enough as those of ethyl acetate and chloroform extracts.

Crude extracts obtained from *Piper longum* root showed mild activity against most of the fungal strains (Table 4). Among them, ethyl acetate extract displayed strong activity against *Fusarium sp.* (17 mm) and found to be inactive against only *Penecillum sp*. while petroleum ether extract exhibited activity against only the organisms *Aspergillus niger* and *Aspergillus fumigatus*. The fungus such as *Penecillum sp*. was resistant against all the crude extracts of *Piper longum* root.

Table 1: Antibacterial activities of different extracts of root of *Piper longum*

Test organisms	Diameter of zone of inhibition in mm				
	PER	EER	CER	MER	STK
Gram positive					
Bacillus megaterium	0	12	11	9	22
Streptococcus β- haemolyticus	0	15	14	12	21
Streptococcus aureus	9	17	14	8	23
Bacillus subtilis	0	9	10	7	25
Sarcina lutea	0	22	9	7	24
Gram negative					
Escherichia coli	0	11	9	7	20
Pseudomonas aeruginosa	0	15	12	9	20
Shigella sonnei	0	21	9	12	22
Shigella dysenteriae	0	14	11	10	24
Salmonella typhi	0	13	10	9	23
Klebsiella species	0	14	0	0	26
Shigella boydii	0	0	0	0	20
Shigella flexneriae	0	16	15	17	21

PER= Petroleum ether extract of root (400 µg/disc); EER= Ethyl acetate extract of root (400 µg/disc); CER= Chloroform extract of root (400 µg/disc); MER= Methanol extract of root (400 µg/disc) and STK = Kanamycin (30 µg/ disc).

Table 2: Antibacterial activities of different extracts of stem of *Piper longum*

Test organisms	Diameter of zone of inhibition in mm				
	PES	EES	CES	MES	STK
Gram positive					
Bacillus megaterium	0	9	10	9	20
Streptococcus β- haemolyticus	0	11	9	18	21
Streptococcus aureus	9	14	15	11	22
Bacillus subtilis	0	13	13	10	24
Sarcina lutea	0	9	11	7	21
Gram negative					
Escherichia coli	0	9	11	7	20
Pseudomonas aeruginosa	0	11	10	9	20
Shigella sonnei	0	9	7	0	22
Shigella dysenteriae	0	0	0	0	24
Salmonella typhi	0	10	10	9	23
Klebsiella species	0	10	9	0	26
Shigella boydii	12	17	13	10	20
Shigella flexneriae	0	17	15	14	25

PES= Petroleum ether extract of stem (400 µg/disc); EES= Ethyl acetate extract of stem (400 µg/disc); CES= Chloroform extract of stem (400 µg/disc); MES= Methanol extract of stem (400 µg/disc) and STK = Kanamycin (30 µg/ disc).

Table 3: Antibacterial activities of different extracts of leaves of Piper longum

Test organisms	Diameter of zone of inhibition in mm				
	PEL	EEL	CEL	MEL	STK
Gram positive					
Bacillus megaterium	0	10	10	9	21
Streptococcus b- haemolyticus	0	12	9	18	23
Streptococcus aureus	9	17	16	15	23
Bacillus subtilis	0	11	10	9	24
Sarcina lutea	0	9	9	7	21
Gram negative					
Escherichia coli	0	12	11	7	20
Pseudomonas aeruginosa	0	11	10	9	20
Shigella sonnei	0	9	10	8	22
Shigella dysenteriae	0	0	0	0	24
Salmonella typhi	0	7	10	9	23
Klebsiella species	0	8	9	0	26
Shigella boydii	0	20	17	17	21
Shigella flexneriae	0	12	15	0	24

PEL= Petroleum ether extract of leaves (400 mg/disc); EEL= Ethyl acetate extract of leaves (400 mg/disc); CEL= Chloroform extract of leaves (400 mg/disc); MEL= Methanol extract of leaves (400 mg/disc) and STK = Kanamycin (30 mg/ disc).

Table 4: Antifungal activities of different extracts of root of *Piper longum*

Test organisms	Diameter of zone of inhibition in mm				
	PER	EER	CER	MER	STF
Penecillum sp.	0	0	0	0	15
Aspergillus niger	8	14	9	10	14
Aspergillus fumigatus	7	7	7	7	9
Mucor sp.	0	9	13	9	14
Fusarium sp.	0	17	11	0	16
Candida albicans	0	11	8	10	14

PER= Petroleum ether extract of root (300 mg/disc); EER= Ethyl acetate extract of root (300 mg/disc); CER= Chloroform extract of root (300 mg/disc); MER= Methanol extract of root (300 mg/disc) and STF = Fluconazole (50 mg/disc).

Results depicted in Table 5, demonstrate that all the extracts (except petroleum ether) obtained from *Piper longum* stem displayed mild activity against most of the fungi, but petroleum ether extract was found to be inactive against all of the fungi tested (except *Aspergillus niger*). Among the extracts, only ethyl acetate extract was found to be active against all the fungal strains and was strongly active against *Fusarium sp.* (16 mm). The fungus such as *Penecillum sp.* was resistant against all the crude extracts (except ethyl acetate) and all the crude extracts (except petroleum ether) showed higher antifungal effect against *Mucor sp.* (12-15 mm).

From the Table 6, it is evident that the extracts (except petroleum ether) of *Piper longum* leaves showed mild to moderate activities against most of the tested fungi. Chloroform extract exhibited comparatively higher activity against most of the tested fungi than that of the other three. All the crude extracts displayed activity against *Candida albicans*. Petroleum ether extract showed inhibitory effect only the fungi *Aspergillus niger* and *Candida albicans*.

From the above experimental results, it is found that the activities of all methanol extracts of *Piper longum* were not sufficiently enough compared to those of ethyl acetate and chloroform extracts against most of the bacteria tested, and all petroleum ether extracts

Table 5: Antifungal activities of different extracts of stem of *Piper longum*

Test organisms	Diameter of zone of inhibition in mm				
	PES	EES	CES	MES	STF
Penecillum sp.	0	9	0	0	15
Aspergillus niger	8	10	9	8	14
Aspergillus fumigatus	0	7	7	0	9
Mucor sp.	0	12	15	14	14
Fusarium sp.	0	16	9	0	12
Candida albicans	0	10	8	9	14

PES= Petroleum ether extract of stem (300 mg/disc); EES= Ethyl acetate extract of stem (300 mg/disc); CES= Chloroform extract of stem (300 mg/disc); MES= Methanol extract of stem (300 mg/disc) and STF = Fluconazole (50 mg/disc).

Table 6: Antifungal activities of different extracts of leaves of *Piper longum*

Test organisms	Diameter of zone of inhibition in mm				
	PEL	EEL	CEL	MEL	STF
Penecillum sp.	0	9	14	0	15
Aspergillus niger	8	0	13	10	14
Aspergillus fumigatus	0	10	10	0	10
Mucor sp.	0	13	11	9	14
Fusarium sp.	0	8	10	11	12
Candida albicans	8	9	14	7	14

PEL= Petroleum ether extract of leaves (300 mg/disc); EEL= Ethyl acetate extract of leaves (300 mg/disc); CEL= Chloroform extract of leaves (300 mg/disc); MEL= Methanol extract of leaves (300 mg/disc) and STF = Fluconazole (50 mg/disc).

displayed activities against a very few bacterial strains. Methanol extracts of stem and leaves of *Piper longum* showed strong activities against gram-positive *Streptococcus b- haemolyticus* (18 mm), but no inhibition was observed against gram-negative *Shigella dysenteriae*. During the screening work, it was found that the ethyl acetate extract of root displayed highest activity against *Sarcina lutea* (22 mm) and *Shigella sonnei* (21 mm). Antifungal activities of all extracts of the plant *Piper longum* were not significant against most of the tested fungal strains. Chloroform extract of leaves showed higher anti-fungal activity as compared to the other extracts of the plant.

In comparison, the activities of all ethyl acetate extracts (400 mg/disc) of the plant *Piper longum* were comparable with those of same extracts (200 mg/disc) of *Oroxylum indicum* root bark against most of the tested bacteria[10]. Moreover, the activities displayed by chloroform extracts (400 mg/disc) of *Piper longum* were close to those of *Mesua ferrea* leaves against most of the tested bacteria[11].

It may, therefore, be concluded from the above investigation that the crude extracts obtained from various portions of *Piper longum* may be used enough as drug to treat the disease caused by those bacteria, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter. However, further and specific studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

REFERENCES

1. Kirtikar, K.R. and B.D. Basu, 1987. Indian medicinal plants, International book distributors, Dehradun, India, 3: 2128-2129.
2. CSIR, 1989. The Wealth of India: A dictionary of Indian Raw Materials and Industrial Products. Council of Scientific and Industrial Research, New Delhi, 8: 96-99.
3. Young-Cheol, Y., L. Sang-Guei, L. Hee-Kwon, K. Moo-Key, L. Sang-Hyun and L. Hoi-Seon, 2002. A Piperidine Amide Extracted from *Piper longum* L. Fruit Shows Activity against *Aedes aegypti* Mosquito Larvae. Journal of Agriculture and Food Chemistry, 50: 3765-3767.
4. Nongyao, S., K. Sawangjaroen and P. Poonpanang, 2004. Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. Journal of Ethnopharmacology, 91: 357-360.

5. Li, X., J. Zhou, H. Li, S. Du, Y. Li, L. Huang and D. Chen, 2005. Study on proliferation effect of extracts of *Piper longum* on mesenchymal stem cells of rat bone marrow and the relationship to chemical functional groups. Zhong-Yao-Cai., 28: 570-574.
6. Reddy, P.S., K. Jamilk, P. Madhusudhan, G. Anjani and B. Das, 2001. Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharmaceutical Biology, 39: 236-238.
7. Barry, A.L., 1980. Procedures for testing antimicrobial agents in agar media. In antibiotic in laboratory medicine, Eds., V. Lorian. Williams and Wilkins Co., Baltimore, USA., pp: 1-23.
8. Bauer, A.W., W.W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45: 493-496.
9. Rios, J.J., M.C. Reico and A. Villar, 1988. Antimicrobial screening of natural products. Journal of Ethnopharmacology, 23: 127-149.
10. Uddin, K., A. Sayeed, A. Islam, A.A. Rahman, S. Khatun, G.R.M. Astaq Mohal Khan and M.G. Sadik, 2003. Biological activities of extracts and two flavonoids from *Oroxylum indicum* Vent. (Bignoniaceae). OnLine Journal of Biological Sciences, 3: 371-375.
11. Abbas Ali, M, M. Abu Sayeed, M.S.A. Bhuiyan, F.I. Sohel and Mst. Sarmina Yeasmin, 2004. Antimicrobial Screening of *Cassia fistula* and *Mesua ferrea*. Journal of Medical Sciences, 4: 24-29.