INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 87-93, 2015

RESEARCH PAPER OPEN ACCESS

A comparative study of antimicrobial activity of the leaf and stem of *Alocasia indica L.*

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Key words: Comparative study, *Alocasia indica*, Antibacterial, Antifungal.

http://dx.doi.org/10.12692/ijb/7.4.87-93 Article published on October 25, 2015

Abstract

Plants are good sources of pharmaceutical compounds, which confer resistance to pathogenic microorganisms. The aim of this study was to investigate the antimicrobial activity of ethanol extract of both leaves and stems of *Alocasia indica* L *in vitro* by the agar disc diffusion method. The plant showed antibacterial and antifungal activity against Gram positive and Gram negative bacteria and pathogenic fungi. Leaf and stem extracts were found to have antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli* and *Klebsiella pneumoniae*. These extracts also showed antifungal activity against *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. The experiments demonstrated similar inhibition of bacterial and fungal growth for leaf and stem extracts. This indicates the potentiality of *Alocasia indica* L as an important source of antimicrobial compound.

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Introduction

Plants are the source of compounds useful for medicinal purposes. Since ancient times, they have been playing an important role in healthcare system. The use of the medicinal plants for curing disease has been documented in history of all civilizations (F.V. Dfeudis.,1991). Recently, the world Health organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary health care. Herbal medicines are popularized due to their effectiveness, easy availability, low cost and comparatively being devoid of toxic ***effect (Ali M. 1998).*** With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi R *et al.*, 2010).Yet, there is an extensive interest in drugs derived from plants. Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which are therefore, should be utilized to combat the disease causing pathogens (HH EL *et al.*,2010). In addition, antimicrobial resistance (AMR) is an increasingly serious threat to global public health. It threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. (Who.,2014). Furthermore some antibiotics have serious undesirable side effects which also limit their application. So, there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side effect and *higher plants represent a potential source of novel* antibiotic prototypes (*Panda SK et al.,2010*). Researchers, therefore, have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs *(Panda SK et al.,2010 and Pavithra PS et al.,2010).*

Alocasia indica L. is one of the most familiar plants, which grow abundantly throughout the year in Bangladesh. It is mainly used as vegetable. The plant contains flavonoids, alkaloids, cyanogenetic glycosides, steroids, gallic acid, succinic acid, ascorbic acid, amino acids, oxalic acid (Anonymous.,1952) and alocasin (Jatinder S *et al.*,1993). Different parts of this plant are traditionally used as hepatoprotective, antioxidant, analgesic, antiarthritic, antiinflammatory, antitumour and antipyretic (Anonymous.,1952). It is also reported to use in the treatment of diabetes mellitus and piles (Pullaiah T *et al.*,2003 and Karim *et al.*,2014). Alcoholic extract of leaves were evaluated for antimicrobial (Mulla A *et al.*,2010), antidiarrhoeal (Mulla A *et al.*,2011), antioxidant and anti-inflammatory (Mulla W *et al.*,2010) and anthelminitic (Mulla *et al.*,2010) properties. Seeds extract is also reported for its antifungal activity (Bhatt *et al.*,1980). Although previous studies have reported the antimicrobial effect of *Alocasia indica*, little attention has been paid to the study of antimicrobial activity of leaf and stem extract of the plant simultaneously.

In the present study, we investigated the effects of ethanolic extract of leaves and stems of *Alocasia indica* to ascertain the scientific basis for the use of this plant against microbial infection.

Material and methods

Collection of Plant Materials

Mature plants were collected locally for the experimental purposes. The leaves and stems were separated manually from the plants and washed several times with running tap water to remove the foreign materials. Fresh leaves and stem were then allowed to dry in the sunlight for four consecutive days followed by heating in an electric oven at 40°C until a constant weight was reached. The dried leaves and stem were finally ground into fine powder, packed and stored in a refrigerator at 4°C prior to analysis.

Extraction of Plant Materials

For solvent extraction (Soxhlet method), 500g powder of leaves and stem were placed into a separate cellulose paper cone and extracted using ethanol in a

5-1 Soxhlet extractor for 8 h (Pena *et al.*,1992). The extract was then recovered by evaporating off the solvent using rotary evaporator and residual solvent was removed by drying in an oven at 60 °C for 1 h.

Microbial Strains and Culture

A total of ten pathogenic bacteria were selected for the antibacterial activity test, five of which were gram positive and the remaining were gram negative. Antifungal activity test was performed against six pathogenic fungi . The pure microbial strais were collected from the microbiological research laboratory of Institute of Biological Science (IBScs), University of Rajshahi, Bangladesh. The test microorganisms are listed in Table1.

Bacteria were grown in nutrient agar (DIFCO) medium (0.005% of bacto peptone, 1% yeast extract, 125mM Nacl, 2% agar, pH 7.2). Fungi were cultured in potato dextrose agar (PDA) medium (200gm peeled and sliced potato, 40gm Dextrose, 20gm Agar, 1000ml Distill water).

Antimicrobial activity

The antimicrobial activity was determined by agar disc diffusion method (Vander and Vlietnck.,1991). Briefly, the crude extract was dissolved in suitable solvent to develop a solution of known concentration (mg/ml). Dried and sterilized filter paper discs (6 mm) impregnated with known concentration of the test material was placed on agar plates which had previously been inoculated with the test microorganisms. Standard antibiotic disc and blank disc (impregnated with solvent) were used as a positive and negative control, respectively. The plates were then kept in a refrigerator at 4°C for about 24 hours in order to provide sufficient time to diffuse the sample and standard antibiotic from the discs to surrounding agar medium. Finally, the plates were incubated at 37°C for 24 hours to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganism and a clear, distinct zone of inhibition was visualized surrounding the disc on the medium. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in terms of millimeter (mm).

The antibacterial activity of ethanol extract was tested against ten bacteria at concentrations of 300 µg/disc and 400 µg/disc. For antifungal activity, the concentrations were 400 µg/disc and 500 µg/disc. To compare the activity with standard antibiotics, *Kanamycin* (30 µg/disc) and *Nystatin* (50 µg/disc) were used for antibacterial and antifungal test, respectively.

Statistical analysis

Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Excel Version 8.0 software. Significance was defined at $P < 0.05$.

Results and discussion

Application of plant extract to inhibit bacterial and fungal growth is a common practice. In this study, the antibacterial activity of the crude ethanol extract was tested against ten bacteria at concentrations of 300 µg/disc and 400µg/disc. Standard antibiotic disc Kanamycin (K-30 µg/disc) was used for comparison. The results obtained are shown in Table 2, Table 3 and Figure 1. Diameters of zone of inhibition for ethanol extract of leaves of *Alocasia indica* against *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Klebsiella pneumoniae* were 9, 10, 9, and 8 mm, respectively at concentration of 300 µg/disc, while at concentration of 400 µg/disc, diameters of zone of inhibition were 13,11, 12 and 11 mm against the same bacteria. Stem extract also showed the activity against *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Klebsiella pneumoniae* and the diameters of zone of inhibition were 10, 9, 9, and 8 mm, respectively at concentration of 300 µg/disc. At concentration of 400 µg/disc, diameters of zone of inhibition against the same bacteria were 13, 11, 12, and 11 mm, respectively. Interestingly, all these extracts had no activity against *Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Sarcina lutea, Shigella dysenteriae,* and *Steptococcus haemolytia*.

The antifungal activities of both leaves and stems extracts against six pathogenic fungi were investigated at concentrations of 400 µg/disc and 500 µg/disc. The standard antibiotic disc of Nystatin (100 µg/disc) was used for positive control. The results of antifungal activity (zone of inhibition) of test materials against respective fungi were given in the Table 4 and Table 5 and Figure 2. It was found that ethanol extracts of leaves and stems of *Alocasia indica* showed higher antifungal activity against *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. At concentration of 500µg/disc, they produced 10, 11 and 12 mm of zone of inhibition, while at concentration of 400 µg/disc, diameters of zone of inhibition were 8, 9, and 10 mm, respectively.

Table 1. List of test microorganisms used for antimicrobial activity.

Bacteria		Fungi
Gram positive	Gram negative	
Staphylococcus aureus	*Pseudomonas aeruginosa*	*Penicilium sp*
Bacillus subtilis	*Salmonella typhi*	*Aspergillus niger*
Sarcina lutea	*Klebsiella pneumoniae*	*Saccharomyces cerevisiae*
Bacillus cereus	*Escherichia coli*	*Candida albicans*
Streptococcus haemolytica	*Shigella dysenteriae*	*Sclerotia rolfsii*
		Rizopus sp

Table 2. *In vitro* antibacterial activity of Ethanol extract of Aolcasia indica leaves and kanamycin.

Test bacteria	Ethanol extract of *Aolcasia indica* leaves(µg/disc)		Kanamycin (µg/disc)
	300	400	30
	Zone of inhibition (diameter in mm.)		
Staphylococcus aureus	9±0.17	13±0.19	21±0.19
Bacillus subtilis	10±0.18	11±0.21	24±0.25
E. coli	9±0.14	12±0.12	33±0.30
Klebsiella pneumoniae	8±0.15	11±0.13	37±0.33
Salmonella typhi	-	-	36±0.29
Pseudomonas aeruginosa	-	-	27±0.16
Bacillus cereus	-	-	25±0.15
Sarcina lutea	-	-	28±0..28
Shigella dysenteriae	-	-	31±0.22
Steptococcus haemolytia	-	-	23±0.17

All values represent means ± S.D. n = 3. $P < 0.05$ indicates values significantly different, "-" indicates No activity.

The ethanol extract of stems produced zone of inhibition of 10, 13 and 9 mm at concentration of 500 µg/disc, while at the 400µg/disc, they produced diameter were 8, 10 and 9 mm, respectively against the same fungi. Surprisingly, ethanol extracts of both leaves and stems showed no zone of inhibition against *Penicilium sp*, *Sclerotia rolfsii*, and *Rizopus sp*.

The results reveal that extracts of leaves and stems of *Alocasia indica* were effective against Gram-positive, gram-negative bacteria and fungi which are associated with microbial disorders.

Table 3. *In vitro* antibacterial activity of Ethanol extract of Aolcasia indica stem and kanamycin.

Test bacteria	Ethanol extract of *Aolcasia indica* stem(µg/disc)		Kanamycin (µg/disc)
	300	400	30
	Zone of inhibition (diameter in mm.)		
Staphylococcus aureus	10±0.12	13±0.17	21±0.19
Bacillus subtilis	9±0.09	11±0.11	24±0.25
E. coli	9±0.13	12±0.18	33±0.30
Klebsiella pneumoniae	8±0.10	11±0.14	37±0.33
Salmonella typhi	-	-	36±0.29
Pseudomonas aeruginosa	-	-	27±0.16
Bacillus cereus	-	-	25±0.15
Sarcina lutea	-	-	28±0..28
Shigella dysenteriae	-	-	31±0.22
Steptococcus haemolytia	-	-	23±0.17

All values represent means ± S.D. n = 3. $P < 0.05$ indicates values significantly different, "-" indicates No activity

Table 4. *In vitro* antifungal activities of ethanol extract of leaves and Nystatin**.**

Test fungi	Ethanol extract of leaves		Nystatin (100µg/disc)
	(400µg/ disc)	(500µg/ disc)	
Penicilium sp	-	-	25±0.13
Aspergillus niger	8±0.11	10±0.12	24±0.15
Saccharomyces cerevisiae	10±0.14	12±0.15	26±0.17
Candida albicans	9±0.10	11±0.13	25±0.16
Sclerotia rolfsii	-	-	24±0.10
Rizopus sp	-	-	23±0.11

Thus, they can be used in the treatment of infectious diseases caused by these microbes. The type and level of biological activity exhibited by any plant material depends on many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions, and post-harvest processing. Surprisingly, extracts of *Alocasia indica stems* had almost the same level of antimicrobial activity as leaves, indicating the equal importance of these parts to be considered for antimicrobial properties.

Table 5. *In vitro* antifungal activities of ethanol extracts of stem and Nystatin.

Test fungi	Ethanol extract of stem		Nystatin (100µg/disc)
	(400µg/disc)	(500µg/ disc)	
Penicilium sp	-	-	25±0.13
Aspergillus niger	8±0.10	10±0.13	24±0.15
Candida albicans	10±0.09	13±0.12	26±0.17
Saccharomyces cerevisiae	8±0.11	9±0.14	25±0.16
Sclerotia rolfsii	-	-	24±0.10
Rizopus sp	-	-	23±0.11

All values represent means ± S.D. n = 3. $P < 0.05$ indicates values significantly different, "-" indicates No activity.

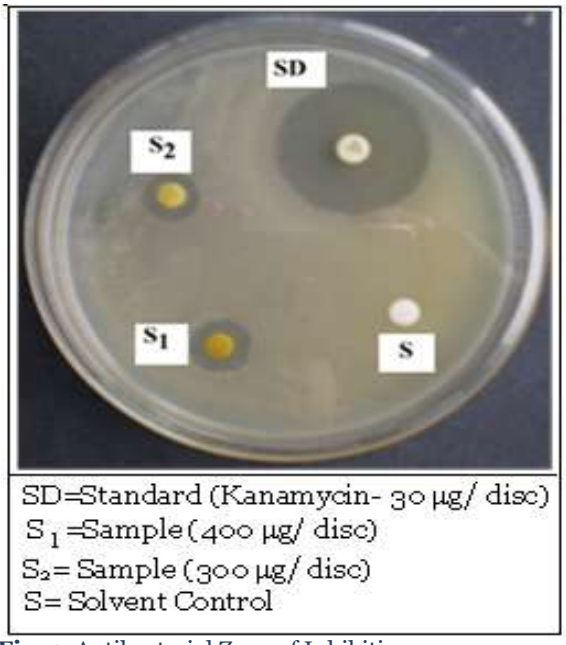

Fig. 1. Antibacterial Zone of Inhibition.

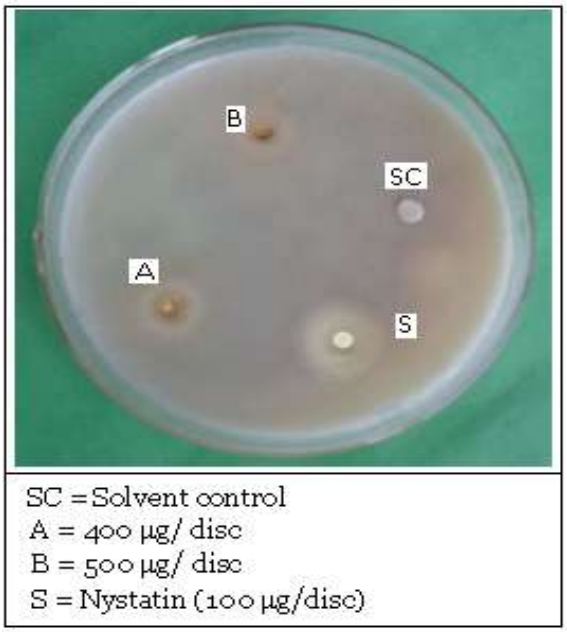

Fig. 2. Antifungal Zone of Inhibition.

Conclusion

The present study suggests that *Alocasia indica* extracts presumably possess compound(s) with antimicrobial properties against gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*), gram-negative (*Escherichia coli and Klebsiella pneumonia*) bacteria, and fungus (*Aspergillus niger, Saccharomyces cerevisiae and Candida albicans)*. Purification of bioactive compounds can, thus, be further studied for the development of novel antimicrobial therapies.

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