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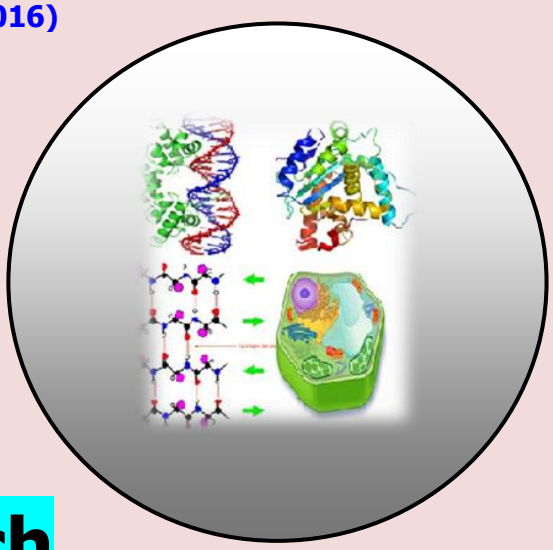

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RESEARCH PAPER

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Antimicrobial Activity of Leaves Extract of *Aristolochia bracteolata* and *Brassica oleracea*

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ABSTRACT

The antimicrobial activities of Brassica oleracea and Aristolochia bracteolata leaves extract against Escherichia coli and Staphylococcus aureus specie of bacteria and fungi of Candida albicans were examined in the present study. The activity was assessed by the formation of inhibition zones. The inhibition zones formed by positive control of antibiotic (Bacteria as Chloramphenicol, fungil as Fluconazole) discs were used to compare. The stronger activity of Brassica oleracea and Aristolochia bracteolata leaves were observed in E. Coli and near to the standard as compared with other microbes. Among the two plants, Aristolochia bracteolata leaves possess considerable antimicrobial activity than Brassica oleracea leaves.

Keywords: Antimicrobial activities, Brassica oleracea, Aristolochia bracteolate, Antibiotic and Candida albicans.

INTRODUCTION

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali *et al*., 1998 and Barbour *et al*., 2004). The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Plant materials are main sources to treat serious diseases in the world (Yasunaka *et al*., 2005). So far a systematic study of plants to determine their antimicrobial active compounds is a comparatively new field. Plant and plant products play a wide range of antimicrobial properties. Hence, In the present study investigates the antimicrobial activity of leaves of *Aristolochia bracteolata* and *Brassica oleracea*

MATERIALS AND METHODS

Plant materials and Preparation of alcoholic extract

The fully mature *Brassica oleracea and Aristolochia bracteolata* leaves were collected in October 2015 from the weekly market, Muthur, Tirupur District, South Tamil Nadu, India. The healthy fresh leaves collected thoroughly washed with distilled water and kept in shade at room temperature about one week to dry. Then made into powder with the help of a Pulveriser and sieved. The Dried powdered samples were Soxhlet extracted with ethanol until the solvent was colorless. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The dried extracts were kept in the refrigerator at 4°C until use.

Antimicrobial activity

Antibiogram was done by disc diffusion method (NCCLS, 1993 and Awoyinka *et al.,* 2007) using plant extract. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli* and *Staphylococcus aureus* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37oC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

RESULTS AND DISSCUSSION

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim, 1997; Towers *et al*., 2001 and Koshy *et al*., 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Murray, 1992; Madunagu *et al*., 2001; Koshy *et al*., 2009 and Senthilkumar and Reetha, 2009) Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance among microorganism and to continue studies to develop new antibiotic and immune modulating compounds with diverse chemical structures and novel mechanisms of action, either synthetic or natural to control pathogenic microorganisms because there has also been an alarming increase in the incidence of new and re-emerging infectious diseases (Ikenebomeh and Metitiri, 1988; Rojas *et al*., 2003 and Geyid *et al*., 2005). This situation forced scientists to search for new antimicrobial substances with plant origin. Plant extract of *Aristolochia bracteolata* and *Brassica oleracea* were screened against *Escherichia coli* and *Staphylococcusaureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Aristolochia bracteolata* and *Brassica oleracea* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones. The inhibitory activities in culture media of the *Aristolochia bracteolata* and *Brassica oleracea* were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

Antimicrobial activity of *Brassica oleracea* leaves extract

The *in vitro* antimicrobial activity of the *Brassica oleracea* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Figure 1. The inhibitory activities in culture media of the *Brassica oleracea* leaves reported in Table 1 were comparable with standard antimicrobiotic viz. chloramphenicol and Fluconazole. *Escherichia coli* and *Staphylococcus aureus*, which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Brassica oleracea* leaves extract was 2.90±0.20mm for 50µl, 5.70±0.39mm for 100µl, 7.80±0.54mm for 150µl for *Escherichia coli*. The mean inhibition zone of *Brassica oleracea* leaves extract was 1.90±0.13mm for 50µl, 4.30±0.30mm for 100µl, 6.50±0.75mm for 150 µl for *Staphylococcus aureus*. The mean inhibition zone for standard is 10.50±0.73and 10.70±0.74 for *Escherichia coli* and *Staphylococcus aureus.*

In addition, *Candida albicans* was strongly influenced with a mean inhibition zone of 2.50±0.17mm for 50µl, 4.80±0.33mm for 100µl, 7.10±0.49mm for 150µl by *Brassica oleracea* leaves extract and 10.40±0.72 for standard. This result is very interesting because *Candida albicans* has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immunocompromised patients (Casalinuovo *et al*., 2004 and Redding et *al*., 1993).

The results showed that the antimicrobial activity was directly proportional to the concentration of *Brassica oleracea* leaves extract. The *Brassica oleracea* leaves extract shows highest antimicrobial activity was observed against *Staphylococcus aureus,* when compared with *Escherichia coli and Candida albicans.* The high doses (150µl) of *Brassica oleracea* leaves extract possess similar activity to standard drug as chloramphenicol for bacteria and Fluconazole for fungi.

Table 1. Antimicrobial activity of *Brassica oleracea* leaves extract.

Microorganisms	(50µl)	(100µl)	(150µl)	Standard (30µl)	Control (solvent) (30µl)
Bacteria					
Escherichia coli (mm)	2.90±0.20	5.70±0.39	7.80±0.54	10.50±0.73	0
Staphylococcus aureus (mm)	1.90±0.13	4.30±0.30	6.50±0.75	10.70±0.74	0
Fungal					
Candida albicans (mm)	2.50±0.17	4.80±0.33	7.10±0.49	10.40±0.72	0

Values were expressed as Mean ± SD

Bacterial standard - Chloramphenicol

Fungal standard - Fluconazole

Escherichia coli ***Staphylococcus aureus*** ***Candida albicans***

Figure 1. Antimicrobial activity of *Brassica oleracea* leaves extract.

Antimicrobial activity of *Aristolochia bracteolata* leaves extract

The *in vitro* antimicrobial activity of the *Aristolochia bracteolata* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Figure2.The inhibitory activities in culture media of the *Aristolochia bracteolata* leaves reported in Table 2 were comparable with standard antimicrobiotic viz. chloramphenicol and Fluconazole.

Escherichia coli and *Staphylococcus aureus*, which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Aristolochia bracteolata* leaves extract was 3.10±0.21mm for 50µl, 6.30±0.44 mm for 100µl, 8.90±0.62 mm for 150µl for *E. coli*. The mean inhibition zone of *Aristolochia bracteolata* leaves extract was 2.00±0.14mm for 50µl, 5.10±0.35 mm for 100µl, 7.70±0.59 mm for 150µl for *Staphylococcus aureus*. The mean inhibition zone for standard is. 11.40±0.79 and 10.80±0.75 for *Escherichia coli* and *Staphylococcus aureus.*

In addition, *Candida albicans* was strongly influenced with a mean inhibition zone of 2.60±0.18mm for 50µl, 5.70±0.39mm for 100µl, 8.50±0.59mm for 150µl by *Aristolochia bracteolata* leaves extract and 11.10±0.77mm for standard. This result is very interesting because *Candida albicans* has been the most extensively studied pathogen in antifungal resistance because of their morbidity and mortality associated with infections in immunocompromised patients (Casalinuovo *et al*., 2004 and Redding et *al*., 1993).

The results showed that the antimicrobial activity was directly proportional to the concentration of *Aristolochia bracteolata* leaves extract. The *Aristolochia bracteolata* leaves extract shows highest antimicrobial activity was observed against *Staphylococcus aureus,* when compared with *Escherichia coli and Candida albicans.* The high doses (150µl) of *Aristolochia bracteolata* leaves extract possess similar activity to standard drug as chloramphenicol for bacteria and Fluconazole for fungi.

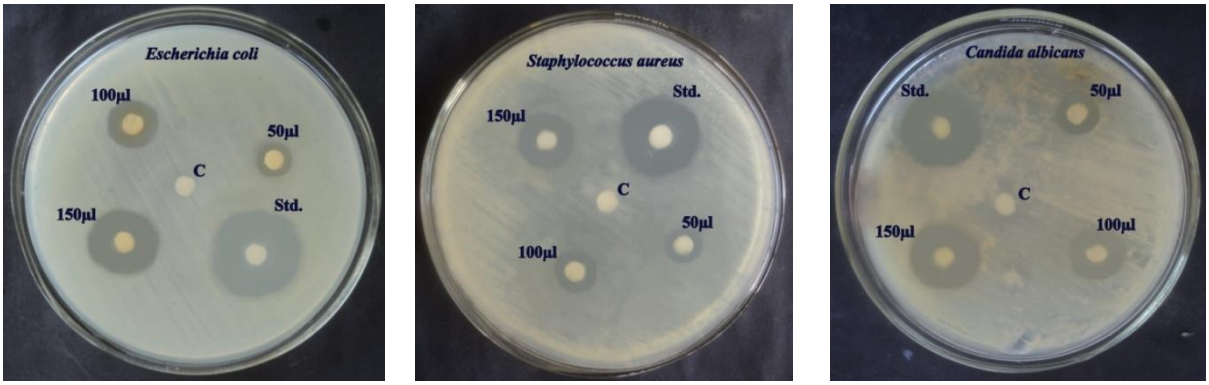
Table 2 shows Antimicrobial activity *Aristolochia bracteolate.*

Microorganisms	(50µl)	(100µl)	(150µl)		Standard (30µl)	Control (solvent) (30µl)
	Bacteria					
Escherichia col (mm)	3.10±0.21	6.30±0.44	8.90±0.62		11.40±0.79	0
Staphylococcus aureus (mm)	2.00±0.14	5.10±0.35	7.70±0.59		10.80±0.75	0
	Fungal					
Candida albicans (mm)	2.60±0.18	5.70±0.39	8.50±0.59		11.10±0.77	0

Values were expressed as Mean ± SD

Bacterial standard : Chloramphenicol ; Fungal standard : Fluconazole

Control : Distilled water



Escherichia coli ***Staphylococcus aureus*** ***Candida albicans***

Fig 2 shows Antimicrobial activity *Aristolochia bracteolate.*

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Paz *et al*., 1995; Vlietinck *et al*., 1995; Kudi *et al*., 1999 and Palambo and Semple, 2001). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Onwuliri and Dawang, 2006 and Mahesh and Sathish, 2008).

CONCLUSION

The extract of *Brassica oleracea* and *Aristolochia bracteolata* leaves were screened for *in vitro* antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and Fungal *Candida albicans* at different concentrations (50, 100 and 150µg/ml). The antimicrobial activity was found to be dose dependent and may be attributed to the presence of bioflavonoids content in *Brassica oleracea and Aristolochia bracteolata* leaves. Among the two plants, *Aristolochia bracteolata* leaves possess considerable antimicrobial activity than *Brassica oleracea* leaves.

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