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Research Article

A COMPARATIVE STUDY OF ANTIMICROBIAL EFFECTS OF *CLITORIS TERNATEA* AND *ANACARDIUM OCCIDENTALE* LEAF EXTRACTS AGAINST THE SELECTED MICROBES

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ABSTRACT

The aim of the present study was to investigate a comparative study of antimicrobial efficacy of *Clitoris ternatea* and *Anacardium occidentale* leaf extract against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and *Bacillus subtilis* (Gram-positive), and *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Salmonella typhi* (Gram-negative) microbial strains. Ethanol extracts of both the leaf were evaluated against the experimental microbial strains using the well diffusion and agar disc diffusion methods. The present investigation showed that some microbial strains were more sensitive to *Clitoris ternatea* as compared to *Anacardium occidentale* and vice versa. But on the whole the *Clitoris ternatea* was found to be more effective than *Anacardium occidentale*. It is concluded that both the extracts showed antimicrobial activity due to the presence of bioactive phytochemicals present in them.

Keywords: *Clitoris ternatea*, *Anacardium occidentale*, Antimicrobial activity, Zone of inhibition well diffusion.

INTRODUCTION

In the recent past decades, the antimicrobial activities of plants have been investigated by a number of researchers worldwide and they have enormous therapeutic potentials for incurable diseases (Choudhary *et al*., 1997). The bioactive compounds of plants have a wide range of biological functions including antimicrobial, antioxidant, anti-inflammatory activities, etc. The development of pharmaceutical drugs derived from plants has allowed the treatment of diseases on a large effective scale. The consumption of antimicrobial drugs may result in unfavorable and untold side effects. Accumulate various secondary metabolites including alkaloids, glycosides, polyphenol, etc. (Sowjanya, 2015). They are effective in the treatment of infectious and incurable diseases with minimal side effects and are associated with synthetic antimicrobials. The usage of drugs has a negative effect on the human body and led to the concurrent resistant bacteria that have become a global issue of public health. Researchers are engrossed in developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, greater attention has been paid to antimicrobial activity, screening and evaluating methods.

Several bioassays such as disc diffusion, well diffusion, and broth or agar dilution are well known and commonly used. To promote the proper use and to determine the potential of plants as a source of new drugs, it is essential to understand their properties, safety, and efficacy as the first and foremost priority. *Clitoris ternatea* Linn (Fabaceae) is a twining climber found abundantly in tropical and subtropical regions, mostly as terrestrial mesophytes which are commonly used in Indian folk medicine. It is popularly known as a "Butterfly pea" in western countries and as Aparajita in the traditional Ayurvedic system of medicine. It is employed in the traditional system of medicine against different diseases such as cathartic, purgative, demulcent, emetic, and anti-inflammatory (Solanki & Jain, 2011). Various parts of it have been reported to have nootropic, anxiolytic, tranquilizing, analgesic, antipyretic, antimicrobial, and

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immunomodulatory activities. It is reported to have brain ionic activity and is popularly known as Shankhapushpi in India (Upadhya & Kumbhojkar, 1993). Therefore, it is worthwhile to evaluate its antimicrobial effects on the selected microorganisms.

Anacardium occidentale Linn (Anacardiaceae) a native of Brazil, having great economic and medicinal value, which is composed of about 74 genera and 600 species. The leaves, stem, and bark extract are utilized widely for the treatment of diarrhea, dysentery, and colonic pain (Dhake, 2009). It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory, and anti-ulcerogenic (Akinpelu & Ojewole, 2001). The leaves are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough intestinal colic, Leishmaniasis, and Syphilis related skin disorders. The nut oil is used topically as an antifungal and for healing cracked heels. Fruit juices from the cashew apple and leaves are known to have antimicrobial, anti-inflammatory, astringent, diuretic, hypoglycemic, and other medicinal properties. The leaves of which contain pharmacological rich components such as alkaloids, essential oils, tannins, saponins, and cardenolides, and are known to have cosmetic, antimicrobial, and antioxidant properties (Sadiq, 2009). The plant *Anacardium occidentale* is very useful, as found by the above-mentioned reports and there is a need to find out more about the potentiality of this plant as an antimicrobial agent against the selected microbial strains.

MATERIALS AND METHODS

Plant material

The plants *Clitoris ternatea* and *Anacardium occidentale* collected from the outskirts of Hyderabad district Telangana state. The plant specimens were identified and the voucher specimens deposited in the department of Botany. The leaves, washed thoroughly many times with running water and then finally sterile water. They are dried in the shade at room temperature for more than three weeks. Dried leaves were made into coarse powder and sieved for crude extraction with ethanol as a solvent.

Extraction

The fine powder was made by pulverizing with clean pestle and mortar and packed in a sealed plastic bottle until extraction. About 20 grams of dried powder were mixed with 350 ml of ethanol (5.7) and extracted by the hot percolated method by the Soxhlet apparatus. The extracts were transferred into a 500 ml each conical flask, tightly closed, and left for 24 hours at room temperature. The collected extracts, concentrated at 40^0C under reduced pressure rotary evaporator to remove as much of the ethanol as possible. The dried extract was then mixed with dimethylsulfoxide (DMSO). At the end of the procedure, the extracts were placed in a sterile glass flask, sealed with paraffin, and then stored at 4^0C until further use.

Microbial strains used

Staphylococcus aureus ATCC 25923, *Streptococcus pneumonia* ATCC 29212, *Mycobacterium tuberculosis* MTCC 12556, and *Bacillus subtilis* UC 564 (Gram-positive), and *Pseudomonas aeruginosa* ATCC 25619, *Klebsiella pneumonia* MTCC109, *Proteus vulgaris* UC 726, and *Salmonella typhi* ATCC12600 (Gram-negative) microbial strains were procured from the Microbial Culture Collection, India.

Antibiotics used

Gentamicin (HIMEDIA Gentamicin GEN 10 susceptibility test disc 10µg/disc and Chloramphenicol (HIMEDIA Chloramphenicol c30 susceptibility test disc 10µg/disc, were used as reference standards for the antibacterial study.

Antimicrobial assay

The ethanolic extracts of *Clitoris ternatea* and *Anacardium occidentale* were screened for their in vitro antimicrobial activity in comparison with experimental standard antibiotics by the well diffusion method (Sunmathi *et al*., 2016). For this activity, a nutrient agar obtained from Himedia Laboratories Ltd Mumbai. The nutrient agar plates were prepared by pouring 15-20 ml of molten media into sterile Petri plates, they were allowed to solidify. 24 hours of bacterial culture spread throughout the medium uniformly with a sterile cotton swab. With the help of a sterile cork, borer wells were made with a diameter of 6mm at equidistant in the medium. The extracts (10, 20, 30, 40mg/ml) were loaded into the wells, and the compound was allowed to diffuse for 2 hours and the plates were kept in incubation at 37^0C for 24 hours. The sensitivity of the organisms was determined by measuring the average diameter of triplicates of the zone of inhibition in mm with the help of a transparent ruler (Rios *et al*., 1988). The zone of inhibition of the extracts was also performed by the agar disc diffusion following Kirby Bauer method at the concentration 10,20,30,40 mg/ml of the extracts in DMSO (Bauer *et al*., 1966). Gentamicin and Chloramphenicol discs were used as reference control and pure solvent control was also maintained throughout the experiment.

RESULTS AND DISCUSSION

Table (1) reveals the antimicrobial activity of ethanolic extracts of C*litoris ternatea* and *Anacardium occidentale.* The mean values of the zone of inhibition of different concentrations against the test organisms were compared with the standard drugs. The results of the zone of inhibition manifest that the extract possesses antimicrobial activity in a concentration-dependent manner. The gram-positive microorganisms were observed to be more susceptible than gram-negative bacteria. There were significant differences in the antimicrobial effects of both the extracts. Figure 1 and 2 depicts the comparative study of extracts between *Clitoris ternatea* and *Anacardium occidentale* showed that the antimicrobial activity is more pronounced in *Clitoris ternatea* than *Anacardium*

occidentale. The major cause of morbidity and mortality, are infectious diseases worldwide. The number of multidrug-resistant microbial strains and the appearance of strains that reduced susceptibility to antibiotics are continuously increasing. Such an increase has been attributed to the indiscriminate use of broad-spectrum antibiotics in various infectious diseases. This situation provided the impetus to the search for new antimicrobial substances from the extracts of various medicinal plants to combat against antimicrobial resistance and for therapeutic treatments. India has a rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha, Unani besides folklore practices. Medicinal plants considered as clinically effective and safer alternatives to synthetic antibiotics. The microorganisms employing new mechanisms to survive (Sekyere & Asante, 2018). From the earlier studies, it is obvious that most of the phytochemicals differ significantly in their structures and properties. These differences may be attributed to the differences in the cell wall constituents of bacteria which vary among the gram-positive and gram-negative bacteria (Yao, 1995). Extracts showed more pronounced activity to gram-positive than gram-negative. The reason is attributed to the sensitivity difference between gram-positive and gram-negative bacteria. This is mainly due to the morphological constitution between these microorganisms. These observations are more likely to be the fact that an outer membrane in gram-negative bacteria, which acts as a barrier to many environmental substances including antibiotics. The significant differences in the antimicrobial effects of both the extracts may be due to the molecular size of the phytochemical compound present in the extracts.

Table 1. Results of antimicrobial activity.

Micro-organisms	(c)Leaf Extracts (mg/ml)	(a)Zone of Inhibiton (mm)					(b) Standards	
		10	20	30	40	Ethanol	Chloramphenicol	Gentamicin
	Gram+^{ve}						10µg/ml	10µg/ml
S aureus	*C ternatea*	0.7	0.8	1	1.2	0.7	1.9	1.8
	A occidentale	0.6	0.7	0.9	0.9	0.6		
S pneumoniae	*C ternatea*	0.8	0.8	0.9	1.3	0.6	1.8	2
	A occidentale	0.7	0.7	0.8	0.9	0.6		
M tuberculosis	*C ternatea*	0.9	1	1.1	1.1	0.7	2	1.9
	A occidentale	0.8	0.8	0.9	1	0.6		
B subtilis	*C ternatea*	1	1.1	1.2	1.2	0.8	1.9	2.1
	A occidentale	0.8	0.9	0.9	1	0.7		
	Gram -^{ve}							
P aeruginosa	*C ternatea*	0.8	0.8	0.9	1	0.7	1.8	1.9
	A occidentale	0.7	0.7	0.8	0.9	0.6		
K pneumoniae	*C ternatea*	0.7	0.8	0.8	0.9	0.7	1.9	2.1
	A occidentale	0.6	0.7	0.7	0.8	0.6		
P vulgaris	*C ternatea*	0.7	0.7	0.8	1	0.8	2	2
	A occidentale	0.7	0.7	0.8	0.9	0.7		
S typhi	*C ternatea*	0.8	0.8	0.9	1	0.8	2.1	2
	A occidentale	0.7	0.7	0.8	0.8	0.7		

CONCLUSION

Based in the preliminary study, the authors concluded as follows: The leaf extracts of *Clitoris ternatea* and *Anacardium occidentale* has a significant antimicrobial activity against the selected microbial strains which may be due to the presence of active phytochemicals in the extracts. From the present study, it is evident the susceptibility to the extracts were more pronounced in gram-positive as compared to gram-negative organisms. This is of the opinion of authors which may be due to the structural configuration of an outer membrane in gram-negative bacteria which acts as a barrier to many phytochemicals present in the extract and the different sizes of organic phytochemical molecules. The antimicrobial activity observed more pronounced in *Clitoris ternatea* than *Anacardium occidentale* this may be due to an absence of more bioactive molecules in the extract of *Anacardium occidentale.* Further, research has to be conducted at the cellular level of the organisms for a wider understanding of the inhibition mechanism. Identification and isolation of specific antimicrobial compounds in their purified forms can pave better ways to control drug-resistant strains of microbes. The results were in accordance with the earlier researchers.

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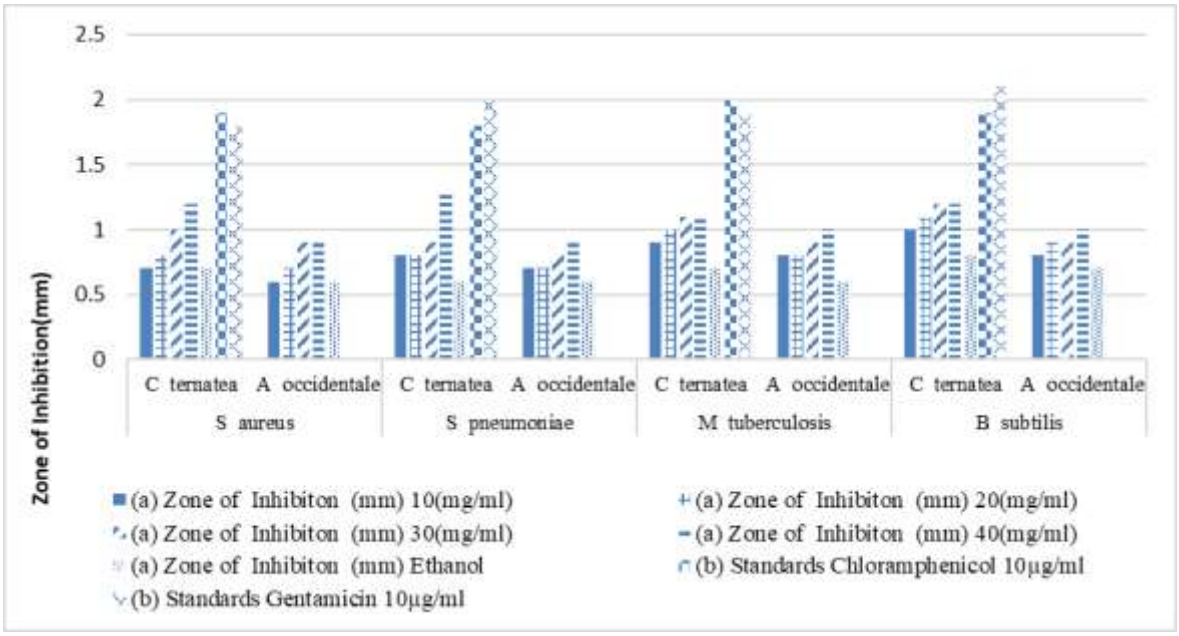

Figure 1. Antimicrobial activity of leaf extracts concentration against gram-positive micro-organisms.

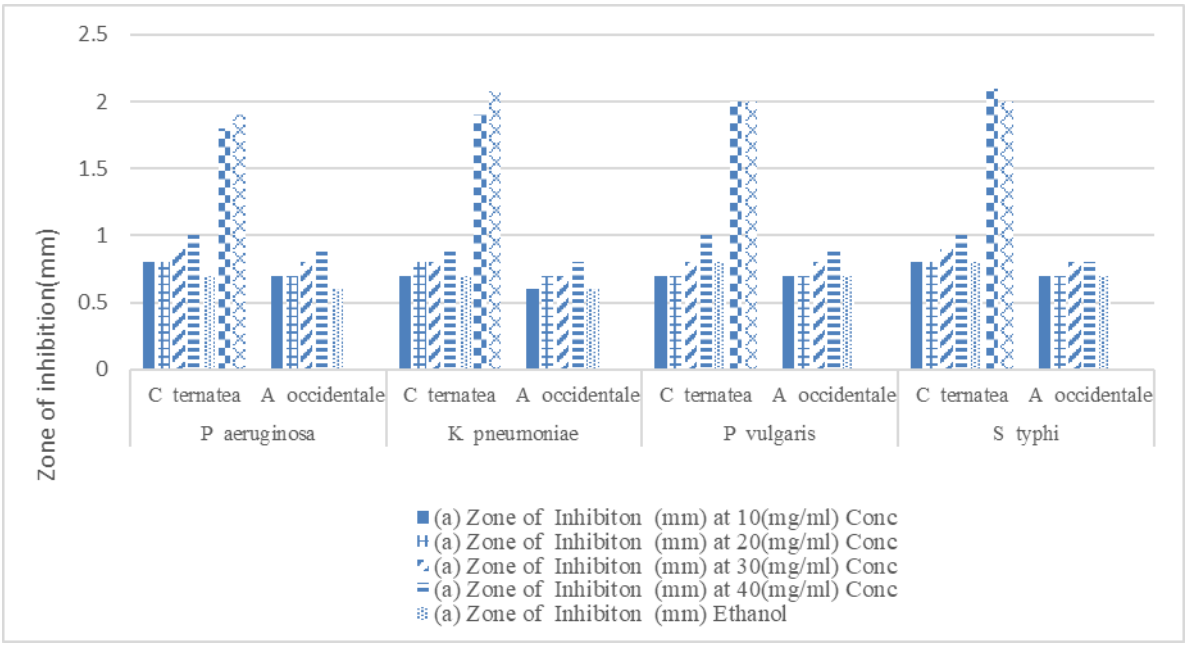

Figure 2. Antimicrobial activity of leaf extracts concentration against gram-negative micro-organisms.

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