ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 4, Issue, 8, pp. 1715-1718, August, 2014

Full Length Review Article

ANTIMICROBIAL ACTIVITY TEST AGAINST ISOLATED BACTERIA

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ARTICLE INFO

Article History:
Received 19th May, 2014
Received in revised form
20th June, 2014
Accepted 18th July, 2014
Published online 31st August, 2014

Key words:
Enterobacter aerogen,
Ocimum sanctum plant extract

ABSTRACT

In the present study aimed to evaluate the growth inhibitory effect of *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* leaves extracts on *Esherichia coli, Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen*. Aquous plant extracts were tested against 4 bacteria. Gel diffusion method, were used in this investigation. The antibacterial activity of *Azadirachta indica* plant extract was exhibited maximum zone of inhibition against *Esherichia coli* 14 mm (Mean value in Dia.), when compared with other medicinal plant extracts. *Ficus religiosa* plant extract was zone of inhibition 13 mm (Mean value in Dia.) against *Esherichia coli* and 11 mm (Mean value in Dia.) against *Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen.* The *Enterobacter aerogen was* highly inhibition 8 mm (Mean value in Dia.) against *Ocimum sanctum* plant extract. At the same time *Hibiscus rosasinensis* plant extract was highly sensitive 13 mm (Mean value in Dia.) against *Staphylococcus aureus*. The present observation, the *Azadirachta indica* and *Ficus religiosa* medicinal plant extracts are having best control of antibacterial activity.

INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs. Natural photochemical are known to contain substances that can be used for therapeutic purposes or as precursor for the synthesis of novel drugs. Nearly 50% modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry. Plants remain the most common source of antimicrobial agents (Bibitha *et al.,* 2002 and Maghrani *et al.,* 2005). Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast (Hulin *et al.,* 1998). Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases (Perumal Samy and Ignacimuthu, 2000). There is an essential need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Therefore, search for medicinal plants with potential secondary metabolites have been extensively investigated as a source of medicinal agents. Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry.

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Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs (De *et al*., 2002 and Ncube *et al.,* 2008). Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases (Natarajan *et al.,* 2003 and Shah *et al.,* 2006). Plant based products extracts are cheaper alternatives to the development of synthetic drugs. The plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness. So returns to natural substances are an absolute need of our time (Swayamjot *et al.,* 2005; Kumar *et al.*, 2007). In the last few years a number of studies have been conducted to verify the effectiveness of plant extracts against bacterial infections (Prashanth *et al.,* 2006; Ung *et al.*, 2010). *Azadirachta Indica* belongs to the family Meliaceae, commonly known as neem. It is used in traditional medicine as a source of many therapeutic agents. *A. indica* (leaf, bark and seed) are known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses (Biswas *et al.,* 2002).

Different parts of neem (leaf, bark and seed oil) have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial, antimutagenic, anticarcinogenic, antiinflammatory, antihyperglycaemic, antiulcer and antidiabetic properties (Talwar *et al*., 1997). The biological activities are attributed to the presence of many bioactive compounds in different parts. The present study evaluated the individual and in combination growth inhibitory effect of 4 medicinal plant extracts against 4 bacteria.

MATERIALS AND METHODS

Plants were collected between the month of June and July 2015 in the Chidambaram area Tamil Nadu, India. Plant leaves were initially dried in an airconditioned, dehumidified room, then further dried in an oven at ca. 40^{o}C for a total of seven days, and then finally ground to a fine powder. Antimicrobial activity test was determined by the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1996). The Antimicrobial activity was tested against isolated 4 bacterial strains. The medicinal plants of *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* leaves extract were tested by the disc diffusion method. The extracts were prepared by reconstituting with aquous.

The test microorganisms were seeded into respective Mueller - Hinton agar medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in Mueller - Hinton agar broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates., *Esherichia coli, Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen* were used for antibacterial test. Erythromycin (10 µg mlG1) used as positive control. The antibacterial assay plates were incubated at 37°C for 24h. After incubation, the results were observed and measured the diameter of inhibition zone (mm) around the each well.

RESULTS AND DISCUSSION

The antibacterial activity of *Azadirachta indica* plant extract was exhibited maximum zone of inhibition against *Esherichia coli* 14 mm (Mean value in Dia.), when compared with other medicinal plant extracts. *Ficus religiosa* plant extract was zone of inhibition 12 mm (Mean value in Dia.) against *Esherichia coli* and 11 mm (Mean value in Dia.) against *Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen.* The *Enterobacter aerogen was* highly inhibition 8 mm (Mean value in Dia.) against *Ocimum sanctum* plant extract.

Table 1. Statistical analysis of mean and standard deviation in Antimicrobial activity test in medicinal plants extract against isolated bacteria

Name of the Species	Zone of inhibition (dia in mm)			
	S1. *Hibiscus rosasinensis*	S2. *Azadirachta indica*	S3. *Ficus religiosa*	S4. *Ocimum sanctum*
Salmonella typhii	10.33 ± 0.58	10.33 ± 1.15	10.33 ± 2.33	9.67 ± 2.08
Staphylococcus aureus	13.00 ± 3.00	10.67 ± 1.53	10.00 ± 3.60	10.00 ± 3.60
E-coli	11.67 ± 2.52	14.33 ± 2.52	12.00 ± 2.00	11.67 ± 2.52
Enterobacter aerogen	9.33 ± 0.58	10.67 ± 0.58	10.00 ± 4.58	8.67 ± 3.51

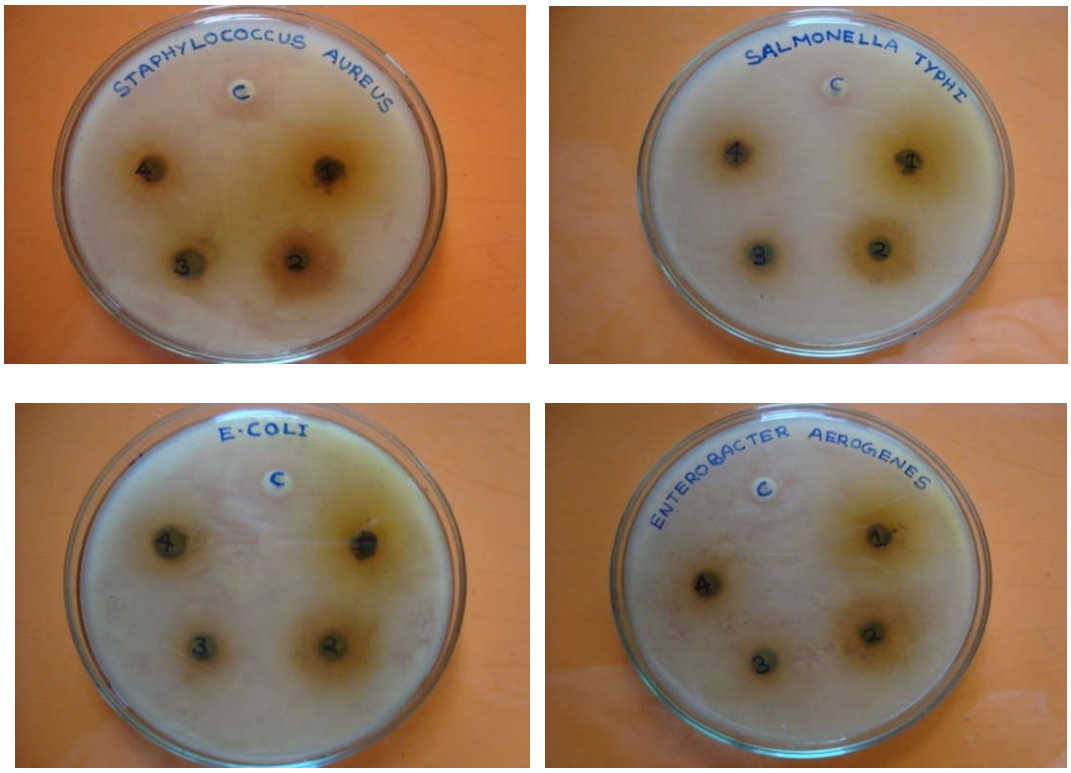

Plate 1. Antimicrobial activity test against isolated bacteria from infected Fresh water carp *Catla catla*

At the same time *Hibiscus rosasinensis* plant extract was highly sensitive 13 mm (Mean value in Dia.) against *Staphylococcus aureus*. The *Azadirachta indica* and *Ficus religiosa* medicinal plant extracts are having best control of antibacterial activity. Antimicrobial acitivity test against five different medicinal plants (leaves) extracts such as *Hibiscus rosasinensis, Azadirachta indica, Ficus religiosa and Ocimum sanctum* (Leaves) were tested against some pathogenic bacteria such as *Esherichia coli, Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen.* The antibacterial activity of *Azadirachta indica* plant extract was exhibited maximum zone of inhibition against *Esherichia coli*, when compared with other medicinal plant extracts. According to Abalaka *et al.* (2012) were studied the antibacterial effects of *A. indica* on the test organisms revealed that *P. aeruginosa* showed the highest zones of inhibition (mm) followed by *S. aureus* while *E. coli* had the least zone of inhibition (mm) at various extract concentrations of 500mg/ml, 50mg/ml and 5mg/ml. The extracts of *A. indica* showed a higher value of zones of inhibition on the tested organisms. In a similar study hexane and aqueous extract of *Azadirachta indica*, inhibited *Es-cherichia coli*, *P. aeruginosa*, *S. pyogenes* and *S.aureus* (El-Mahmood *et al.*, 2010).

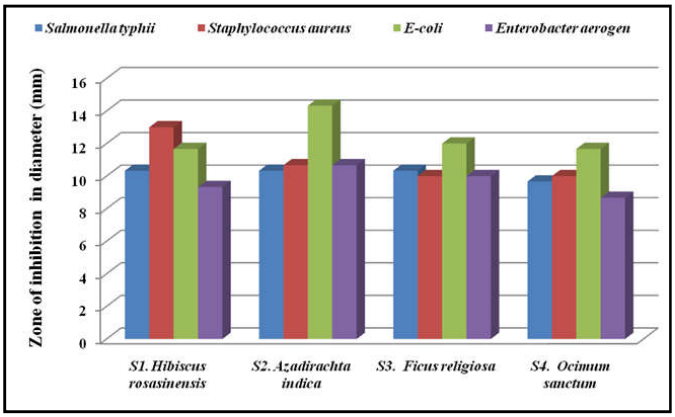

Fig. 1. Antimicrobial activity test against isolated bacteria from infected carp *Catla catla*

Methanol extract of the leaves of *Azadirachta indica* exhibited pronounced activity (28mm) against *Bacillus subtilis*, high activity (18mm) against the Gram positive *Staphylococcus aureus* and the Gram-negative organisms *Proteus vulgaris* (18 mm) and *Salmonella typhi* (20 mm), low activity (14mm) against *Pseudomonas aeruginosa* and inactive against *Escherichia coli* were reported by Nishant Rai *et al.*, (2011). The methanol extract of *Hibiscus* has got phytomedical property it may be due to the nature of biologically active compounds present in *hibiscus* whose activity are enhanced in the presence on methanol and also methanol has an stronger extraction capacity which could have produced greater number of active constituents responsible for antibacterial activity (Barker *et.al.*, 1995). Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The methanol, ethanol, ethyl acetate and chloroform and aqueous extracts of the leaves of *A. aspera, A. parviflora, A. indica,* and *C. odorata* were subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria *E. coli* and *S. aureus.* High activity against the Gram-positive organism *E. coli* was found in aqueous and all tested solvent extracts of *A. indica.* In case of human pathogenic *S. aureus*, maximum inhibition of 8 mm was obtained in aqueous extracts of *A. indica.* Similar observations were reported from nimbolide isolated from neem seed oil showing antibacterial activity against *S. aureus* and Staphylococcus coagulase (Nazma and Rao, 1977). In the present investigation, the antibacterial activity of *Azadirachta indica* plant extract was exhibited maximum zone of inhibition against *Esherichia coli* 14 mm (Mean value in Dia.), when compared with other medicinal plant extracts. *Ficus religiosa* plant extract was zone of inhibition 13 mm (Mean value in Dia.) against *Esherichia coli* and 11 mm (Mean value in Dia.) against *Salmonella typhii, Staphylococcus aureus* and *Enterobacter aerogen.* The *Azadirachta indica* and *Ficus religiosa* medicinal plant extracts were exhibited higher antibacterial activity.

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