INDIAN JOURNAL OF APPLIED MICROBIOLOGY

Volume 15 Number 1 January-June 2012, pp. 49-55.

In vitro studies on Antibacterial activities of bark extract of *Terminalia arjuna* (roxb.) against selected Pathogenic bacteria

Arunagirinathan N*, V.Santhosh, N.Vijaykanth and S.Balakrishnan

PG and Research Department of Microbiology & Biotechnology, Presidency College (Aut.), Chennai–600 005, India.

Abstract: The antibacterial effect of aqueous, acetone and ethanol extracts of bark of Arjuna on four bacteria namely, *Staphylococcus aureus, Escherichia coli, Proteus sp.*and *Pseudomonas aeruginosa* were determined by Agar well diffusion, Disc diffusion and Microdilution methods. The results revealed that acetone was the best solvent in extracting the constituents conferring antimicrobial properties of bark of Arjuna followed by ethanol and aqueous extracts. In the Agar well diffusion method, the zones of inhibition caused by acetone extract at 0.5, 1.0, 1.5 and 2.0 mg concentrations respectively were 14, 16, 18.5 and 20 mm (against *S. aureus*); 13.5, 14.5, 16 and 17 mm (against *E. coli*) 12.5, 15, 16.5 and 17.5 mm (against *Proteus spp*.) and 14, 16, 18.5 and 20 mm (against *P. aeruginosa*) were. In the Disc Diffusion method the zones of inhibition caused by acetone extract at 0.5, 1.0, 1.5 and 2.0 mg concentrations respectively were 13, 16.5, 19.5 and 21.5 mm (against *S.aureus*) 13, 14, 15.5 and 17.5 mm (against *E. coli*) 13, 15, 16.5 and 19.5 mm (against *Proteus spp.*) and 11, 13.5, 16 and 18 mm (against *P. aeruginosa*). Acetone extract showed varying degree of bactericidal activity against *S. aureus* (at 25µg/ml), *E. coli* and *Proteus sp.*(each at 12.5µg/ml) and against *P. aeruginosa* (at 6.25µg/ml). The Arjuna plant product showed significant bactericidal activity against all the 4 clinical strains as the inhibition obtained by the acetone extract ranged from 6.25 - 25 µg/ml. These findings therefore established a good support for the use of *Terminalia arjuna* in traditional medicine.

Key words: *Terminalia arjuna*, Acetone extract, Antibacterial activity, MIC

*Author for Correspondence. E-mail: n_arunagiri@yahoo.co.in

Introduction

Medicinal plants have been a major source of therapeutic agents since ancient times for treating human diseases. Nowadays, there is several manifold increase of medicinal plant based industries, estimated at a rate of 7-15% annually, due to the growing interest on the use of medicinal plants around the world which are growing [1]. Since 1980, the World Health Organization has been encouraging the scientific community to identify and develop the traditional medicine and phytotherapy. The main Indian traditional systems of medicine namely Ayurveda and Siddha are primarily plant based systems. The evaluation of new drugs, especially phytochemically obtained materials has opened up a vital area for research and development. According to WHO, globally about 80% of the population rely on the traditional medicine for the treatment of various diseases [2,3]**.**

Terminalia arjuna (Roxb.) Wt. and Arn. is a large evergreen tree distributed across the greater part of the Indian Peninsula along rivers and found in Sub-Himalayan tract, Chota Nagpur, Orissa, West Bengal, Punjab, Deccan and Konkan [4,5]. In the Indian System of Medicine, the applications of barks of this plant is a long list which includes astringent, coolent, aphrodisiac, cardiotonic, tonic in fractures, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumour, excessive perspiration, asthma, inflammation, skin disorders, etc. [6,7]. Arjunolic acid, Tomentosic acid, β-sitosterol, ellagic acid, (+)-leucodelphinidin, Arjungenin, Arjunglucoside I-IV, Tannins containing catechin, Gallocatechin, Epicatechin and Epigallocatechin are the main chemical constitutions present in the bark of Arjuna [8].

Materials and Methods

Plant material

The bark of Arjuna plant was procured from a store located in a market area of Parrys Corner, Chennai, India.

Bacterial Cultures

Four clinical isolates of pathogenic bacteria namely, *Staphylococcus aureus*, *Escherichia coli*, *Proteus sp.* and *Pseudomonas aeruginosa* were used as test organisms in this study. They were obtained from the Department of Microbiology Dr. M.G.R Janaki College of Arts and Science for women, Adyar, Chennai. The isolates were subcultured on nutrient agar (Himedia, Mumbai, India) and stored at 4°C until use.

Preparation of extracts

Twenty g of powder of *T. arjuna* stem bark was dissolved well in 100 ml of double distilled water (ratio 1:5). The suspension was filtered by using a Seitz filter of pore size 0.2 ìm. The sterile extract was then transferred to lyophilization flask and kept in deep freezer at – 80°C for 4 h. The frozen extract was then processed in Lyophilizer and the lyophilized powder was then transferred to sterile 5ml vials and

stored for further use. The acetone and ethanol extract were prepared by using 100 ml of 70% of acetone / Ethanol instead of 100 ml of double distilled water.

Determination of Antibacterial activity

Antibacterial activities of *T. arjuna* bark extract were studied by using agar diffusion and disc diffusion method described by Bauer *et al*. [9]. From the stock solution (1mg/ml) of the extracts and different dilutions of the drug containing 0.5, 1.0, 1.5 and 2.0 µg/ml were prepared in dimethyl sulfoxide (DMSO). The inoculums of the cultures were prepared and standardized to the Mc Farland standard 0.5 Scale and swabbed on Mueller Hinton agar plates so as to obtain lawn cultures. Discs impregnated with the extracts were placed on the plates along with as standard reference drugs (100 µg) namely, Chloramphenicol, Oxacillin, Gentamycin and Tetracycline. The plates were incubated at 37°C for 48 hours and observed antibacterial activity. The zone of inhibition caused by the drugs was measured in mm and compared with that of standard antibiotic discs.

Determination of MIC

Minimum inhibitory concentration (MIC) was determined by microdilution method [10] using Mueller Hinton broth. From the stock solution (1mg/ml) of the extracts different dilutions of the drug containing 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 and 0.7812 µg/ml were prepared in nutrient broth. Hundred µl of each dilution was added in the respective wells and 100 µl of nutrient broth served as control in the microtitre plate. Chloramphenicol, Oxacillin and Gentamycine were used as standard reference drugs (100 µg/ml). All the wells were inoculated with respective test organisms. The microtitre plate were incubated at 37°C for 18-24 hrs. The lowest concentration / highest dilution of the drug that showed no growth of the bacteria was interpreted as the value of MIC.

Results

Antibacterial activity by Disc diffusion method

Antibacterial activities of aqueous, acetone and ethanolic extracts of Arjuna against 4 clinical bacterial strains by Disc diffusion method are shown in Table-1. Zones of inhibition of 12, 13.5, 15.5 and 17 mm against *S. aureus,* 9.5, 10.5, 11.5 and 13.5 mm against *E. coli* were obtained with aqueous extract. The acetone extract caused the zones of inhibition of 14, 16, 18.5 and 20 mm against *S. aureus* and *P.aeruginosa* and 13.5, 14.5, 16 and 17 mm against *E. coli.* Zones of inhibition of 12.5, 13.5, 15.5, 18.5 mm against *E. coli* and 11.5, 12.5, 14, 16mm against *Proteus sp.*were obtained for ethanolic extract at the concentrations of 0.5, 1.0, 1.5 and 2.0 mg respectively

Antibacterial activities of three standard antibiotics viz, Chloramphenicol, Gentamycin and Oxacillin against the 4 clinical strains were also checked by disc diffusion method. Maximum zones of inhibition of 26 mm against *S. aureus* and 25mm against *Proteus sp.*by Oxacillin, 24mm against *E. coli* by chloramphenicol and 21mm against *P. aeruginosa* by Gentamycin were recorded.

Table 1. Antibacterial activity of extracts of *T. arjuna* (Disc diffusion method)

S. No	Test Organisms	Zone of inhibition in mm in mg/ml											
		Aqueous				Acetone				Ethanol			
		0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
1	*S. aureus*	12.0	13.5	15.5	17.0	14.0	16.0	18.5	20.0	13.0	14.5	16.5	17.5
2	*E. coli*	9.5	10.5	11.5	13.5	13.5	14.5	16.0	17.0	12.5	13.5	15.5	18.5
3	*Proteus sp.*	10.5	12.0	14.0	15.5	12.5	15.0	16.5	17.5	11.5	12.5	14.0	16.0
4	*P. aeruginosa*	9.5	11.0	13.0	15.0	14.0	16.0	18.5	20.0	11.0	13.5	15.5	17.0

Table 2. Antibacterial activity of extracts of *T. arjuna* (Agar Well diffusion method)

S. No	Test Organisms	Zone of inhibition in mm in mg/ml											
		Aqueous				Acetone				Ethanol			
		0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
1	*S. aureus*	12.5	13.5	15.5	16.5	13.0	16.5	19.5	21.5	13.0	14.0	16.0	17.0
2	*E. coli*	11.5	14.0	15.0	16.0	13.0	14.0	15.5	17.5	13.5	14.5	16.5	18.0
3	*Proteus sp.*	12.0	14.0	15.5	17.5	13.0	15.0	16.0	19.5	13.0	13.5	15.0	17.5
4	*P. aeruginosa*	12.0	14.0	16.5	16.5	11.0	13.5	16.0	18.0	13.0	13.5	15.5	16.5

Antibacterial activity by Agar Well diffusion method

Zones of inhibition of 11.5, 14, 15 and 16 mm against *E. coli*, 12, 14, 15.5 and 17.5mm against *Proteus sp.*were obtained with the aqueous extract. Zones of inhibition of 13, 16.5, 19.5 and 21.5mm against *S. aureus*, 13, 14, 15.5 and 17.5mm against *E. coli* were obtained with acetone extract. The ethanolic extract at 0.5, 1.0, 1.5 and 2.0 mg concentrations respectively caused the zones of inhibition of 13.5, 14.5, 16.5, 18mm against *E. coli* and 13, 13.5, 15.5 and 16.5mm against *P. aeruginosa* (Table 2; Fig.1).

Microdilution Method

Antibacterial activities of three extracts (Aqueous, Acetone and Ethanol) against the 4 clinical bacterial isolated by Microdilution method are depicted in Fig. 2. Aqueous extract exhibited bactericidal activity against *S. aureus* and *P. aeruginosa* at $\geq$100µg/ml, against *Escherichia coli and Proteus sp.*at $\geq$50 µg /ml. Acetone extract showed bactericidal activity against *S. aureus* at $\geq$25µg/ml and against *P.aeruginosa* at $\geq$6.25µg/ml. Ethanolic extract exhibited bactericidal activity against *Proteus sp.*at 100µg/ml and against *S. aureus* $\geq$12.5µg/ml.

Antibacterial activities of standard antibiotics chloramphenicol and Oxacillin against 4 clinical strains were checked by Microdilution Method indicated that both these antibiotics exhibit bactericidal activity at ≥1.56µg/ml against the test organisms.

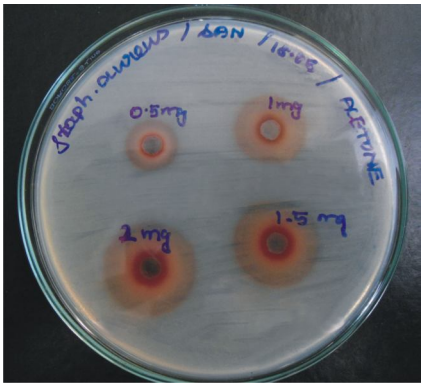

Fig. 1. Inhibition of *S. aureus* by the Acetone extract of *T. arjuna*

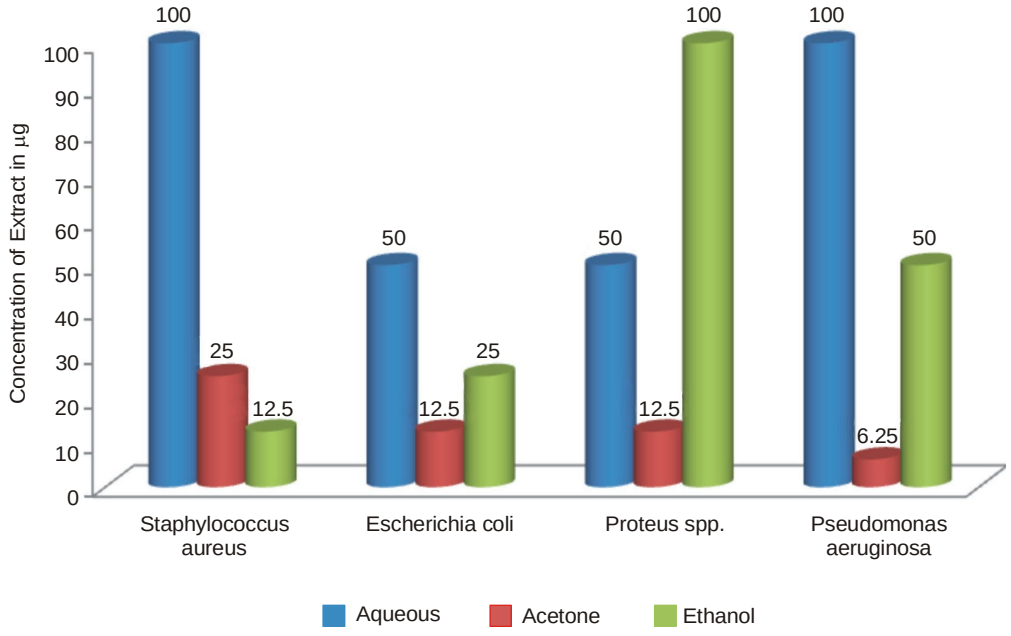

Fig. 2. Antibacterial activity of extracts of *T. arjuna* (Microdilution method)

Discussion

Among the three Arjuna extracts used (Aqueous, Acetone and Ethanol), while the acetone extract showed maximum bactericidal activity, the aqueous extract showed least activity against the test organisms as determined by well diffusion, disc diffusion and microdilution methods. A zone of inhibition of bacterial growth for more than 10 mm by the plant extract was interpreted as significant bactericidal activity [11]. In the present study most of the organisms caused zones of inhibition of more than 10mm. The findings of the present study are in agreement with Shinde *et al.* [12] who demonstrated the antibacterial activity of acetone, hexane and dichloromethane leaf extracts of five Terminalia species by agar-well-diffusion method against *E.coli, P.aeruginosa, Bacillus subtilis, S. aureus and Staphylococcus epidermidis*. They also reported that the extracts from the *T. arjuna* exhibited potent antibacterial activity *against E.coli, Klebsiella aerogenes, Proteus vulgaris and P. aerogenes* (gram-negative bacteria) at 1000-5000 ppm as determined by the disc diffusion method. Similar results have been obtained by Shinde *et al.* [12]. Chaudhari and Mengi [13] , who studied that the effect of topical application of phytoconstituents from a hydroalcohol extract of Arjuna bark, recorded the antimicrobial activity against *P. aeruginosa, E. coli, S. aureus, Streptococcus* pyogenes, but not with *Candida albicans.* The results of their study strongly advocated the beneficial effects of fraction I, consisting mainly of tannins of *T. arjuna*, in the acceleration of the healing process as well as the astringent effect of tannins. Rani and Khullar [14] reported the higher antibacterial activity of the methanol extracts of *T.arjuna* against multi-drug resistant *Salmonella typhi* in their study.

Khan *et al.* [15] had studied the Antimicrobial activity of the crude ethanol extracts of five plants against multidrug resistant (MDR) strains of *E. coli, K. pneumoniae, C. albicans* and ATCC strains of *S. aureus, P. aeruginosa, E. coli, K.pneumoniae* and *C. albicans*. According to Berghe *et al*. [16] if an inhibition is obtained by 1-10 mg of plant extract/ml then it can be considered as a potential drug further investigations. In the present study the acetone extracts showed better minimum inhibitory concentrations ranging from 6.25 – 25 µg/ml and hence the Arjuna plant product could be considered to have significant antibacterial activity. The active principle in this plant product can be further studied before practically applying it for effective control of pathogenic bacteria.

Conclusion

Arjuna plant product showed significant bactericidal activity against all the 4 clinical isolates used in the study as the inhibition of organisms by the Acetone extract ranged from 6.25 - 25 µg/ml. These findings therefore established a good support for the use of *Terminalia arjuna* in traditional medicine. Further investigation needs be carried out in order to analyze the active principle of Arjuna for effective utilization of this plant product to control vital human pathogenic bacteria.

References

1. Padmaa, M. P., Leena J. P. and Angelin, S. T., 2008, "Genius Salacia: A comprehensive review", *J. Nat. Remedies*, 8, pp.116-131.
2. Padmaa, M. P., 2009, "A phyto-pharmacological review of *Gmelina arborea* Roxb", *Herbal Heritage*, 1, pp.8-17.
3. Salim, K. P. and Padmaa, M. P., 2009, "A phyto-pharmacological review of *Syzygium cumini* skeels", *Pharmacol online Newsletter*, 2, pp.110-122.
4. Anonymous, 1999, "The Ayurvedic Pharmacopoeia of India. Part 1", Ministry of Health and Family Welfare, New Delhi.
5. Kirtikar, K. R and Basu, B. D., 1989, "Indian Medicinal Plants", L M Basu Publication, Allahabad.
6. Dwivedi, S. and Udupa, N., 1989, "*Terminalia arjuna*: Pharmacognosy, phytochemistry, pharmacology and clinical use: A review", *Fitoterapia*, 60, pp.413-420.
7. Warrier, P. K., Nambiar, V. P. K. and Ramankutty, C., 1994, "Indian Medicinal Plants. A Compendium of 500 Species", Vol. 1, Orient Longman Pvt. Ltd., Madras, India.
8. Rastogi, R. P. and Mehrotra, B. N., 1993, "Compendium of Indian Medicinal Plants" Vol. 1, 2 & 3, CSIR, New Delhi.
9. Bauer, R., Kirby, M., Sherris, J. and Turch, M., 1966, "Antibiotic susceptibility testing by a standardized single disc method", *Am. J. of Clin. Pathol.*, 36, pp.439-496.
10. Rajarajan, S., 2002, "In vitro antibacterial and antifungal properties in the leaf extract of Henna (*Lawsonia inermis* .L)" *Ind. J. of Appl.Microbiol.*, 2(1), pp.59-61.
11. Dobrowoiski, M., 1991, "Antibacterial, antifungal, antiamoebic, anti-inflammatory and antipyretic studies on propolis bee products" *Journal of Ethnopharmacology*, 35, pp.77-82.
12. Shinde, S. L., Junne, S. B., Wadje, S. S. and Baig, M. M. V., 2009, "The diversity of antibacterial compounds of *Terminalia species* (Combretaceae)" *Pak. J. Biological Sci.s*, 12, pp.1483-1486.
13. Chaudhari, M. and Mengi, S., 2006, "Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats", *Phytotherapeutic Research*, 20, pp.799-805.
14. Rani, P. and Khullar, N., 2004, "Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*" *Phytotherapeutic Research*, 18, pp.670-673.
15. Khan, R., Islam, B., Akram, M., Shakil, S. and Ahmad, A. *et al.*, 2009, "Antimicrobial activity of five herbal extracts against Multi Drug Resistant (MDR) strains of bacteria and fungus of clinical origin" *Molecules*, 14, pp.586-597.
16. Berghe, V., Cos, P., Hermans, N., Bruyne, S., Apers, J. B., Sindambiwe, D., Pieters, L. and Vlietinck, A. J., 1991, "Further evaluation of Rwandan medicinal Plant extracts for their antimicrobial and antiviral activities" *J. Ethnopharmacology*, 79(2), pp.155-163.