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Antimicrobial screening of free and bound flavonoid from the bark of *Terminalia arjuna*

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ABSTRACT

Continuous use of antibiotics results in the increased number of antibiotic resistant strains of microorganisms. Thus, a diverse arsenal of new antimicrobial agents is urgently needed to combat the diminishing efficacy of existing antibiotics. Almost all parts of plants (roots, stem, leaves, flowers, fruits and seeds) have been reported to have one or the other important bioactive compound but very little has been reported from bark of the plant except for few. Present study is confined to explore bark of *Terminelia arjuna* for some bioactive compounds. *T. arjuna* bark was collected, air dried and soxhlet extracted by using standard method for flavonoid extraction. Bound and free flavonoid extracts were then screened for antimicrobial activity using Disc Diffusion Assay. MIC, MBC and TA of each extract was also calculated. Both bound and free flavonoid showed activity against all the selected pathogens but the maximum inhibition zone was observed against *Agrobacterium tumifacians* (IZ= 19mm, AI=1.461±0.010) & Bacillus subtilis (IZ= 16mm, AI= 1.230±0.098) by the bound and free flavonoid extract of the plant respectively. Results obtained advocates the use of bark of the selected plant for pharmaceutical purpose to combat upcoming resistant pathogens.

Keywords: Flavonoid, Antimicrobial, MIC, MBC, TA.

INTRODUCTION

Medicinal plants contain ample amount of ingredients which can be used for the synthesis and development of drug. Nowadays due to regular use of antibiotics against diseases, microorganisms are developing resistance against several antibiotics which are regularly prescribed by physicians and are becoming multidrug resistant. Besides antibiotics have also been reported to affect our normal metabolism adversely if are used continually. Hence there is an urgent need to develop new herbal drugs to cure diseases.

In India *Terminelia arjuna* Wight & Arn. traditionally has been used to treat many diseases like diabetes, cardiovascular ailments and many more. The tree commonly known as Arjuna belongs to family *Combretaceae*, is a deciduous and evergreen tree found throughout India, Srilanka and Bangladesh. It has been considered as a cardiac tonic by Ayurvedic physicians [1]. Antioxidant, antimutagenic, antimicrobial and hepatoprotective activities of this plant have also been reported by Kaur *et al.* [2]. It is also speculated that most of the pharmacological, wound healing and antimicrobial activities of this plant is due to the presence of tannin in the bark of plant [3-5]. Antimicrobial activity of aqueous extracts of bark, root, leaves and fruits has also been reported for some microorganisms [6]. Cytotoxic and antimicrobial activity of ethanolic extract of the bark of this plant has also been reported by Morshed MA *et al*. [7] and Aneja KR *et al*. [8]. In one study, the antimicrobial activity of bark extract against multi resistant *Vibrio cholerae* has also been revealed [9]. Flavonoids like luteolin, kaempferol and quercetin have been identified in the bark of terminelia arjuna [10].

Screening of crude extract of the bark of *Terminelia arjuna* for antimicrobial activity has already been done. Literature reveals that meager work has been done related to the free and bound flavanoids of the bark, hence present investigation is to validate the antimicrobial activity of free and bound flavanoids of the bark.

MATERIALS AND METHODS

Plant collection and authentication

Bark of *Terminalia arjuna*, was collected from the eastern region of Rajasthan (Jaipur). Plants were got identified by the senior taxonomist of the Department of Botany, University of Rajasthan and Voucher specimen no: RUBL211458 was submitted to the Herbarium, Botany department, University of Rajasthan.

Test Pathogens

Pathogens screened were *Escherichia coli* (MTCC no.46), *Pseudomonas aruginosa* (MTCC no.1934), *Raoultella planticola* (MTCC 2271), *Enterobacter aerogenes* (MTCC 2822), *Bacillus subtilis* (MTCC 121), *Agrobacterium tumefaciens* (MTCC-431). Pathogens were procured from IMTECH (Chandigarh, Punjab, India). These bacterial strains were grown and maintained on Muller-Hinton Agar medium.

Preparation of extract

Flavonoid extraction: Bark of *Terminalia arjuna* was collected; shade dried, finely powdered and extracted using the method of Subramanian & Nagarjan [11]. Hundred grams of finely powdered sample was soxhlet extracted with 80% hot methanol (500ml) on a water bath for 24 h and filtered. Using separating funnel the filtrate obtained was re- extracted successively with petroleum ether (fraction I), ethyl ether (fraction II), and ethyl acetate (fraction III). Fraction of petroleum ether was discarded due to being rich in fatty substances, where as fractions of ethyl ether and ethyl acetate were further analyzed for free and bound flavonoids respectively. Using 7% H_2SO_4, ethyl acetate fraction of the sample was refluxed for the hydrolysis for 2 h (for removal of bounded sugars) and again filtrate was extracted in separating funnel with ethyl acetate. Ethyl acetate extract thus obtained was washed with distilled water to neutrality. Ethyl ether (free flavonoids) and ethyl acetate fractions (bound flavonoids) were dried in vacuo and weighed (Table 2). The extracts were stored at 4°C and were re-suspended in acetone to get 10mg/ ml concentration for antimicrobial assay.

Antimicrobial assay

Antimicrobial screening was done by "Disc Diffusion Assay" [12]. Each Muller-Hinton Agar medium base plates were seeded with different bacterial inoculum (inoculum's size 1×10^8 CFU/ml for bacteria.). Discs of sterile Whatmann no.1 filter paper (6mm in diameter) were impregnated with 100µl of bound and free flavonoid extract of 10mg/ml concentration to give a final concentration of 1 mg/disc. To remove residual solvent, which might interfere with the determination process, discs were left to dry in vaccuo. These dried discs impregnated with two different extracts were then placed on the corresponding seeded agar plates. Each extract was tested in comparison with streptomycin (1mg/disc) as standard for bacteria. For diffusion of extracts, plates were kept at 4 °C, thereafter were incubated at 37 °C for bacteria for 24h. Using standard formula, for "Activity index" for each extract was calculated. (Table 1).

Activity index = IZ produced by extract/ IZ produced by standard Where, IZ = inhibition zone.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Minimum inhibitory concentration (MIC) for each extract was calculated using the broth dilution method [13]. For determination of MIC, plant extracts were once again resuspended in acetone (acetone has no activity against the test microorganisms) to make its final concentration of 10 mg/ml. In the broth media of 96-wells two fold serially diluted extracts were added. Thereafter 100µl inoculum (for bacteria 1×10^8 CFU/ ml) was added to each well. Negative and positive controls have also been kept. Bacterial suspension was used as negative control, while broth containing standard drug was used as positive control. Micro titer plates were then incubated at 37 °C for 24 h for bacteria. Each extract was assayed in duplicate therefore every time two sets of micro plates were prepared. One plate was kept for incubation at 37 °C while another was kept at 4 °C. The lowest concentration which showed no turbidity after incubation was taken as MIC values. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. Minimum bactericidal concentration (MBC) was determined by sub culturing 50 µl from each well showing no apparent growth (Table 1). Least concentration of extract showing no visible growth on sub culturing was taken as MBC.

Total activity (TA) determination

Total activity is the volume upto which the test extracts can be diluted without losing the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g [14] Table 2.

Statistical Analysis

Mean value and standard deviation were calculated for each test bacteria. Data were analyzed by one-way ANOVA and *P* values were considered significant at $P>0.005$. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

RESULTS

Antibacterial activity

Antibacterial activity (assessed in terms of IZ in mm and activity index) of the flavonoid extract of bark of *Terminelia arjuna* tested against six different pathogenic bacteria, data obtained for the antimicrobial potential of plant extract are tabulated in table 1 & 2. In the present study, free and bound flavanoid extracts were tested for their bioactivity, all the extracts showed significant antimicrobial potential against test microbes. Maximum antibacterial activity of both extract was observed against one of the selected pathogens [Figure 1].

Agrobacterim tumifacians

Bound flavonoid extract showed the maximum inhibition zone against *Agrobacterium tumifacians* (IZ= 19mm, AI=1.461±0.010)

Bacillus subtilis

Free flavonoid extract showed highest activity against *Bacillus subtilis* (IZ= 16mm, AI=1.230±0.098)

MIC and MBC

The range of MIC and MBC of extracts recorded was 0.078 - 1.250mg/ml & 0.078 - 0.625mg/ml respectively. In the case of bound flavonoid, bactericidal effect was observed for *E. aerogens* whereas in case of free flavonoid bactericidal effect was observed for *R. planticola.*

Total activity

Total activity as a measure of potency was also determined. Maximum total activity was observed for bound flavonoid against *A. tumifacians.* Overall, the test pathogens were more sensitive to the bound flavanoid than to the free flavonoid.

Table 1: Antimicrobial assessment of bound and free flavonoid extract of bark of *Terminalia arjuna* against different pathogenic bacteria

Test pathogen	Extract	IZ	AI	MIC	MBC
Enterobector aerogens	F1	11	0.733±0.016	0.312	0.312
	F2	--	--	--	--
Bacillus subtilis	F1	17	1.308±0.023	0.156	0.078
	F2	16	1.230±0.098	0.156	0.078
Routella planticola	F1	16	0941±0.031	0.156	0.078
	F2	14	0.823±0.018	0.312	0.312
Agrobacterium tumifacians	F1	19	1.461±0.010	0.078	0.039
	F2	--	--	--	--
Escherichia coli	F1	13	0.928±0.018	0.312	0.156
	F2	10	0.714±0.030	0.625	0.312
Pseudomonas aeruginosa	F1	16	1.231±0.019	0.156	0.078
	F2	8	0.615±0.027	1.250	0.625

IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc), AI= Activity Index (IZ developed by extract/ IZ developed by standard), SEM, (--) = No activity, Values are average of three, IZ of streptomycin against *E. aerogens* (15mm), *B. subtilis* (13mm), *R. planticola* (17mm), *A. tumefaciens* (13mm), *E. coli* (14), *P. aeruginosa* (13mm). MIC = Minimum Inhibitory Concentration (mg/ml) MBC = Minimum Bactericidal (mg/ml), F1=Bound flavonoids, F2= Free flavonoids

Table 2: Total activity of the bark extracts of *Terminelia arjuna*

Test pathogen	Extract	Quantity of extract mg/g dried plant part	Total activity (ml/g)
Enterobector aerogens	F1	6.0	19.23
	F2	1.5	--
Bacillus subtilis	F1	6.0	38.46
	F2	1.5	9.61
Routella Planticola	F1	6.0	38.46
	F2	1.5	4.81
Agrobacterium tumifacians	F1	6.0	76.92
	F2	1.5	--
Escherichia coli	F1	6.0	19.23
	F2	1.5	2.40
Pseudomonas aeruginosa	F1	6.0	38.46
	F2	1.5	1.20

Total activity= Extract per gram dried plant part/MIC

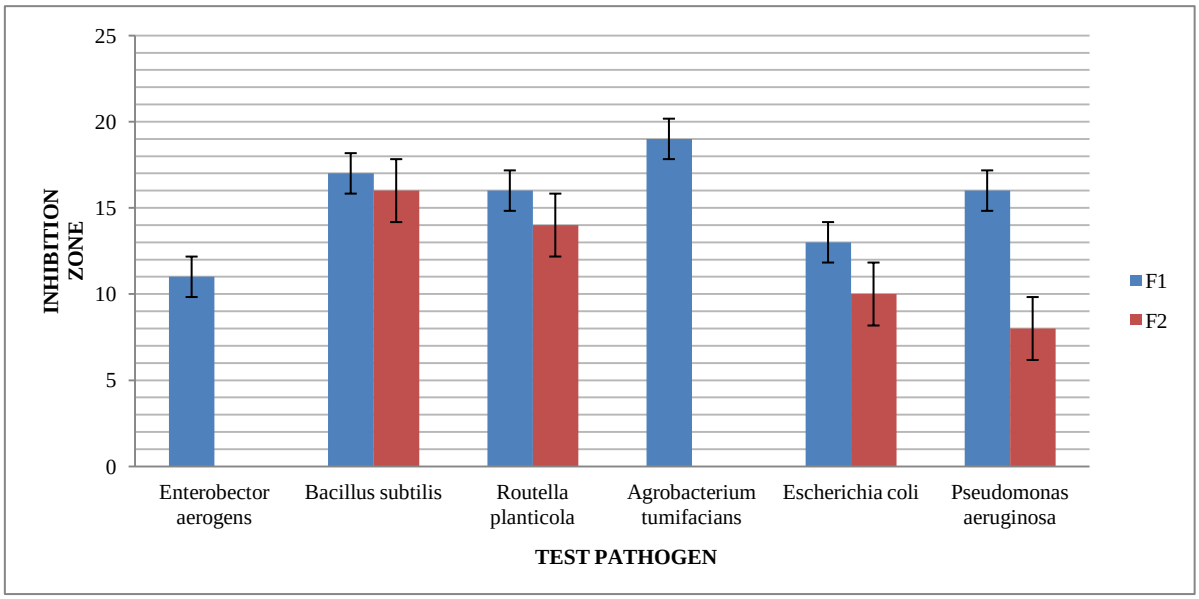

Figure 1: Graph showing antimicrobial activity of bound and free flavonoids of bark of *Terminelia arjuna*

DISCUSSION

Results of the present study showed that both bound and free flavanoid showed antimicrobial activity against test pathogen indicating broad- spectrum nature of *T. arjuna*. Bound flavonoid extract of *T. arjuna* bark express maximum antibacterial activities by suppressing the growth of all microbes under investigation. In the present study, bound flavanoid and free flavonoid were found to be potent inhibitors of tested organisms except *E. coli* and *A.tumifacians.* Results reveal that bound and free flavonoid extacts adversely affect the growth of *Agrobacterim tumifacians* and *Bacillus subtilis* respectively. As compare to free flavonoid, bound flavonoid inhibited the selected pathogens more effectively. Excellent antimicrobial activities were observed for bound flavanoid extract, shown by low MIC and MBC values. MBC values were found higher than the

MIC values of the extracts against microorganisms tested, indicating the bacteriostatic effects of the extracts.

Terminelia arjuna revealed its antimicrobial potential against test pathogens which are involved in number of human diseases. Aqueous extracts of bark/ stem, root, leaves and fruits of *T. arjuna* has previously been studied for antibacterial activity, but still the literature available is meager. High inhibitory potential of methanolic extract [15] and anti-bacterial activity of the crude extract of *Terminalia arjuna* against multi antibiotic resistant *Vibrio choleraea* [9] has also been determined earlier. Antimicrobial activity of *T. arjuna* methanolic bark extract has previously showed greater result against gram negative bacteria than gram positive, [16] in present study greater activity against gram negative organisms showed by bound flavanoid. Screening of the plant under investigation (*T. arjuna*) so far has not been worked out for bound and free flavonoids. Mostly the crude extracts have been screened, that too without MIC, MBC and TA determination. Such studies could only indicate their antimicrobial potential but are not helpful in establishing them as an antibiotic, hence cannot replace the existing antibiotics. In the present study IZ, AI, MIC, MBC/MFC and TA have been evaluated for each extract. MIC values recorded were very low, indicating strong bioefficacy of the plant. It is worth mentioning that IZ of bound flavonoid of *Terminelia arjuna* bark extract, against most of the tested pathogens, found to be more as compared to standard drugs. Due to continuous use of antibiotics microorganisms are becoming resistant against the antibiotics which are in use, therefore the present investigation is of great significance as far as the future drugs are concerned and advocates the use of selected plant for pharmaceutical purpose and to for prepare flavonoids based antimicrobial drugs for resistant pathogens, ofcourse after clinical trials.

CONCLUSION

Terminalia arjuna, traditional medicinal plant of India, is the rich source of bioactive compound. As now, little work has been done on the biological activity and hence extensive investigation is needed to exploit the bioactive compounds for medicinal purpose. The results of the above study revealed that the flavonoid extract of *T. arjuna* was exhibit antibacterial activity which might be helpful in preventing the progress of various diseases and can be used in alternative system of medicine.

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REFERENCES

1. Dwivedi S., Putative uses of Indian cardiovascular friendly plants in preventive cardiology. Ann Natl Acad Sci 1996; 32: 159- 175.
2. Kaur K.S., Arora S., Kumar., Nagpala A. Antimutagenic activities of acetone and methanol fractions of *Terminalia arjuna*. Food Chem Toxicol.2002; 40: 1475- 1482. doi:10.1016/S0278-6915(02)00078-9
3. Patnaik T., Dey R.K., Gouda P. Isolation of triteroenoid glycoside from bark of *Terminalia arjuna* using chromatographic technique and investigation of pharmacological behavior upon muscle tissue. E J. Chem.2007; 4: 474-479. doi: 10.1155/2007/538932
4. Chaudhari M., Mengi S. Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. Phytother Res.2006; 20: 799-805. doi: 10.1002/ptr.1857
5. Bele A.A., Jadhav V.M., Kadam VJ. Potential of tannins: A review. Asian J Plant Sci.2010; 9: 209-214. doi: 10.3923/ajps.2010.209.214
6. Ramya S., Kalaivani T., Rajasekaran C., Jepachanderamohan P., Alaguchamy N., Kalayansundaram M *et al.* Antimicrobial activity of aqueous extracts of bark, root, leaves and fruits of *Terminalia arjuna* Wight & Arn. Ethnobotanical Leaflets. 2008;12: 1192-97.
7. Morshed M.A., Uddin A., Rahman A., Hasan T., Roy S., Amin A.A., Ahsan R., Islam. *In vitro* antimicrobial and cytotoxicity screening of *Terminalia arjuna* ethanol extract. International Journal of Biosciences. 2011; 1(2):31-38.
8. Aneja K.R., Sharma C., Joshi R. Antimicrobial activity of *Terminalia arjuna* Wight & Arn.: An ethnomedicinal plant against pathogens causing ear infection. Braz J otorhinolaryngol. 2012; 78(1):68-74. doi.org/10.1590/S1808-86942012000100011
9. Fakruddin M., Alam K.M.A., Mazumdar R.M., Islam S., Nipa M.N., Iqbal A., Bhuiyan HR. Anti-bacterial activity of the extract of *Terminalia arjuna* against multi antibiotic resistant vibrio cholerae. J Sci Res.2011; 3 (1):129-137. doi:10.1186/1472-6882-12-232
10. Sharma P. Antimicrobial activity of flavonoids from in vitro tissue culture and plant parts of medicinally important tree *Terminalia arjuna*. Int. J. Pharm. Sci. Rev. Res. 2012; 17(2); 17, 90-92.
11. Subramnian S.S., and Nagarjan S. Flavonoids of the seeds of *Crotolaria retusa* and *Crotolaria striata*. Current Sciences (India) 1969;3:38:65.
12. Andrews J.M. BSAC standardized disc susceptibility testing method. J. Antimicrob Chemoher. 2011; 4:43-57. doi: 10.1093/jac/48.suppl_1.43
13. Basri D.F., Fan S.H. The potential of aqueous and acetone extracts of gall of *Quercus infectoria* as antibacterial agents. Indian Journal of Pharmacology. 2005; 37:26-29. doi: 10.4103/0253-7613.13851
14. Eloff J.N. Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. Phytomedicine 2004;11(4):370-371.
15. Patil U.H., Gaikwad D.K. Phytochemical evaluation and bactericidal potential of *Terminalia arjuna* stem bark. International journal of pharmaceutical science and research. 2011; 2(3): 614-619.
16. Mandal S., Patra A., Samanta A., Roy S., Mandal A., Mahapatra T. D. *et al*. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. Asian Pac J Trop Biomed 2013; 3(12): 960-966.

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