http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (22): 1483-1486, 2009
ISSN 1028-8880

The Diversity of Antibacterial Compounds of *Terminalia* Species (Combretaceae)

[1]S.L. Shinde, [2]S.B. Junne, [3]S.S. Wadje and [3]M.M.V. Baig
[1]Department of Botany, Shri Shivaji College, Parbhani, Maharashtra, India
[2]Department of Chemistry, Yeshwant Mahavidyalaya, Nanded, Maharashtra, India
[3]Department of Botany, Yeshwant Mahavidyalaya, Nanded, Maharashtra, India

Abstract: The antibacterial activity of acetone, hexane, dichloromethane leaf extract of five *Terminalia* species (*Terminalia alata* Heyne ex Roth., *Terminalia arjuna* (Roxb.) Wt. and Arn., *Terminalia bellerica* (Gaertn.) Roxb., *Terminalia catappa* L. and *Terminalia chebula* Retz.) were tested by Agar-well-diffusion method against human pathogens *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The Rf values and relative activities of separated compounds were tested. Hexane and dichloromethane extracts have shown more antibacterial components than the acetone extract indicating the non-polar character of the antibacterial compounds. The non-polar character of the antibacterial compounds was confirmed from the Rf values. It indicated that the antibacterial activity was not due to tannins. *Terminalia catappa* found to possess the compounds which are more antibacterial. *Terminalia arjuna* and *T. catappa* plants were found most promising for isolating antibacterial compounds.

Key words: Combretaceae, *Terminalia* species, antibacterial activity, Rf value

INTRODUCTION

Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites. Plant derived medicines are widely used because first, they are relatively safer than the synthetic alternatives and they are easily available and cheaper. Second, the public is becoming increasingly aware of problems with the over prescriptions and misuse of traditional antibiotics. Third, time to time discovery of new pathogens and forth, remarkable abilities of microbes to develop resistance against antibiotics.

The species of *Terminalia* are very well known for their therapeutic values since long and has proved by many researchers to be useful as anticancer (Kandil *et al*., 1999), antigenotoxic (Chu *et al*., 2007), anti-inflammatory (Fan *et al*., 2004) etc. The species of *Terminalia* also exhibit antimicrobial activity. Fruits and leaves of *T. chebula* showed antibacterial activity against ten pathogenic bacteria (Malekzadeha *et al*., 2001). Ethanol extract of *T. catappa* showed antibacterial activity against nine bacterial strains with minimum inhibitory concentrations (MICS) ranging from 0.25 to 16 mg mL^{-1} (Kloucek *et al*., 2005). The bark extracts of *T. arjuna* exhibited antibacterial activity (Samy and Ignacimuthu, 2001).

In view of its high medicinal potential and previous findings, we tried to isolate various fractions from the leaves following antibacterial activity against various species so that it's further possible uses in medicines, therapeutics and food preservations would be determined.

MATERIALS AND METHODS

Collection of plant materials: The study was carried out during the year 2007-08, 2008-09 in the Department of botany, Yeshwant Mahavidyalaya, Nanded. The leaves of test plants like *Terminalia alata*, *Terminalia arjuna*, *Terminalia bellerica*, *Terminalia catappa* and *Terminalia chebula* were collected from Nanded region.

Source of microorganisms: The bacteria selected for study were common human pathogens like *E. coli* (ATCC-10412), *Pseudomonas aeruginosa* (ATCC-27853), *Bacillus subtilis*, *Staphylococcus aureus* (ATCC-103207) and *Staphylococcus epidermidis*. The above cultures were obtained from Marathwada Agricultural University, Parbhani (MS).

Standardization of microorganisms: For standardization of microorganism exactly 0.5 mL of overnight cultures of each organism was dispended into 20 mL of sterile nutrient broth and incubated for 3-5 h to standardize the culture to 10^6 cfu mL^{-1}. A loop-ful of the standard cultures was used for the antibacterial activity (Collins *et al*., 1995).

Corresponding Author: S.L. Shinde, Department of Botany, Shri Shivaji College, Parbhani, Maharashtra, India

Extract preparation: For testing efficacy of plant extracts, acetone, methanol and hexane extracts of these plant parts were prepared. Five grammas of dried plant material were extracted with 50 mL of solvent. The extraction was allowed to proceed for 48 h. The alcoholic and ethyl acetate extracts were decanted and the solvent removed by evaporation at room temperature (28±2°C) to obtain the extract. The air dried extracts were stored for 48 h in sterile universal bottles to room temperature. The sterility of the extracts was confirmed before use.

Phytochemical analysis: Chemical constituents of the extracts were analyzed by Thin Layer Chromatography (TLC) using aluminium-backed TLC plates (Merck, Silica gel 60 F_{254}). The TLC plates were developed under saturated conditions with one of the three eluent systems developed (Masoko *et al*., 2005) for the separation of components of Combretaceae plant extracts i.e., ethyl acetate/Methanol/water (40:5.4:5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5:4:1) : [CEF] (intermediate polarity/acidic); benzene/ethanol/ ammonium hydroxide (90:10:1) [BEA] (non-polar/basic). The plates were observed under UV light (detecting agent) and location of the separated compounds were ascertained and marked. Silica gel was scrapped from marked area. The compounds were eluted in sterile distilled water. These eluted compounds were used for assessing antibacterial activity.

Assessment of antibacterial activity of plant extracts: Antibacterial activities of the eluted compounds were evaluated by Agar well-diffusion method and expressed by diameter zone of inhibition in mm. The bioassay was carried out by using 1 mL of inoculum prepared from an overnight culture for given test bacteria, 1 mL of the resultants bacterial cell suspension was poured in the Petri plate and the plates were poured with respective medium. The medium was allowed to solidify and wells were prepared using sterilized cork borer (diameter 5 mm). Wells of 5 mm diameter were made in the solidified inoculated medium. The wells were filled with 0.5 mL of eluates. Plates were then incubated aerobically at 28±2°C for 24 h. The experiments were conducted with three replications.

Statistical analysis: The data were analyzed for significant differences ($p<0.05$) of effects using a one-way Analysis of Variance (ANOVA).

RESULTS

Zone of inhibition method was used to screen the antibacterial compounds in different extracts. Bacteria free zone around the well was recorded as zone of inhibition in mm.

Three separation systems i.e., EMW, BEA and CEF were used to separate components of *Terminalia.* The result obtained in BEA and CEF are presented here and not of EMW. It is because antibacterial compounds being non-polar, they could not be separated properly in EMW. With the exception of two ethanol extracts from *T. bellerica* and two from *T. catappa*, no antibacterial compound could be observed in the EMW system.

Table 1 shows the results of acetone/ hexane/dichloromethane extract of five *Terminalia* species separated with BEA possessed few compounds with high antibacterial activity against tested bacterial pathogens. Acetone extracted compounds of all the five species of *Terminalia* showed less antibacterial activity compared to hexane and dichloromethane extracts. Maximum antibacterial activity was shown by acetone extracted compounds against *B. subtills* and *E. coli* and least against *S. epidermidis* where as *P. aeruginousa* and *S. aureus* could not be inhibited by any of these compounds.

Hexane and dichloromethane extracts of all *Termialia* species showed number of antibacterial compounds that were very active against all tested bacterial pathogens. Hexane extracts of *T. catappa* and *T. chebulla* showed more compounds which were antibacterial. In *T. alata* compounds of 0.36 to 0.45 Rf values and in *T. catappa* of 0.78 Rf value showed maximum zone of inhibition against *B. subtilis* and *P. aeruginosa*, *T. alata* and *T. arjuna* hexane extracts did not show activity against *S. epidermidis.* Hexane and dichloromethane extracts of all *Terminalia* species tested showed more inhibitory compounds against *B. subtilis* and *E. coli*, followed by *P. aeruginosa*, *S. aureus* and *S. epidermidis*. Dichloromethane extracts of *T. alata* and *T. arjuna* showed more compounds which were antibacterial.

Acetone, hexane and dichloromethane extracts of *Terminalia* species were also separated with CEF and the results obtained are presented in Table 2. Acetone extracts of *T. catappa* and *T. chebulla* showed compounds with 0.97 and 0.94 Rf value which were more active against *B. subtilis* and *E. coli.* Acetone (CEF) extracts of *Terminalia* species did not show activity against *P. areuginosa*, *A. aureus* and *S. epidermidis* with exception of one compound of *T. alata* for *P. aeruginosa* and *T. arjuna* for *S. aureus* and *S. epidermidis.* Hexane and dichloromethane extracts displayed number of compounds, which were very active against *B. subtilis* and *E. coli.* Hexane and dichloromethane extracts separated with CEF showed similar compounds in all *Terminalia* species tested.

Table 1: Antibacterial activities of fractions of *Terminalia* species leaves extracts separated by TLC with BEA as eluent

	Zone of inhibition in mm														
	Acetone/BEA					Hexane/BEA					Dichloromethane/BEA				
Compounds with Rf values	Bs	Eo	Pa	Sa	Se	Bs	Eo	Pa	Sa	Se	Bs	Eo	Pa	Sa	Se
T. alata															
0.45	09	08	-	-	-	10	08	09	09	-	10	08	08	-	
0.36	-	-	-	-	-	08	-	10	09	-	09	08	-	-	
0.20	-	-	-	-	-	-	-	-	-	-	10	09	-	-	
0.16	08	08	-	-	-	08	09	08	08	-	09	-	-	-	
T. arjuna															
0.87	10	08	-	-	-	08	07	08	08	-	10	08	07	-	-
0.53	-	08	-	-	07	08	08	08	09	-	10	-	08	07	07
0.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.28	-	-	-	-	-	09	07	-	-	-	07	08	07	07	08
0.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.12	-	08	-	-	-	07	08	-	-	-	07	07	08	-	-
T. bellerica															
0.79	08	-	-	-	-	07	08	-	08	07	09	10	08	-	-
0.53	-	-	-	-	-	08	08	-	-	-	09	09	08	-	-
0.44	-	-	-	-	-	07	07	-	-	-	-	-	07	-	07
0.28	-	-	-	-	-	08	-	-	09	-	-	-	07	-	07
0.21	08	09	-	-	-	-	08	08	08	08	08	09	09	07	-
T. catappa															
0.78	10	10	-	-	-	10	09	10	10	08	08	09	09	07	07
0.73	-	08	-	-	07	08	08	07	07	07	08	08	09	07	-
0.53	-	-	-	-	-	07	07	08	07	07	07	07	-	-	07
0.28	-	07	-	-	-	07	07	07	-	08	08	08	-	07	-
T. chebulla															
0.55	07	-	-	-	-	09	08	-	08	-	09	09	-	-	07
0.46	09	08	-	-	-	08	07	08	-	-	08	07	-	-	07
0.38	-	08	-	-	-	08	-	08	-	-	-	07	-	07	-
0.10	09	-	-	-	07	-	08	-	-	-	-	-	-	07	-

Bs: *Bacillus subtilis,* Eo: *E. coli* (ATCC 10412), Pa: *Pseudomonas aeruginosa* (ATCC 27853), Sa: *Staphylococcus aureus* (ATCC 103207), Se: *Staphylococcus epidermidis.* BEA: Benzene, ethyl acetate, ammonium hydroxide

Table 2: Antibacterial activities of fractions of *Terminalia* species leaves extracts separated by TLC with CEF as eluent

	Zone of inhibition in mm														
	Acetone/CEF					Hexane/CEF					Dichloromethane/CEF				
Compounds with Rf values	Bs	Eo	Pa	Sa	Se	Bs	Eo	Pa	Sa	Se	Bs	Eo	Pa	Sa	Se
T. alata															
0.94	08	08	07	-	-	08	09	09	-	-	08	09	-	09	-
0.54	08	07	-	-	-	-	08	-	-	-	-	-	07	-	-
0.50	-	-	-	-	-	-	-	-	-	-	08	07	-	08	-
0.24	07	08	-	-	-	10	10	-	09	07	08	09	07	-	-
T. arjuna															
0.95	-	-	-	-	-	08	10	10	08	09	10	08	-	-	08
0.85	08	07	-	-	-	09	10	10	07	-	10	07	08	-	09
0.81	07	-	-	07	-	-	07	-	-	08	07	08	-	-	07
0.75	08	07	-	07	07	07	08	-	09	07	07	-	08	-	-
0.55	-	07	-	-	-	08	-	08	-	08	07	08	-	07	-
T. bellerica															
0.94	07	08	-	-	-	09	-	-	09	-	08	-	07	07	07
0.90	-	-	-	-	-	08	-	07	-	-	07	08	-	-	-
0.86	-	08	-	-	-	08	08	-	-	-	08	07	-	-	08
0.75	-	07	-	-	-	07	-	-	08	-	08	08	-	-	-
0.50	-	-	-	-	-	07	-	08	-	08	07	-	07	08	-
T. catappa															
0.97	10	10	-	-	-	08	07	-	07	08	08	09	09	-	07
0.95	-	08	-	-	-	08	08	07	07	07	-	08	08	08	08
0.90	-	-	-	-	-	07	-	-	-	-	09	08	08	-	09
0.86	08	-	-	-	-	07	08	09	07	07	08	07	-	07	07
0.74	-	08	-	-	-	09	-	09	-	-	08	08	09	-	-
T. chebula															
0.94	10	-	-	-	-	10	10	10	07	09	09	09	09	08	-
0.90	08	08	-	-	-	08	07	08	-	-	07	07	-	07	-
0.85	-	-	-	-	-	-	08	-	-	-	08	08	-	07	-
0.55	-	07	-	-	-	-	08	-	-	-	-	07	-	-	-

Bs: *Bacillus subtilis*, Eo: *E. coli* (ATCC 10412), Pa: *Pseudomonas aeruginosa* (ATCC 27853), Sa: *Staphylococcus aureus* (ATCC 103207), Se: *Staphylococcus epidermidis*. CEF: Chloroform, ethyl acetate, formic acid

DISCUSSION

In all *Terminalia* species *T. catappa* and *T. bellerica* showed more antibacterial components than other tested species of *Terminalia. T. chebula* is customary traditional medicine used by villages and tribals of many states in India including Tamil Nadu for curing fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection, wound infections (Dash, 1991). Similar results were obtained earlier by Babayi *et al*. (2004) and Shahina *et al*. (2007). The present studies showed that *Terminalia* species possessed number of antibacterial compounds. Similar results were reported by Kannan *et al*. (2009) and Phulan and Khullar (2004) Absences of antibacterial activity in present studies in *Terminalia* species cannot be attributed to tannins as was previously noted by Baba-Moussa *et al*. (1999). The results obtained here are in line with previous study of Kannan *et al*. (2009) and Kloucek *et al*. (2005).

CONCLUSION

Terminalia species have been found to be effective against some pathogenic microorganisms involved in wounds, burns and skin infections. Similar results were earlier obtained by Babayi *et al*. (2004). Thus, species of *Terminalia* can be used in the treatment of these ailments. The extracts of these plants proved to be active against *Bacillus subtilis* and *E. coli*, followed by *Pseudomonos aeruginosa*, *S. aureus* and *Staphylococcus epidermidis*.

REFERENCES

Baba-Moussa, F., K. Akpagana and P. Bouchet, 1999. Antifungal activities of seven West African Combretaceae used in traditional medicine. J. Ethnopharmacol., 66: 335-338.

Babayi, H., I. Kolo, J.I. Okogun and U.J.J. Ijah, 2004. The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. Biokemistri, 19: 106-111.

Chu, S.C., S.F. Yang, S.J. Liu, W.H. Kuo, Y.Z. Chang and Y.S. Hsie, 2007. *In vitro* and *in vivo* antimetastatic effects of Terminalia catappa L. leaves on lung cancer cells. Food Chem. Toxicol., 45: 1194-1201.

Collins, C.H., M. Patricia and J.N. Lyne, 1995. Microbiological Methods. 1st Edn., Butterworth Heinmann Ltd., Oxford, pp: 121.

Dash, V.B., 1991. Materia Medica of Ayurveda. B. Jain Publishers Pvt., New Delhi, India, pp: 61.

Fan, Y.M., L.Z. Xu, J. Goa, Y. Wang, X.T. Tang, X.Z. Zhao and Z.X. Zhang, 2004. Phytochemical and anti-inflamatory studies on *Terminalia catappa*. Fitoterapia, 75: 253-260.

Kandil, F.E., A.M. Soliman, S.R. Skodack and T.J. Mabry, 1999. A new anticancer tannin and known tannins from *Terminalia catapa*. Asian J. Chem., 11: 1001-1004.

Kannan, P., S.R. Ramadevi and W. Hopper, 2009. Antibacterial activity of Terminalia chebula fruit extract. Afr. J. Microbiol. Res., 3: 180-184.

Kloucek, P., Z. Polesny, B. Svobodova, E. Vlkova and L. Kokoska, 2005. Antibacterial screening of some Peruvian medicinal plants used in Callerßa District. J. Ethnopharmacol., 99: 309-312.

Malekzadeh, F., H. Ehsanifar, M. Shahamat, M. Levin and R.R. Colwell, 2001. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobacter pylori*. Int. J. Antimicrob. Agents, 18: 85-88.

Masoko, P., J. Picard and J.N. Eloff, 2005. Antifungal activities of six south African *Terminalia* species (Combretaceae). J. Ethnopharmacol., 99: 301-308.

Phulan, R. and N. Khullar, 2004. Antimicrobial evaluation of some medicinal plants. Phytoterapy Res., 18: 670-673.

Samy, R.P. and S. Ignacimuthu, 2001. Antibacterial effects of the bark of *Terminalia arjuna* justification of folklore beliefs. Pharmaceutical Biol., 39: 417-420.

Shahina, N., S. Ahemad and A.R. Sheikh, 2007. *In vitro* antibacterial activity of the extracts derived from *Terminalia catappa*. Res. J. Microbiol., 2: 180-184.