EVALUATION OF ANTIBACTERIAL POTENTIAL OF TERMINALIA CHEBULA AGAINST PATHOGENIC ORGANISMS OF LACRIMAL SYSTEM

Charmi P Shah*, U D Vachhani[1], M N Trivedi[1], V J Joshi[2], D D Santani[1]

*Jodhpur National University, Narnadi, Jahnawar road, Jodhpur, Rajasthan. India.
[1]Rofel Shri G M Bilakhia College of Pharmacy. Vapi, Gujarat. India.
[2]Alkem Lab Ltd. Dabhel, Daman. India
Email ID - charmishah15@yahoo.co.in

Summary

The presence of organic constituents in the medicinal plants plays a vital role in exhibiting antimicrobial activity. In the present study six pathogenic isolates of lacrimal system are used to determine its sensitivity towards various plant extracts of different polarities of *Terminalia chebula*. Antibacterial activity of the plant extracts were determined using agar well diffusion method. The results showed good antibacterial activity of all the plant extracts against the test isolates. Semi polar extracts were more effective compared to polar and non polar extracts. Gram positive isolates were more sensitive than gram negative isolates. Thus from the results of the present study it could be concluded that *T. chebula* can be used in treating the lacrimal disorders caused by the organisms studied.

Key words: Antibacterial activity, *Terminalia chebula*, Agar well diffusion method, Lacrimal system.

Introduction

Infectious diseases account for approximately one-half of all deaths in tropical countries caused by microorganisms. It is born with man and drugs came in to existence since a very early period to relive the pain from diseases and to cure them.[1] Thus the story or history of drugs is as old as mankind. Antibiotics are widely used as therapeutic agents and are important to reduce the bioburden of various diseases. In recent years antibiotic resistance has posed a major therapeutic problem by diminishing the effectiveness of the drugs in both developed and developing countries.[2] To overcome this situation it is necessary to search new antimicrobial compounds from various sources such as plants.

In traditional Indian system of Ayurveda and Siddha, medicinal plants are widely used to combat various diseases because of their higher safety margin, easy availability at affordable price, easily biodegradable and pose minimum environmental hazards.[3] The organic compounds obtained from plants are extensively used in many of the pharmaceutical preparations either in pure form or as extracts. According to WHO 80% of the world population rely on plants based products to meet their health care needs. [4]

Terminalia chebula Retz is a medicinal plant belonging to family Combretaceae. It is commonly called as black myrobalan. It is native to India, Srilanka, Pakistan, Nepal and China. The fruits of *T. chebula* are commonly used in treatment of various ailments such as allergy, vomiting, urinary tract infections, cardiac diseases, digestive problems, bleeding, cancer, skin disorders, and diabetes mellitus.[5] It also possesses antioxidant activity and free radical scavenging property. Antimicrobial activitiy of *T. chebula* have also been reported in many research publications.[6-8] To the best of our knowledge there are no reports available for antibacterial activity of fruits of *T. chebula* against the

pathogenic organisms selected in the present study. In view of these reported values of the medicinal plants the aim of the present work is to study antibacterial potential of fruits of *T. chebula* against the pathogenic bacterial strains of lacrimal system.

Materials and Methods

Plant Material: The fresh fruits of *T. chebula* were collected from the local market of Vapi, India and further their taxonomic identities were confirmed by the Botanist Dr. Y. J. Thanki, Department of Bioscience, VNSGU, Surat. The fruits of selected plant were washed thoroughly thrice with distilled water and were surface sterilized with absolute alcohol. These were air dried under the shade at room temperature to get completely a dried product. The dried fruits were then milled in an electrical mixer grinder to get fine powder and stored in air tight bottle at 4 – 8^{o}C till further use.

Plant Extraction: Plant extracts were prepared by maceration.[9] 10 g of the plant material was extracted with 100 ml each of petroleum ether, water and ethanol in a flask. The flasks were kept on a flask shaker for 24 h at room temperature. The solvents from non polar (ether) and semi polar (ethanol) macerates were evaporated and to each remaining residue 100 ml of sterile distilled water was added. They were further filtered and centrifuged at 5,000 rpm for 10 min. Each supernatant obtained were sterilized using 0.2µ disposable filter.

Culture Media: The microbiological media used for the study were Nutrient agar, Muller Hinton Agar and Nutrient broth. They were purchased from HiMedia Pvt Ltd. Mumbai, India.

Bacterial Cultures: Pathogenic strains of both gram positive and gram negative organisms were isolated from the samples collected from the patients who suffered from the infection of lacrimal system. The samples used in the study were collected at Desai Eye Hospital, Bilimora, Gujarat. The gram positive organisms used were *Staphylococcus aureus* (29 isolaes), Streptococci species (15 isolates) and Coagulase Negative Staphylococci (17 isolates). The gram negative organisms used were *E. coli* (20 isolates), *Pseudomonas aeruginosa* (25 isolates) and Klebsiella species (16 isolates). All the isolated strains were identified using published guidelines[10] and were maintained on Nutrient agar slant. Controls were also maintained using reference organisms (*Staphylococcus aureus* ATCC 19615, *Streptococcus pyogens* ATCC 19615, *Staphylococcus epidermidis* ATCC 12228 *E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 12657).

Inoculum: Suspension of organism was prepared as per 0.5 McFarland standard. Isolated bacterial strains were inoculated in Nutrient broth for 24 hours at 37^{o} C. The turbidity was adjusted using normal saline in such a manner, that it contained approximately 1.5x 10^{8} cells/ml.

Evaluation of Antibacterial Activity: Agar well diffusion method was employed to determine antibacterial activity.[11] The method is based upon the diffusion of the plant extract to such extent that it inhibits the growth of organisms around the hole containing plant extract. 0.1 ml of each test bacterial strain was seeded with 25 ml sterile molten Muller Hinton agar and was poured in sterile petri plates (20 x 90 mm). The bores of 7 mm were prepared using sterile borer in to agar plates. The bores were filled with 0.1 ml of each plant extract. The plates were allowed to stand at room temperature for 15 minutes to allow the diffusion of plant extracts in to the medium and were then incubated at 37^{o} C for 24 h. Controls were also maintained using reference standard organisms. The antibacterial activity was determined by indicating mean zones of inhibition in mm for each species isolate were plotted using a ruler on the underside of the plate.

Results and Discussion

Plants have provided a source of inspiration for novel drug compounds as plant-derived medicines and have made significant contribution towards human health. The results of *in vitro* antibacterial activity of the selected plant extracts against the bacterial isolates are summarized in Fig.1.

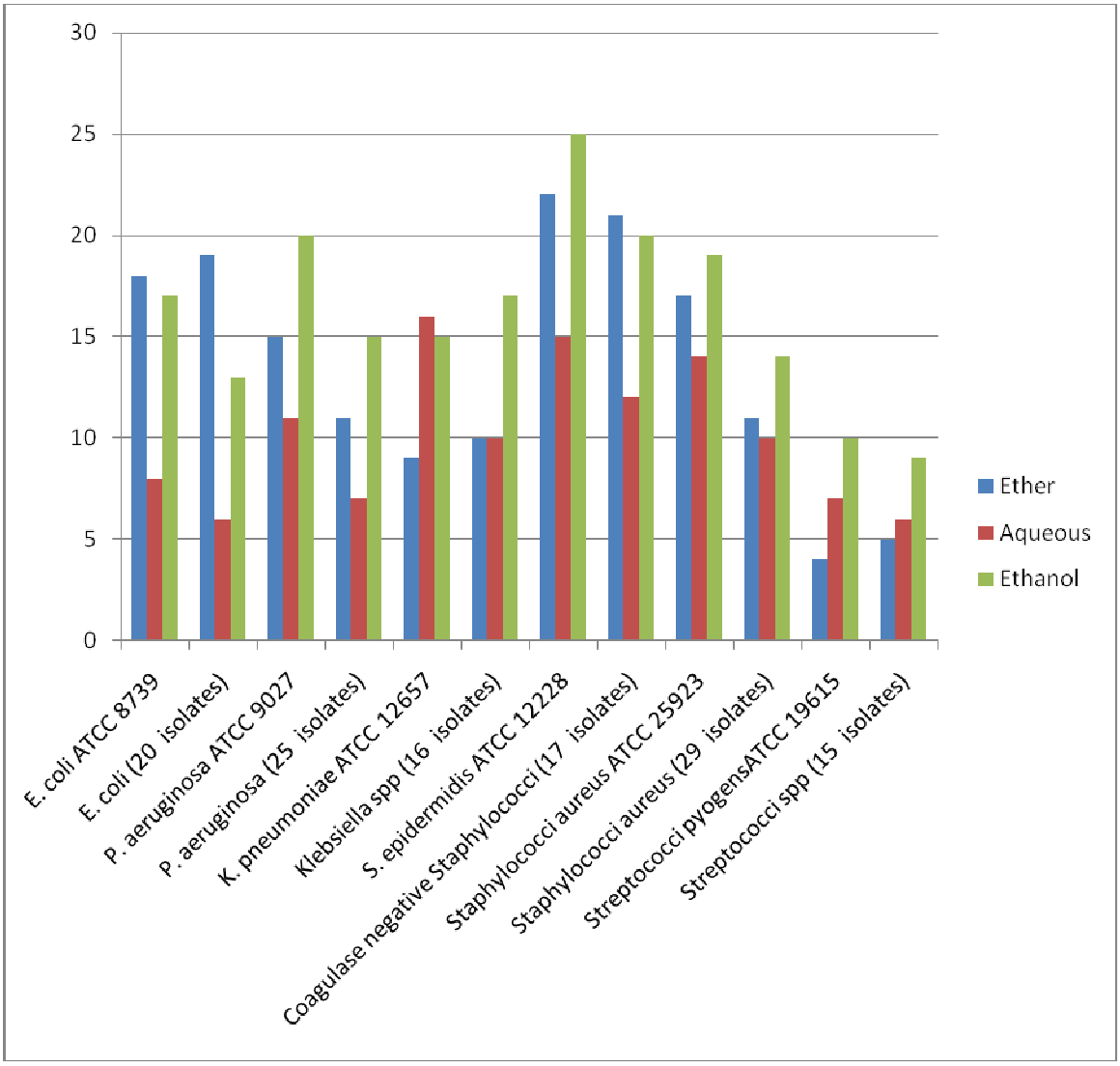

Fig. 1: Antibacterial activity of *T. chebula* against the bacterial isolates causing dacryocystitis

Great variation was observed among the different test bacterial strains when screened for the sensitivity against plant extracts varied. All the three plant extracts were effective to both gram positive and gram negative organisms. This is in agreement with the results of previous reports.[12] But the gram positive organisms exhibited more effectiveness than gram negative organisms. The difference may be due to the presence of outer membrane (lipopolysaccharides) of gram negative bacteria. Traditional healers used primarily water as a solvent as well as there were also evidences that as the polarity increases antibacterial activity increases. But, the obtained data showed that semi polar extracts (ethanol) were more effective than non polar (petroleum ether) and polar extracts (water). Similar results were obtained by the reports available in past.[13] This result can be attributed to the presence of botanical compounds in more amounts in the ethanol extracts compared to other extracts. It was also observed that the isolates of *S. aureus* were found to be most sensitive organisms by exhibiting greater zone of inhibition than other bacterial isolates.

Conclusion

T. chebula exhibited good *in vitro* antibacterial activity against the test isolates but *in vivo* studies on this plant is necessary to know its pharmaco-kinetic properties and their toxicity at different body sites. Further studies are also required on the plant extracts to identify the active antibacterial constituents.

References

1. Dutta D, Chakraborty P. Orphan drugs- Its pros and cons. Research journal of pharmaceutical dosage form and technology 2009; 1(2): 59-66.
2. Ajaybhan P, Chauhan N, Chauhan A. Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. Report and Opinion 2010; 2(6): 37-42.
3. Sharma KK, Sangraula H, Mediratta, PK. Some new concepts in antibacterial drug therapy. Indian J Pharmacol 2002; 34: 390.
4. Caufield C. In the rain forest. The Oxford University Press. Chichago. 1991.
5. Chatttopadhyay RR, Bhattacharyya SK. Terminalia chebula: An update. Pharmacognosy Reviews 2007; 1(1): 151-156.
6. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their antientericpotential against multi-drug resistant *Salmonella typhi.* Phytother Res 2004; 18(8): 670-73.
7. Kim HG, Cho JH, Jeong EY, et al. Growth inhibitory activity of active component from *Terminalia chebula* fruits against intestinal bacteria. J Food Prot 2006; 69(9): 2205-9.
8. Vonshak O. Barazani P. Sathiyomoorthy R, et al. Screening of South-Indian medicinal plants for anti-fungal activity. Phyther. Res 2003; 17(9): 1123-5.
9. Pradip P, Leena P, Achaleshwar B. Antibacterial activity of Athyrium pectinatum (Wall.) Presl. Natural Product Radiance 2006; 5(4): 262-65.
10. Murray PR, Baron EJ, Pfaller MA, et al. Manual of clinical microbiology, 7th edn. Washington, DC: American society for microbiology, 2002.
11. Mirghani MES, Yosuf F, Kabbashi NA, et al. Antibacterial activity of mango kernel extracts. J Applied Sci 2009; 9: 3013-19.
12. Kannan P, Ramadevi SR, Waheeta H. Antibacterial activity of Terminalia chebula fruit extract. African Journal of Microbiology Research 2009; 3(4): 180-184.
13. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62(2): 183-193.