Original article

Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens

Kamal Rai Aneja, Radhika Joshi

Department of Microbiology, Kurukshetra University, Kurukshetra-136119, Haryana, India

Received: July 2009 **Accepted:** August 2009

Abstract

Introduction and objective: Dental caries is a common oral bacterial pathology that has been associated with *Streptococcus* spp., mainly *Streptococcus mutans* and *Lactobacillus* spp. Antibacterial activities of *Terminalia chebula* extracts against several bacterial strains have been reported. The aim of this study was to evaluate the possible antimicrobial potential of *T. chebula* fruit extracts (acetone, ethanol, methanol, cold and hot aqueous) against five dental caries causing microorganisms.

Materials and methods: For this purpose, three bacteria *S. mutans, Staphylococcus aureus, Lactobacillus acidophilus* and two yeasts *Candida albicans* and *Saccharomyces cerevisiae* were tested. Antimicrobial activity was tested using agar well diffusion method. All the tested extracts showed antibacterial activity against two bacteria *S. mutans* and *S. aureus* but no antimicrobial activity was observed against *L. acidophilus, C. albicans* and *S. cerevisiae.*

Results: The highest activity was shown by the acetonic extract with a mean diameter of inhibition zone being 25.32mm and a minimum inhibitory concentration (MIC) of 25mg/ml against *S. mutans* and a mean diameter of 32.97mm and a MIC of 12.5mg/ml against *S. aureus* followed by ethanolic, hot aqueous, cold aqueous and methanolic extracts.

Conclusion: These promising findings suggest the presence of antibacterial activity in the tested plant material, exhibited by its bioactive compounds, and serving them as an alternative antimicrobial agent against dental caries causing microorganisms.

Keywords: Dental caries, *Terminalia chebula*, antimicrobial activity, agar well diffusion, inhibition zone

Introduction

Dental caries is a common oral bacterial pathology caused by a biofilm consisting of microorganisms present on the tooth surface [1,2]. It is a disease that has been associated with *Streptococcus* spp., mainly *Streptococcus mutans* and *Lactobacillus* spp. [3,4]. Herbal remedies have long history of use for gum and tooth problems. In many traditional cultures, there are no plastic-bristle brushes, rather, the use of herbal chewing sticks for relieving dental problems is common [5].

Terminalia chebula Retz, (family *Combretaceae*) is a flowering evergreen tree attaining a height up to 30m, with widely spreading branches and a brown roundish crown. The leaves are elliptical, oblong, with an acute tip, cordate at the base, margins entire, glabrous above with a

yellowish pubescence below. The flowers are monoecious, dull white to yellow, with a strong unpleasant odour, borne in terminal spikes or short panicles. The fruits are glabrous, ellipsoid to ovoid drupes, and yellow to orange brown in colour. It is native to India, Pakistan, Nepal, South West of China and Srilanka. *T. chebula* is called the 'King of Medicine' in Tibet and is always listed at the top of the list in Ayurvedic Materia Medica due to its extraordinary power of healing [6].

The dried ripe fruits have traditionally been used in the treatment of asthma, sore throat, vomiting, hiccup, bleeding piles, gout, heart and bladder diseases [7]. Its paste with water is found to be anti-inflammatory, analgesic and having purifying and healing capacity for wounds [8]. Its powder is used as an astringent in loose, bleeding gums and oral ulcers. It is used to increase the appetite, liver stimulant, as stomachic, as gastrointestinal prokinetic agent, in chronic diarrhea and mild laxative [9,10]. Being a mild laxative, it is mild herbal colons cleanse [10]. It is used in various weaknesses, nervous irritability. It promotes the receiving power of five senses [11]. It is good for chronic cough, coryza, sore throat and asthma. It is helpful in renal calculi, dysurea and retention of urine [11,12]. It is useful in skin disorders with discharges like allergies, urticaria and other erythematous disorders [13]. It is given as adjuvant herb in chronic fever [9,14]. It reduces the ill effects of the fat rich, creamy and oily food [9].

Antibacterial activities of *T. chebula* extracts against several bacterial strains have been reported [6,15-17]. It is effective in inhibiting *Helicobactor pylori* [17], *Xanthomonas campestris* pv. *citri* [18] and *Salmonella typhi* [19,20]. In view of these reported medicinal values, the present work was carried out to examine the antimicrobial potential of five different extracts of *T. chebula* fruits against dental caries causing microorganisms.

Materials and methods

The fresh matured fruits of *T. chebula* were collected from the local market of Delhi, India. Dr. Vashishta (Botany Department) Kurukshetra University, Kurukshetra confirmed the identification of the specimen.

Extraction

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30°C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in air-tight bottles. Four different solvents namely ethanol, methanol, acetone and aqueous (hot and cold) were used for extraction.

A 10g amount of pulverized fruit was separately soaked in 100ml of acetone, ethanol, methanol, and cold sterile distilled water for 24h. In addition, the same amount (i.e. 10g) of pulverized fruit was immersed in 100ml of hot sterile distilled water (100°C) and allowed to stand for 30min on a water bath with occasional shaking, and kept undisturbed for 24h. Each preparation was filtered through a sterilized Whatman No.1 filter paper and the filtered extract was concentrated under vacuum below 40^{o}C using Heidolph, VE-11 rotaevaporator [6, 21]. The dried extract thus obtained was exposed to UV rays for 24h and checked for sterility on nutrient agar plates and stored in labelled sterile bottles in a freezer at 4°C until further use [22].

Tested microorganisms

Three dental caries causing bacteria *Streptococcus mutans* (MTCC*497), *Staphylococcus aureus* (MTCC 740), *Lactobacillus acidophilus* (MTCC *447) and two yeasts *Candida albicans* (MTCC

227) and *Saccharomyces cerevisiae* (MTCC 170) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. The microorganisms were subcultured on the specific media recommended for different microorganisms such as Brain heart infusion agar (*S. mutans*), Nutrient agar (*S. aureus*), Lactobacillus MRS agar (*L. acidophilus*), Malt yeast agar (*C. albicans* and *S. cerevisiae*) and incubated aerobically at 37°C. The media were procured from Himedia Laboratory Pvt. Ltd., Bombay, India. Identification of all the strains was confirmed by standard biochemical and staining methods [23-25].

Screening for antimicrobial activity

The acetone, methanol, ethanol, cold and hot water *T. chebula* fruit extracts were used for the screening. Antimicrobial activity of various extracts was determined by the agar well diffusion method [26]. In this method, pure isolate of each microbe was subcultured on the recommended specific media for each microorganism at 37°C for 24h. A plate of each microorganism was taken and a minimum of four colonies were touched with a sterile loop and transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 cells/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay.

100µl of inoculum of each test organism was spread onto the specific media plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells or cups of 8mm were made with a sterile borer in the inoculated agar plates and the lower portion of each well was sealed with a little specific molten agar medium. The extracts were reconstituted in 20% dimethylsulphoxide (DMSO) for the bioassay analysis [27]. A 100µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated specific media agar plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h [28,29]. Sterile DMSO served as the negative control and ciprofloxacin (for bacteria) and amphotericin B (for fungi) served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm [30]. The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h [31]. The MIC for the extracts was determined by following the modified agar well diffusion method [26]. A twofold serial dilution of each extract was prepared by first reconstituting the powder in 20% DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml.

A 100µl volume of each dilution was introduced into wells (triplicate) in the specific media agar plates already seeded with 100µl of standardized inoculum (10^6 cells/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 hrs and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition, considered as the MIC, was recorded for each test organism [22].

Results

The results of antimicrobial activity of acetone, ethanol, methanol and aqueous

(hot and cold) extracts of *T. chebula* by agar well diffusion method revealed that all the five extracts of *T. chebula* showed antimicrobial activity against two dental caries causing bacteria only i.e. *S. mutans* and *S. aureus*. Highest mean diameter of inhibition zone was produced by the acetonic extracts {25.32mm and a MIC of 25mg/ml (Table1) against *S. mutans* and 32.97mm and a MIC of 12.5mg/ml against *S. aureus*} followed by ethanolic {22.65mm against *S. mutans* and 31.32mm against *S. aureus*}, hot aqueous {21.96mm against *S. mutans* and 27.97mm against *S. aureus*}, cold aqueous {21.65mm against *S. mutans* and 25.32 against *S. aureus*} and methanolic {20.96mm against *S. mutans* and 23.65mm against *S. aureus*} extracts. No antimicrobial inhibitory activity was shown by any of the five tested extracts of *T. chebula* against *L. acidophilus*, *C. albicans* and *S. cerevisiae*. The positive bacterial control ciprofloxacin showed an antimicrobial inhibitory zone of 27.32mm against *S. mutans*, 34.66mm against *S. aureus* and 25.65mm against *L. acidophilus* and the antifungal positive control amphotericin-B produced a mean diameter of inhibition zone of 13mm against *C. albicans* and 11.94mm against *S. cerevisiae* while the negative control i.e. DMSO produced no observable zone against any of the tested microorganism.

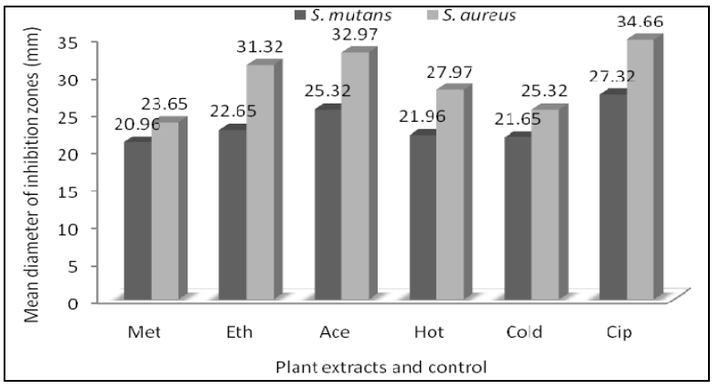

Fig. 1: Comparative antibacterial activity of five extracts of *T. chebula* against dental caries causing *S. mutans* and *S. aureus* along with the positive control (ciprofloxacin)
Met, Methanol; Eth, Ethanol; Ace, acetone; Hot, hot aqueous; Cold, cold aqueous; Cip, Ciprofloxacin

Table 1: Minimum Inhibitory Concentration (MIC) of *T. chebula*

T. chebula extracts	MIC (mg/ml)	
	S. mutans	*S. aureus*
Acetone	25	12.5
Methanol	25	12.5
Ethanol	25	12.5
Cold aqueous	25	12.5
Hot aqueous	25	12.5

Discussion

We chose *S. mutans*, *L. acidophilus* and *C. albicans* as test microorganisms for our study because they have been implicated in dental caries [32,33]. *Candida albicans* is also the most common yeast isolated from the oral cavity, and is associated with fungal oral infections, endocarditis and septicemia [34]. *S. aureus*, a major human pathogen, is responsible for a number of hospital-acquired infections and propagates mainly in mouth and hands acquired in the hospital environment [35-37]. *Saccharomyces*

cerevisiae considered to be an opportunistic pathogen in the oral cavity, may induce significant oral risks by acting as a tertiary colonizer in dental caries and causing both superficial and invasive infections [38].

Results of our foregoing findings reveal that all the tested extracts of *T. chebula* exhibited growth inhibitory activity against two dental caries causing bacterial strains evaluated. The susceptibility of the positive control for bacteria i.e. ciprofloxacin towards the acetonic extract of *S. aureus* was more or less the same. It is also observed that the acetonic extract of *T. chebula* was more potent against both *S. mutans* and *S. aureus* compared to other tested extracts. The acetone and ethanol extracts of fruits of *T. chebula* showed greater antimicrobial activity than the corresponding water and methanolic extracts. This finding is interesting, because in the traditional method of treating a microbial infection, decoction of the plant parts or boiling the plant in water was employed. Whereas, according to the present study, preparing an extract with an organic solvent (acetone and ethanol) shows a better antimicrobial activity [39,40].

The result further revealed that antibacterial potency of the bioactive compounds was not affected when extracted in boiling water indicating that the plant material contains thermo stable bioactive compounds. It is also important to note that susceptibility of the pathogens varied with solvent extract and aqueous extract. This indicates the involvement of more than one active principle of biological significance. Moreover, the potential for developing antimicrobial drugs from plants appears rewarding, as it will lead to the development of a phytomedicine that will act more effectively against microorganisms. Therefore, such screening experiments form a primary platform for further phytochemical and pharmacological studies that may open the possibilities of finding new clinically effective antimicrobial compounds. Thus, results from the previous and present studies have established that *T. chebula* is a potential candidate plant, which could be incorporated into orodentrifice.

Conclusion

Since all the tested extracts of *T. chebula* were highly effective against two of the tested dental caries causing bacteria, purification and toxicological studies of the plant and *in vivo* trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental caries causing bacteria. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *T. chebula* should be emphasized for the control of dental caries.

Acknowledgment

We would like to thank Dr. BD. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, for rending help in confirmation of the identification of the plant, Dr. Tapan Chakrabarti, Institute of Microbial Technology, Chandigarh, for providing the microbial cultures and the Chairperson of the Department of Microbiology for providing laboratory facilities.

References

1) Allaker RP, Douglas CWI. Novel anti-microbial therapies for dental plaque-related diseases. *Int J Antimicrob Agents*. 2009; 33(1): 8-13.
2) Ambrosio SR, Furtado NAJC, De Oliveira DCR, Da Costa FB, Martins CHG, De Carvalho TC, Porto TS, Veneziani RCS. Antimicrobial activity of kaurane

diterpenes against oral pathogens. *Z Naturforsch.* 2008; 63c, 326-330.
3) Chung JY, Choo JH, Lee MH, Hwang JK. Anticariogenic activity of macelignan isolated from *Myristica fragrans* (nutmeg) against *Streptococcus mutans*. *Phytomedicine.* 2006; 13: 261-6.
4) Hirasawa M, Takada K. Susceptibility of *Streptococcus mutans* and *Streptococcus sobrinus* to cell wall inhibitors and development of a novel selective medium for *S. Sobrinus*. *Caries Res.* 2002; 36: 155-160.
5) Bo A. Importance of herbs for dental health. World dental.org, 2008.
6) Bag A, Bhattacharyya SK, Bharati P, Pal NK, Chattopadhyay RR. Evaluation of antibacterial properties of Chebulic myrobalan (fruit of *Terminalia chebula* Retz.) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli. Afr JPS.* 2009; 3(2): 025-9.
7) Kirtikar KR, Basu BD. *Terminalia chebula.* In: Indian Medicinal Plants, 2nd ed., Allahabad, India, Lolit Mohan Basu Publication, 1935; 1020-23.
8) Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, CSIR, 1956.
9) Sharma PV. Dravya Guna Vigyana, Vol. 2, Chaukhamba Bharati Academy, 1995; 753-8.
10) Nadkarni AK. *Terminalia chebula.* In: Dr. K.M. Nadkarni's Indian Materia Madica, 3rd ed., Bombay, Popular Prakashan Pvt. Ltd., 1976; 1202-11.
11) Das B. Materia Medica of Ayurveda. New Delhi, Jain Publishers, 1991; 8.
12) The Wealth of India - Raw Materials, Vol. 10, Publication and Information Directorate. New Delhi, CSIR, 1950; 171-7.
13) Dastur JF. *Terminalia chebula.* In: Medicinal Plants of India and Pakistan. Bombay, D.B. Taraporevala Sons & Co. Pvt. Ltd., 1962; 162-3.
14) Wealth of Asia. CD-ROM (D-2.3) NISCOM, New Delhi, 1996.
15) Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobactor pyloli*. *Int J Antimicrob Agents.* 2001; 18, 85-8.
16) Kim HG, Cho JH, Jeong EY, Lim JH, Lee SH. Growth inhibitory activity of active component of *Terminalia chebula* fruits against intestinal bacteria. *J Food Prot.* 2006; 69(9): 2205-9.
17) Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S, Datta S, Pal NK. Antibacterial activity of black myrobalan (Fruit of *Terminalia chebula* Retz.) against uropathogen *Escherichia coli*. *Phcog Rev.* 2007; 11: 212-5.
18) Afzalakhtar M, Rabber-Bhatti MH, Aslam M. Antibacterial activity of plant diffusate against *Xanthomonas compestris pv*. citri. *Int J Pest Mgt.* 1997; 43(2): 149-53.
19) Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi. Phytother Res.* 2004; 18: 670-3.
20) Kannan PI, Ramadevi SR, Waheeta H. Antibacterial activity of *Terminalia chebula* fruit extract. *Afr J Microbiol Res* 2009; 3(4), 180-4.
21) Ogundiya MO, Okunade MB, Kolapo AL. Antimicrobial activities of some Nigerian chewing sticks. *Ethnobot Leaf.* 2006; 10: 265-71.
22) Nkere CK, Iroegbu CU. Antibacterial screening of the root, seed and stem bark extracts of *Picralima nitida. Afr J Biotechnol* 2005; 4(6): 522-6.
23) Aneja KR. Experiments in Microbiology Plant Pathology and Biotechnology. New Delhi: New Age International Publishers, 2003.
24) Benson HJ. Microbiological Applications: Laboratory Manual in General Microbiology. USA: McGraw Hill Publication, 2004.
25) Cappuccino JG, Sherman N. Microbiology Lab Manual. USA: Benjamin-Cummings Publishing Company, 1995.
26) Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for

antibacterial activity. *J Ethnopharmacol.* 2001; 78: 119-27.
27) Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, Siva R, Jayakumararaj R. Investigations on antibacterial activity of leaf extracts of *Azadirachta indica* A. Juss (Meliaceae): a traditional medicinal plant of India. *Ethnobot Lealf.* 2008; 12: 1213-7.
28) Khokra SL, Prakash O, Jain S, Aneja KR, Dhingra Y. Essential oil composition and antibacterial studies of *Vitex negundo* Linn. extracts. *Indian J Pharmt Sci.* 2008; 70(4): 522-6.
29) Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. *J Ethnopharmacol.* 1980; 23: 127-49.
30) Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J App Microbiol.* 1999; 86: 985-90.
31) Thongson C, Davidson PM, Mahakarrchanakul W, Weiss J. Antimicrobial activity of ultrasound-assisted solvent-extracted spices. *Lett Appl Microbiol.* 2004; 39: 401-6.
32) Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices: Results of an in vitro diffusion method study. *J Am Dent Assoc.* 2004; 135: 1133-41.
33) Joshi AR, Joshi K. Ethnobotany and Conservation of Plant Diversity in Nepal. Kathmandu, Nepal, Rub Rick, 2005.
34) Bagg J. Essentials of Microbiology for Dental Students. New York, Oxford University Press, 1999.
35) Knighton HT. Study of bacteriophage types and antibiotic resistance of *Staphylococci* isolated from dental students and faculty members. *J Dent Res.* 1960; 39: 906-11.
36) Lowy FD. *Staphylococcus aureus* infections. *N Eng Med.* 1998; 339(8): 520-32.
37) Piochi BJ, Zelante F. Contribution to the study of *Staphylococcus* isolated in the mouth.III. *Staphylococcus* isolated from dental plaque. *Rev Fac Odontol Sao Paulo.*1975; 13(1): 91-7.
38) Bonjar GHS. Anti yeast activity of some plants used in traditional herbal medicine of Iran. *J Biol Sci.* 2004; 4: 212-5.
39) Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999; 12: 564-82.
40) Nair R, Kalariya T, Sumitra C. Antibacterial activity of some selected Indian medicinal flora. *Turk J Biol.* 2005; 29: 41-7.

Address for correspondence:
Radhika Joshi, Department of Microbiology, Kurukshetra University, Kurukshetra-136119, Haryana, India
Tel: 9355566163; Fax: 009111 23557580
Email: joshi_radhika31282@yahoo.com