Pharmacognosy Communications
Volume 1 | Issue 2 | Oct-Dec 2011
www.phcogcommn.org

Research Letter

Screening of Methanolic Bark Extract of *Albizia odoratissima* for Antimicrobial Activity

Dinesh Kumar[1]*, Sonia Kohli[1], Sunil Kumar[1], Jyoti Gupta[1] Pranay Jain[2] and Ram Kumar Pundir[3]

[1]Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, Haryana, India. [2]Department of Biotechnology, U.I.E.T., Kurukshetra University, Kurukshetra-136119, Haryana, India. [3]Department of Biotechnology, A.C.E. & A.R., Ambala-133101, Haryana, India

ABSTRACT: *Introduction: Albizia odoratissima* Benth., [Family: Mimosaceae] is commonly known as Black Siris. In traditional Indian Medicine, bark of *A. odoratissima* is used in the treatment of leprosy, ulcers and cough. The objective of this study was to screen methanolic extract of *A. odoratissima* bark for antimicrobial activity owing to its ethnomedicinal use. *Method:* In agar well diffusion method, plate count agar (PCA) plates were inoculated with 100µl of each pathogenic microorganism adjusted to standardized inoculum (1.5 × 10^8 CFU/ml) in triplicates and spread with sterile swabs. After incubation for 24 hrs at 37 °C, the plates were observed. The zone of inhibition was measured and expressed in millimeters. *Result and Discussion:* The extract showed good antibacterial activity only against Gram positive bacteria with zone of inhibition ranging from 12 mm to 21 mm. Maximum zone of inhibition of 21 mm at 80 µg/ml concentration was observed against *S. mutans*. The extract did not show any inhibitory activity against Gram-negative bacterium (*P. auriginosa*). The test antibiotic ciprofloxacin showed activity with zone of inhibition ranging from 29 mm to 34 mm while ketoconazole showed zone of inhibition of 28 mm against *C. albicans.* Phytochemical analysis of the extract showed the presence of steroids, tannins, phenolics, saponins. *Conclusion:* From the study, it can be concluded that the methanolic extract of *A. odoratissima* bark possesses significant antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus, Staphylococcus mutans*) and yeast (*Candida albicans*) which might be due to the presence of steroids, saponins and phenolics in the bark, with no effect on Gram negative bacteria.

KEYWORDS: *Albizia odoratissima,* Diffusion, Folk, Incubation, Pathogenic.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Enteric bacteria are major causes of food-borne illnesses and gastrointestinal problems in the developing countries and human beings around the world.[1] Clinically, antibiotics produced by soil microorganisms and higher plants have been known sources of antibiotics.[2] According to Charles and Simmon (1992), the use of medicinal plants also contributes significantly to primary health care in various parts of the world.[3] Numerous studies have reported that medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens.[4,5] Therefore, medicinal plant extracts are emerging as alternatives to conventional natural preservatives for the control of the growth of food borne pathogens and food spoilage bacteria as they are generally safe to humans, and environmentally friendly.[6]

Albizia odoratissima Benth. (family: Mimosaceae) commonly known as Black Siris (Hindi), Bhusirisah (Sanskrit) Karmaru (Punjabi), Cinduga (Telgu), Karuvagai (Tamil) and Ceylon rose-wood (Eng.), is a large erect tree distributed in the sub-Himalayan tracts, slopes and valleys up to 1,500 m, common in peninsular India, Assam, West Bengal and also throughout the western ghats of South India.[7-9] It is a large deciduous tree, 15-25 m tall with dark grey bark, leaves bipinnate, leaflets 4-15 pairs, obliquely oblong whereas flowers being pale yellowish white, fragrant in terminal heads and fruits are oblong pod, redish brown at maturity.[9] In traditional Indian Medicine, the bark of *A. odoratissima* is used in the treatment of leprosy, ulcers and cough.[10] The bark is astrigent, acrid, cooling, depurative, expectorant and useful in skin diseases, rheumatism, erysipelas cough, bronchitis, diabetes and burning sensation. The bark contains condensed tannins such as D-catechin, isomers of leucocyanidin, melacidin and melanoxitin.[11] In the current wave of antimicrobial resistance against

*Correspondence: Email: dineshbarbola@yahoo.co.in; Contact no: +91-1744-239617(office). +91 9466772500 (Mobile) Fax: +91-1744-238277
DOI: 10.5530/pc.2011.2.9

chemotherapeutic drugs, there is need to search for plants that could be resistance-free and affordable. The objective of this study was to screen methanolic extract of *A. odoratissima* bark for antimicrobial activity owing to its ethnomedicinal use.

MATERIALS AND METHODS

Procurement and identification of Plant Material

The bark of plant was collected from the Herbal Nature Park, Yamuna Nagar, during November 2009 and identified as *Albizia odoratissima* (Family: Mimosae) by Dr. H.B. Singh, Scientist In charge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (No.: NISCAIR/RHM 1382/184) has been deposited.

Preparation of Extracts

Shade dried plant bark was powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and methanol using soxhlation method. The extract was concentrated to dryness using Rotary evaporator (Heidolph, model-4011, USA). The yield of the extract was found to be 20.34% w/w. The extract was preserved in a refrigerator at 4 °C.

Phytochemical study

Methanolic bark extract of the plant was screening for the presence of active constituents as per reported methods.[12]

Test microorganisms

A total of four test bacteria namely *Staphylococcus aureus* MTCC-7443, *Streptococcus mutans* MTCC-570 (Gram-positive bacteria) and *Pseudomonas aeruginosa* MTCC-2295 (Gram-negative bacterium) and one yeast *Candida albicans* MTCC-227 were obtained from MTCC, IMTECH, Chandigarh.

Agar well diffusion method for antimicrobial activity

In agar well diffusion method, plate count agar (PCA) plates were inoculated with 100µl of each pathogenic microorganism adjusted to standardized inoculum (1.5×10^8 CFU/ml) in triplicates and spread with sterile swabs. Wells or cups of 8 mm size were made with sterile cork borer into agar plates containing the microbial (bacterial and yeast) inoculum and the lower portion was sealed with a little molten agar medium. 100µl volume of the four different concentrations (80µg/ml to 20µg/ml**)** of methanolic extract of *Albizia odoratissima* was poured into a well of inoculated plates. Ciprofloxaxin (antibacterial) and ketoconazole (antifungal drug) were used as positive control which was introduced into a well instead of a extract. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37 °C, the plates were observed. Antimicrobial activity was indicated by an inhibition zone surrounding the well containing the extract. The zone of inhibition was measured and expressed in millimeters. Antimicrobial activity was recorded if the zone of inhibition was greater than 8 mm.[13,14]

RESULTS AND DISCUSSION

The methanolic extract of *Albizia odoratissima* was evaluated against three human pathogenic bacteria and one yeast by agar well diffusion method as depicted in Table 1. The extract showed antibacterial activity only against Gram-positive bacteria, with zone of inhibition ranging from 12 mm to 21 mm. A maximum zone of inhibition of 21 mm at 80 µg/ml concentration was observed against *S. mutans*. The extract did not show any inhibitory activity against Gram-negative bacterium (*P. auriginosa*). The extract was found to be effective at the three concentrations (80 µg/ml, 60 µg/ml and 40 µg/ml) against yeast, *C. albicans*

Table 1: Antimicrobial activity of methanolic extract of Albizia odoratissima against human pathogenic microorganisms

Chemical compound	Concentrations	Diameter of zone of growth inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	Yeast
		Staphylococcus aureus (MTCC-74430	*Streptococcus mutans* (MTCC-5700	*Pseudomonas aeruginosa* (MTCC-2295)	*Candida albicans* (MTCC-227)
Methanolic bark extract of *Albizia odoratissima*	80 µg /ml	14 mm	21 mm	NA	18 mm
	60 µg /ml	13 mm	20 mm	NA	17 mm
	40 µg /ml	13 mm	17 mm	NA	11 mm
	20 µg /ml	12 mm	17 mm	NA	NA
Ciprofloxacin disc (Positive control)	5.0 µg	29 mm	29 mm	34 mm	NA
Ketoconazole disc (Positive control)	10.0µg	NA	NA	NA	28 mm

NA- No activity

with diameter of zone of growth inhibition ranging between 11 mm and 18 mm. This extract may be used to control the growth of Gram-positive bacteria and yeast. The test antibiotic ciprofloxacin showed antimicrobial activity with zones of inhibition ranging from 29 mm to 34 mm while ketoconazole showed zone of inhibition of 28 mm against *C. albicans*. The methanolic extract showed good activity against Gram-positive bacteria and yeast. Zaika also reported that Gram-negative bacteria were less susceptible to the antimicrobial agents than Gram-positive bacteria.[15] This may possibly be due to the presence of high lipid content in the cell walls of Gram-negative bacteria. Furthermore, the outer membrane of Gram-negative bacteria is known to present a barrier to penetration of numerous antibiotic molecules, and the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside.[16] Phytochemical analysis of the extract showed the presence of steroids, tannins, phenolics, saponins in the bark. Earlier, phenolic compounds from *Olea europaea* L.,[17] saponins from *Sorghum bicolor* L.[18] and steroids from *Jatropha padagrica*[19] were reported for antimicrobial activity. Therefore, the antimicrobial effect shown by methanolic extract of the plant might be due to the presence of these constituents. It can also be concluded from the present study that the antimicrobial activity of the extract is directly proportional to the concentration used and is thus concentration-dependent and hence, extract *Albizia odoratissima* might be used as an alternative to the antibiotic used in pharmaceuticals on the basis of inhibition exhibited by the methanolic extract against the pathogenic microorganisms.

CONCLUSION

From the study, it can be concluded that the methanolic extract of *A. odoratissima* bark possesses significant antimicrobial activity against Gram positive bacteria (*Staphylococcus aureus, Staphylococcus mutans*) and yeast (*Candida albicans*) with no effect on Gram negative bacteria.

REFERENCES

1. Stainer RY, Ingraham JL, Wheelis ML. Painter PR General Microbiology. Macmillan Press 1987; 5th ed: p.21-40.
2. Trease G, Evans W. Pharmacognosy. Saunders and London 1972; 15th ed: p.161-163.
3. Charles DJ. A new geranoil chemotype of *Ocimum gratissimum*. J. Essential Oil Res 1992; **4**:231-234.
4. Mari M, Bertolini P, Pratella GC. Non-conventional methods for the control of postharvest pear diseases. J. Appl. Microbiol 2003; **94**:761-766.
5. Obagwu J, Korsten L. Control of citrus green and blue molds with garlic extracts. European J. Plant Pathol. 2003; 109:2-7.
6. Thangavelu R, Sundararaju P, Sathiamoorthy S. Management of anthracnose disease of banana caused by *Colletotrichum musae* using plant extracts. J. Horti. Sci. Biol. 2004; 79:664-668.
7. The Wealth of India-Raw materials. National Institute of science communication, CSIR, New Delhi, 2006; 1:128
8. Gamble JS. Flora of the Presidency of Madras. Botanical Survey of India. Calcutta 1956; 2:306.
9. Pullaiah T. Encyclopedia of World Medicinal Plants. Regency publications. New Delhi, 2006; 1:p.101-102.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants, Lalit Mohan Basu, Allahabad; 1935:p.939.
11. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants: Complete Source Book. Agrobios. Jodhpur: India; 2003:p. 27.
12. Khandelwal, KR. Practical Pharmacognosy. 18th ed., Pune 2007: Nirali Prakashan, pp. 10-14.
13. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plants extracts. *Int. J. Antimicrob. Agents* 2001; **17**:527-529.
14. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol* 1988; **23**:127-49.
15. Zaika, LL. Spices and herbs: Their antimicrobial activity and its determination. *J. Food Safety* 1988; **9**:97-118.
16. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol* 1999; **86**:985-990.
17. Pereira AP, Ferreira ICFR, Marcelino F, Valentao P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA. Phenolic Compounds and Antimicrobial Activity of Olive (*Olea europaea* L. *Cv.* Cobrançosa) Leaves. *Molecules* 2007; **12**:1153-1162.
18. Soetan KO, Oyekunle MA, Aiyelaagbe OO, Fafunso MA. Evaluation of the antimicrobial activity of saponinsextract of *Sorghum Bicolor* L. Moench. Afr. J. Biotechnol. 2006; 5, 2405-2407.
19. Odebiyi OO. Antimicrobial and antifungal properties of the extractives of *Jatropha padagrica*. Fitoterapia 1985; **56**:297-299.