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RESEARCH ARTICLE

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COMPARATIVE PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES ON THE BARK OF *WRIGHTIA TINCTORIA* AND *WRIGHTIA ARBOREA*

M. S. KHYADE*[1] AND N. P. VAIKOS[2]

[1]Post Graduate Department of Botany, Sangamner Nagarpalika Arts, D.J. Malpani Commerce and B.N. Sarda Science College, Sangamner-422 605 (MS), India.
[2]Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (MS), India.
Email: mskhyade@rediffmail.com, drvaikos@gmail.com

ABSTRACT

Comparative phytochemical and antibacterial activities of bark of *Wrightia tinctoria* and *Wrightia arborea* were investigated. The bark extracts showed the presence of alkaloids, phenolics, saponins and tannins in both the species. The antibacterial activities of bark of *W. tinctoria* and *W. arborea* in successive different solvent were tested against gram +ve and gram -ve organisms. The chloroform extracts of *W. arborea* showed the broader spectrum of antibacterial activity when compared with *W. tinctoria*. However, *Wrightia tinctoria* is a widely used medicinal plant.

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KEY WORDS

Comparative, bark, *Wrightia* species, phytochemical, antibacterial

INTRODUCTION

In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases. Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs [1-3]. Plant extracts have been used for a wide variety of purposes for many thousands of years [4]. In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies [5]. Antimicrobials of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [6].

Wrightia tinctoria R.Br. and *Wrightia arborea* (Densst.) Mabb. belong to the family Apocynaceae. They are distributed in all districts of deciduous forest of India [7] *W. tinctoria* is commonly called as "Indrajav" and locally as Pandhara kuda, while *W. arborea is* known as Tambda kuda. These species have been important in the traditional healing. However, the former one is widely recognized medicinal plant.

The bark of *W. tinctoria* is considered for antidiarrhoeal, aphrodisiac, anthelmintic, febrifuge, stomachic, toothache, tonic and dog bite [8-10]. It is employed in seminal weakness and flatulence [10], also used in piles and skin diseases [11]. Whereas, a preparation of the bark made from *Wrightia arborea* is found useful in menstrual and renal complaints [8, 9]. Moreover, several studies viz. pharmacognosy, antimicrobial activity and phytochemistry on different parts of these two species studied earlier[11-26]. This work however, is designed to evaluate the comparative account of phytochemical components and antibacterial activity of bark of both species on selected bacterial strains.

MATERIALS AND METHODS

Collection of Plant materials

The fresh barks of *W. tinctoria* were collected in the month of October [2006] from forest area of Aurangabad district (M.S.) and that of W. *arborea* from the Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The plant species were identified with the help of Flora of Marathwada[27] and voucher specimens have been deposited at the Botany department of the university. Plant samples were washed and shade dried at room temperature for 15 days.

Preparation of extracts and phytochemical screening

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 gm of powdered material was extracted in soxhlet extraction apparatus successively with 250 ml of each of the following solvents viz. petroleum ether, chloroform, acetone and methanol[28]. The extracts obtained with each solvent were filtered through Whatman filter paper No.1and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator and were suspended in dimethyl sulphoxide (DMSO) for prior to use.

The extracts of bark of both species were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods[29-31]. The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

Test culture

The test bacteria used for the screening antimicrobial activity were *Bacillus subtilis, Bacillus megaterium, Escherichia coli, Klebsiella planticola, Micrococcus luteus,* MTCC 106, *Pseudomonas aeruginosa* MTCC 2488, *Staphylococcus aureus,* and *Salmonella typhi .,* The cultures were obtained from Microbial Type culture Collection (MTCC), IMTEC, Chandigarh, India. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4ºC. All cultures were checked for viability and purity by regular plating. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37±1ºC for 24 hours.

Screening for antibacterial properties

The antibacterial activities of the successive bark extracts of petroleum ether, chloroform, acetone and methanol of both the plant species were tested by Agar well diffusion method [32]. The culture plates were prepared by pouring 20 ml of sterile nutrient agar. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (8 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 100mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37ºC during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Ampicillin (40 µg/ml) was used as standard antibiotic. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the bark of both species of *Wrightia* are shown (Table 1). It indicates the presence of alkaloids, phenolics, saponins and tannins. The presence of terpenoids is indicated only in *Wrightia tinctoria* while flavonoids are found alone in *W. arborea.* However, steroids, triterpenoids, flavonoids and glycosides were reported to occur[33,34] in *W. tinctoria* and triterpenoids, cardiac glycosides in W. arborea[35]

Table 1
Phytochemical constituents of bark of W. tinctoria and W. arborea

Phytochemicals	*Wrightia tinctoria*	*Wrightia arborea*
Alkaloids	+	+
Anthraquinones	—	—
Cardiac glycosides	—	—
Coumarins	—	—
Flavonoids	—	+
Leucoanthocyanins	—	—
Simple phenolics	++	++
Steroids	—	—
Saponins	++	++
Tannins	+++	+++
Terpenoids	+++	—

Note: *(+) weak, (++) moderate, (+++) strong and (-) absent.*

The antibacterial activities of *W. tinctoria* and W. *arborea* in petroleum ether, chloroform, acetone and methanol extracts examined in the current study and their potency were qualitatively assessed by the presence or absence of inhibition zones and zone diameter

(Table 2). The results showed that the extracts of *W. tinctoria* mediated some degree of activity against bacteria. Except, *K. planticola* other strains like *S. typhi, B.subtilis, B. megaterium, E. coli, P. aeruginosa* and *M. luteus* are inhibited by chloroform, acetone and methanol extracts, while petroleum ether indicated negative inhibition at test concentration.

The antibacterial activitiy of *W. arborea* extracts were the most prominent in the activities against all bacteria tested at test concentration (table 2). Except petroleum ether extracts, others such as chloroform, acetone and methanol showed broader spectrum of activity. However, chloroform extracts of *W. arborea* exhibited significant antibacterial activities against all the bacteria tested but, particularly, to *M. luteus, S.aureus S. typhi and P. aeruginosa.*

Table 2
Antibacterial activity of bark extracts of W.tinctoria and W.arborea

Bacterial strains	Gram stain + / -	Dose (mg/ml)	Inhibition zone in diameters (mm / sensitive strains)								DMSO	Ampicillin (40 μg/ml
			Wrightia tinctoria				***Wrightia arborea***					
			A	B	C	D	A	B	C	D		
Staphylococcus aureus	+	X	—	11	10	14	—	15	14	15	0	23
Bacillus subtilis	+	X	—	13	11	10	—	9	16	14	0	21
Bacillus megaterium	+	X	—	15	13	11	—	13	11	16	0	25
Micrococcus luteus	+	X	—	16	13	11	—	17	15	15	0	30
Escherichia coli	-	X	—	10	9	10	—	14	13	13	0	17
Salmonella typhi	-	X	—	13	11	13	—	15	12	12	0	19
Pseudomonas aeruginosa	-	X	—	11	0	10	—	15	10	11	0	16
Klebsiella planticola	-	X	—	0	0	0	—	13	11	0	0	21

Note: - *X-100mg/ml (100µl/well); A- Petroleum ether, B - Chloroform, C- Acetone, D -Methanol, — no inhibition, Figures are diameter of zone of inhibition (triplicate).*

Based on these results, it is possible to conclude that of two species of *Wrightia*, it is *W. arborea* which exhibited broader range of antibacterial activity to varying degrees, as it is a less known medicinal plant in the Indian literature. Particularly, the chloroform extract of *W. arborea* showed significant antibacterial activities and it can be used as a good antimicrobial agent in new drugs for therapy

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REFERENCES

1. Ahmed, Mohommed Z and Mohammed F, Screening of some Indian Medicinal plants for their antimicrobial properties. Journal Ethnopharmacol, 62,183-193, (1998).
2. Kokosha L, Polesny Z, Rada V, Nepovin A and Vanek T, Screening of some Siberian medicinal plants for antimicrobial activity. Journal Ethnopharmacol, 62, 51-53, (2002).
3. Jones FA, Herbs-useful plants- Their role in history and today. Eur J Gastroenterol Hepatol, 8, 1227-1231, (1996).
4. Hammer KA, Carson CF and Riley TV, Antimicrobial activity of essential oils and other plant extracts. Journal Appl Microbiol, 86,985-990, (1999).
5. Werner F, Okemo P and Ansorg R, Antibacterial activity of East African Medicinal Plants. Journal Ethnopharmacol, 60, 79-84, (1999).
6. Prumal Samy R and Ignacimuthu S, Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. Journal Ethnopharmacol, 69, 63-71, (2000).
7. Sivarajan VV and Balachandran I, Ayurvedic Drugs and Their Plant Sources, Oxford and IBH Publishing Co. PVT. Ltd. New Delhi: 267-269, (1999).
8. Chopra RN, Nayar SI and Chopra IC, Glossary of Indian Medicinal Plants, CSIR, New Delhi, India:259,(1956).
9. Agarwal CK, Economic Plants of India, Kailash Prakashan, Culcutta: 406, (1986).
10. Joshi SG, Medicinal plants, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi: 51,(2000).
11. Khare CP, Indian medicinal plants, Springer-Verlag Berlin, Heidelberg, Newyork: 721-722, (2007).
12. Prasad S and Kaul PN, Pharmacognostical studies of *Holarrhena antidysenterica* and *Wrightia tomentosa* barks, Indian J Pharm, 18, 423-432, (1956).
13. Narayan Aiyer K and Kolammal M, *Wrightia tomentosa* Roem & Schult. Pharmacognosy of Ayurvedic drugs Kerala, Vol 1, Department of Pharmacognosy University of Kerala. Trivendrum: 59-60, (1960).
14. Atal CK and Sethi PD, *Wrightia tinctoria* R.Br. bark an adulterant of kurchi. Journal Pharm Pharmacol, 14, 41-45, (1962).
15. Wahi SP, Wahi AK, Khosa RL and Sharma M, A simple method of distinguishing *Holarrhena antidysenterica* from its adulterant *Wrightia tomentosa.* Indian J Pharm, 39 (1): 25, (1977).
16. Daniel M, Sabnis SD, Chemotaxonomical studies on Apocynaceae. Indian J Ex Biol, 16 (4): 512-513, (1978).
17. Krishnamurthy JR, Kalaimani S and Veluchamy G, Clinical study of Vetapalai (*Wrightia tinctoria*) oil in the treatment of Kalanjagapadai (Psoriasis). Journal Res Ayur Siddha, 2(1): 58-66, (1981).
18. Malvia P., Pharmacognostical investigation of seeds of *Wrightia tinctoria* and *Holarrhena antidysentrica.* Indian J Pharm Educat, 9, 25, (1975).
19. Daniel M. and Sabnis S.D., A chemotaxonomic appraisal of the status of Apocynaceae and Asclepiadaceae. Indian Bot Reptr, 1(2): 84-90, (1982).
20. Parvathi A and Santha Kumari, Chemotaxonomical studies of some Apocynaceae and Asclepiadaceae. Indian J Bot, 7 (2): 198-200, (1984).
21. Ghosh D, Thejmoorth P and Veluchamy G, Anti-inflammatory, analgesic and antipyretic activities of 777 oil-a siddha medicine. Bull Med Ethnobot Res, 6(2-4): 141-154, (1985).

22. Lin LJ, Topcu G, Lotter H, Ruangrungsi N, Wagner H, Pezzuto JM and Cordell GA, Wrightiadione from *Wrightia tomentosa*. Phytochemistry, 31 (12): 4333-4335, (1992).
23. Jolly CI and Nisha RM, Comparative pharmacognostical, Physiochemical and Antibacterial studies on seeds of *Holarrhena antidysenterica* wall and *Wrightia tinctoria* R. Br. Indian J Pharm Sci, 58 (2): 51-54, (1994).
24. Reddy YS.R, Sama Venkalesh T, Ravichandran TS and Sueresh B, Pharmacognostical studies on *Wrightia tinctoria* bark. Pharm Biol, 37(4): 291-295, (1999).
25. Gupta AK, Quality standards of Indian medicinal plants, Vol.1, Indian Council of medical Research, New Delhi: 109-116,(2003).
26. Khyade MS and Vaikos NP, Antibacterial evaluation and phytochemical analysis of *Wrightia tinctoria* R. Br. Leaves. Pharmacologyonline**,** 2, 808-813, (2009**).**
27. Naik VN, Flora of Marathwada, Vol 1, Amrut Prakashan, Aurangabad (M.S), India: 834,(1998).
28. Vogel AI, Elementary practical organic chemistry (Second edition), Orient Longman Limited: 45-168, (1988).
29. Harborne JB, Phytochemical methods, Chapman Hall, London: 100 – 101, (1984).
30. Ajaiyeoba EO, Phytochemical and antimicrobial studies of *Gynandropsis gynandra* and *Buchholzia coriaceae* extracts. Afri J Biomed Res, 3, 161-165, (2000).
31. Edeoga HO, Okwu DE and Mbacbie BO, Phytochemical constituents of some Nigerian medicinal plants. Afri J Biotech, 4, 685-688, (2005).
32. Kavanagh F, Analytical microbiology, Part II. Academic Press, New York: 126, (1972).
33. Rangaswami S, Nageswara RM, Crystalline chemical components of the bark of *Wrightia tinctoria* R. Br. Proceeding of Indian Academy of Science 57A, 115-20,(1963).
34. Reddy YSR,Venkatesh S, Ravichandran T, Suburajau T, Sueresh B, Pharmacognostical studies on *Wrightia tinctoria* bark. Pharm Biol, 37, 291-295, (1999).
35. Kaneria M,Baravalia Y,Vaghasia Y and Chanda S,Determination of Antibacterial and Antioxidant Potential of Some Medicinal Plants from Saurashtra region, India. Ind J Pharm Sci,71(4):406-412, (2009).