Available online on 15.01.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

An investigation of *Baccharoides anthelmintica* (L.) Moench seed extract for antibacterial and antioxidant activities

Dr. Ved Prakash*

Department of Botany, Bhagat Singh Govt. P.G. College, Jaora (MP) 457226 India

Article Info:

Article History:

Received 16 Oct 2022
Reviewed 19 Dec 2022
Accepted 28 Dec 2022
Published 15 Jan 2023

Cite this article as:

Prakash V, An investigation of *Baccharoides anthelmintica* (L.) Moench seed extract for antibacterial and antioxidant activities, Journal of Drug Delivery and Therapeutics. 2023; 13(1):29-32

DOI: http://dx.doi.org/10.22270/jddt.v13i1.5901

*Address for Correspondence:

Dr. Ved Prakash, Department of Botany, Bhagat Singh Govt. P.G. College, Jaora (MP) 457226 India

Abstract

Use of herbal remedies is on the rise in developed and developing countries. Plant kingdom is a gold mine for novel and affordable antimicrobial and antioxidant compounds. The medicinal properties of plants are due to metabolites especially secondary compounds produced by plant species around the globe. The current study was designed to investigate the seed extract of *Baccharoides anthelmintica* (L.) Moench for its antibacterial and antioxidant activities. The antibacterial activity of the acetone, aqueous and methanol seed extracts was determined *in-vitro* against medically important pathogens such as *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus* and *Yersinia pestis* by agar-well diffusion method using different concentrations (25, 50, 75 and 100%). Results showed low to significant antibacterial activity against the mentioned pathogenic bacterial species. The methanol extract of *B. anthelmintica* showed maximum zone of inhibition (20.40±0.68 mm) in the growth of *L. monocytogenes* which was followed by *P. aeruginosa* (19.10±1.77 mm), *S. aureus* (18.55±2.20 mm), *E. coli* (16.00±0.60 mm) and *Y. pestis* (16.00±0.00 mm) at 100% of its concentration respectively. Methanol seed extract was found to be more effective against selected pathogenic bacterial species as compared to acetone and aqueous seed extracts. Further the seed extract inhibited gram-positive bacteria more efficiently than gram-negative bacteria. The antioxidant capacity of the different seed extracts (methanol, acetone and aqueous) of *B. anthelmintica* was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay at different concentrations (20, 40, 60, 80 and 100 µg/mL). The plant showed 14.40±0.05 and 13.20±0.55 % DPPH radical scavenging potential in methanol and acetone extracts respectively at 100 µg/mL and exhibited no radical scavenging potential in case of aqueous extract. Therefore, the seed extracts of this plant can be selected for further investigation to discover or determine their ultimate therapeutic potential.

Keywords: *Baccharoides anthelmintica*, seed extract, agar well diffusion, zone of inhibition, DPPH

INTRODUCTION

Medicinal plants are known to contain physiologically active principles/compounds that over the years have been exploited in traditional medicine for the treatment of various diseases because of their anti-microbial properties [1,2]. The development of bacterial resistance to presently available antibiotics has aroused the need to search for new antibacterial agents from plant-based sources.

Free radicals, well known as reactive oxygen species (ROS), radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers are generated continuously resulting in extensive damage to tissues and biomolecules leading to various diseased conditions[3]. The antioxidant-based drug formulations are being widely used for the prevention and treatment of complex diseases such as Alzheimer's disease, cancer, atherosclerosis, stroke, diabetes etc.[4] So, the medicinal plants having antioxidant potential are usually being employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress[5,6]. Further, it has been established that the antioxidant effect of plant and plant-based products is mainly attributed to phenolic compounds such as flavonoids and phenolic acids[7,8]. Phenolic compounds present in medicinal plants exhibit strong antioxidant activity and may help to protect the cells against the oxidative damage caused by reactive oxygen[9].

Baccharoides anthelmintica (Syn: *Vernonia anthelmintica* (L.) Willd.) is widely distributed in different parts of the world i.e. India, Pakistan, Nepal, Sri Lanka, Zimbabwe, Zambia, Botswana, Malawi and Congo (Kinshasa). *B. anthelmintica* is a large annual herb about 60-100 cm tall in height. The stem is robust, erect and leafy with velvety branches with alternatively arranged leaves, 5-8 cm long, usually obovate to lance-shaped. Flower heads are borne at the end of the branches in 10-20 cm clusters where seed pods are 4-6 cm long, ribbed and oblong[10].

As the scientific name of the plant indicates, *Baccharoides anthelmintica* contains compounds that can be used as valuable anthelmintic medicines. This plant is also used for the treatment of asthma, hiccup, inflammatory swellings, sores and itching of the eyes as well[11]. The seeds of this plant are of great repute as a medicine for white leprosy (leucoderma) and other skin diseases[12]. Because of above mentioned useful properties, I planned to carry out antibacterial and antioxidant studies of seed extracts of *Baccharoides anthelmintica*.

MATERIALS AND METHODS

Collection of plant material

The seeds of *Baccharoides anthelmintica* were collected from Devamanal area of District Sirmaur Himachal Pradesh.

Processing of plant material

After treatment with 2% Mercuric chloride, seeds were allowed to shade dried for 15-20 days. After drying plant material was crushed to form a fine powder with the help of pestle mortar and was stored at room temperature in air tight containers.

Preparation of Acetone and Methanol extracts

3 g of fine powder of *B. anthelmintica* was taken in three different Erlenmeyer flasks to which 30 mL of the acetone/methanol/water were added. After covering these flasks with aluminium foils, they were allowed to stand for 3-5 days for extraction purposes. After this, the extracts were separated with the help of the Whatman filter paper no. 1 and by using rotary evaporator at 40°C. The dried extracts were collected and weighted and finally stock solutions of conc. 50 mg/mL were prepared.

Procurement of bacteria

Bacterial strains used for antibacterial studies were *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa, Staphylococcus aureus* and *Yersinia pestis* which were procured from IGMC, Shimla and Department of Microbiology & Biotechnology, Himachal Pradesh University, Summer Hill, Shimla. The collected pathogens were eventually revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening the Antibacterial Activity of *Baccharoides anthelmintica*

Screening of plant extracts (methanol, acetone and aqueous) of *B. anthelmintica* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptones 5 g, Agar 20 g, Distilled Water 1000 mL) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and then poured into Petri plates. Bacterial strains were grown in nutrient broth for 24 hours. A 100 µL of bacterial suspension was spread on each nutrient agar plate and agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25, 50, 75 and 100% concentration of prepared seed extracts of *B. anthelmintica.* The petri plate kept as a control had pure solvent only. The plates were incubated at 37± 2^{0}C for 24 h in the incubation chamber. Eventually, the zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates used and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as a standard[13,14].

$$\text{Percentage of growth inhibition (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \text{x } 100$$

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets.

Antioxidant Activity Test

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of seed extracts was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Blois[15]. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of seed/test extract, 1 mL of DPPH (0.1 mM in methanol) was added. Corresponding blank sample was prepared and ascorbic acid was used as a reference standard. Mixture of 1 mL methanol and 1 mL DPPH solution (without plant extract) was used as a control. All the tests were carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark by using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}\right) \times 100$$

Where, $A_{control}$ is the absorbance of control; A_{sample} is the absorbance of sample

RESULT AND DISCUSSION

Agar well diffusion method is the most widely used method for screening the antimicrobial activity of plant extracts. The antimicrobials present in the plant extract are allowed to diffuse out into the medium where they interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular since there will be a confluent lawn of bacterial/microbial growth[16,17].

The results of agar well diffusion method for determining antibacterial activity of various plant extracts have been shown in Table 1.1. Methanol extract of *Baccharoides anthelmintica* inhibited all the test bacteria except *B. cereus*. The diameter of zone of inhibition (ZOI) ranged from 8.40 to 20.40 mm (maximum ZOI = 20.40±0.68 mm against *L. monocytogenes*). Acetone extract showed inhibition (18.66±0.76 mm) against *L. monocytogenes* only. Similarly, aqueous extract was found to be effective against *Y. pestis* and *L. monocytogenes* only and exhibited no inhibition against rest of the bacteria. The finding agrees with previously published results of Bernaitis[18]; Eloff[19]; Schlegelova[20] and Vicki[21] with slight variations.

Furthermore, extracts of *B. anthelmintica* in three different solvents (methanol, acetone and aqueous) were tested for their free radical scavenging ability by using DPPH assay and it was observed that the plant extracts showed moderate potency for scavenging free radicals as shown in Table 1.2. The extracts were tested on a concentration range of 20-100 µg/mL and it was established that the activity altogether increased with increase in concentration of plant extracts. In all the cases, methanol extract was found to have better antioxidant potential (14.40±0.05 %) than the corresponding acetone extract (13.20±0.55 %). Apart from this, aqueous extracts did not show any antioxidant activity at all the concentrations. A continuous pattern of increasing antioxidant activity with increasing polarity has been reported[22]. The higher activity of methanol and acetone extracts can be attributed to the presence of higher polyphenols as compared to aqueous or water extracts which may be due to the better solubility of their active components in organic solvents[23].

Table 1.1 Zones of inhibition (ZOI) produced by leaf extracts of *Baccharoides anthelmintica* at different concentrations

Plant Extract	Concentration in %	Inhibition zone diameter (in mm± S.E.)					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanol	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	10.00±0.00	**8.40±1.33**	11.22±0.22	0.00±0.00	12.00±1.20	11.10±0.70
	50	11.90±0.50	11.70±0.50	13.40±0.55	0.00±0.00	13.70±2.22	13.00±0.41
	75	13.00±0.00	13.50±0.60	16.15±0.22	0.00±0.00	16.20±0.05	15.40±0.78
	100	16.00±0.60	16.00±0.00	19.10±1.77	0.00±0.00	**20.40±0.68**	18.55±2.20
Acetone	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**11.00±0.50**	0.00±0.00
	50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	13.55±0.99	0.00±0.00
	75	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.78±1.30	0.00±0.00
	100	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	**18.66±0.76**	0.00±0.00
Aqueous	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	0.00±0.00	10.40±0.34	0.00±0.00	0.00±0.00	**8.00±0.17**	0.00±0.00
	50	0.00±0.00	12.60±0.45	0.00±0.00	0.00±0.00	10.00±0.10	0.00±0.00
	75	0.00±0.00	14.00±0.00	0.00±0.00	0.00±0.00	11.00±0.18	0.00±0.00
	100	0.00±0.00	**15.88±2.08**	0.00±0.00	0.00±0.00	13.70±1.00	0.00±0.00

Table1.2 Free radical (DPPH) scavenging activity (%) of *B. anthelmintica* at different concentrations

Concentration (µg/mL)	Methanol extract	Acetone extract	Aqueous extract	Ascorbic acid
20	8.80±1.12	5.32±1.22	0.00±0.00	35.24±0.50
40	9.96±1.45	6.87±2.25	0.00±0.00	50.54±0.42
60	11.45±2.66	8.32±0.22	0.00±0.00	62.35±1.20
80	12.78±0.45	11.10±0.70	0.00±0.00	74.14±0.00
100	14.40±0.05	13.20±0.55	0.00±0.00	83.26±2.20

Values are given as mean ± SD

CONCLUSION

It was concluded from the above experimental observations that the seed extracts of *Baccharoides anthelmintica* showed significant antibacterial and antioxidant activity at different concentrations. The methanol leaf extract was found to be more effective followed by acetone and aqueous leaf extracts in terms of antibacterial potential. Furthermore, the seed extracts of this plant showed more inhibitory effects in gram-positive bacteria than in gram-negative bacteria. This study also reveals that the plant extracts possess potent antioxidant activity in methanol and acetone solvents, which might be helpful in preventing the occurrence of various oxidative stress-related diseases caused by free radicals. Further investigations on the isolation and identification of antioxidant component (s) of *B. anthelmintica* may lead to chemical entities with potential for clinical or pharmaceutical use.

ACKNOWLEDGEMENTS

Author wants to put on record his gratitude to Indian Council of Medical Research (ICMR), New Delhi for providing financial support to conduct this study.

COMPETING INTERESTS

Authors hereby declare that no competing interests exist.

REFERENCES

1. Adebanjo AO, Adewumi CO, Essein EE, Anti-infective agents of higher plants. In: International Symposium of Medicinal Plants. Fifth ed., University of Ife, Nigeria; 1983; 152-158.

2. Sokmen A, Jones BM, Erturk M, The in vitro antibacterial activity of Turkish medicinal plants. Journal of Ethnopharmacology, 1999; 67:79-86. https://doi.org/10.1016/S0378-8741(98)00189-5

3. Proestos C, Boziaris IS, Nychas GJE, Komaitis M, Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity, Food Chemistry, 2006; 95:664-67. https://doi.org/10.1016/j.foodchem.2005.01.049

4. Devasagayam TPA, Tilak JC, Boloor KK, Review: Free radical and antioxidants in human health, Current Status and Future Prospects, 2004; 53:794-804.

5. Roja G, Rao PS, Anticancer compounds from tissue cultures of medicinal plant, Journal of Herbs, Spices and Medicinal Plants, 2000; 7:71-102. https://doi.org/10.1300/J044v07n02_08

6. Nithya N, Balkrishnan KP, Evaluation of some medicinal plants for their antioxidant properties, International Journal of Pharma Tech Research, 2011; 3: 381-385.

7. Pietta PG, Flavonoids as anti-oxidants, Journal of Natural Products, 2000; 63:1035-1042. https://doi.org/10.1021/np9904509

8. Silva BA, Ferreres F, Malva JO, Dias ACP, Phytochemical and antioxidant characterization of Hypericum perforatum alcoholic extracts, Food Chemistry, 2005; 90:157-167. https://doi.org/10.1016/j.foodchem.2004.03.049

9. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M, Antioxidant activity of plant extracts containing phenolic compounds, Journal of Agricultural and Food Chemistry, 1999; 47:3954-3962. https://doi.org/10.1021/jf990146l

10. Ngeh JT, Rob V, A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae), Journal of Ethnopharmacology, 2013; 146:681-723. https://doi.org/10.1016/j.jep.2013.01.040

11. Dogra NK, Kumar S, Kumar D, Vernonia anthelmintica (L.) Willd.: An ethnomedicinal, phytochemical, pharmacological and toxicological review, Journal of Ethnopharmacology. 2020; 256:112777. https://doi.org/10.1016/j.jep.2020.112777

12. Lei H, Wei YQ, Syed HH, Kun G, Mohammad A, Highly oxygenated stigmastane type steroids from the aerial parts of Vernonia anthelmintica Willd., Steroids, 2012; 77:811-818. https://doi.org/10.1016/j.steroids.2012.03.003

13. Kannan P, Ramadevi SR, Hopper W, Antibacterial activity of Terminalia chebula fruit extract, Afr. J. Microbiol, 2009; 3:180-184.

14. Hemashenpagam N, Selvaraj T, Antibacterial potential of different extracts of Solanum xanthocarpum Chard and Wendt, Plant Archives, 2010; 1:387-390.

15. Blois MS, Antioxidant determination by the use of stable free radicals, Nature, 1998; 26:1199-1200. https://doi.org/10.1038/1811199a0

16. Magaldi S, Mata-Essayag S, Capriles HC, Well-diffusion for antifungal susceptibility testing, International Journal of Infectious Diseases, 2004; 8:39-45. https://doi.org/10.1016/j.ijid.2003.03.002

17. Valgas C, De Souza SM, Smania EFA, Screening methods to determine antibacterial activity of natural products, Brazilian Journal of Microbiology, 2007; 38:369-380. https://doi.org/10.1590/S1517-83822007000200034

18. Bernaitis L, Shobha KL, Ashok M, Revathi PS, Mathew J, Khan DM, Comparative evaluation of the antimicrobial activity of ethanol extract of Taxus baccata, Phyllanthus debilis and Plectranthus amboinicus against multi drug resistant bacteria, International Journal of Pharmaceutical Sciences and Research, 2013; 4:3147-3150.

19. Eloff JN, A sensitive and quick microplate method to determine the minimal inhibitory concentration of the plant extracts for bacteria, Planta Medica, 1998; 64:711-713. https://doi.org/10.1055/s-2006-957563

20. Schlegelova J, Brychta J, Klimova E, Napravnikova E, Babak V, The prevalence of and resistance to antimicrobial agents of Bacillus cereus isolates from food-stuffs, Vet Med-Czech, 2003; 48:331-338. https://doi.org/10.17221/5787-VETMED

21. Vicki AL, Debra SK, Jenny G, Andrew CC, Philip TA, Jacqueline C, Susceptibility of Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides and Bacillus thuringiensis to 24 antimicrobials using sensititre automated microbroth dilution and agar gradient diffusion methods, Journal of Chemotherapy, 2007; 60:555-567. https://doi.org/10.1093/jac/dkm213

22. Goze I, Alim A, Tepe AS, Sokmen M, Sevgi K, Tepe B, Screening of the antioxidant activity of essential oil and various extracts of Origanum rotundifolium Boiss. from Turkey, Journal of Medicinal Plant Research, 2009; 3:246-254.

23. Boer D, Antifungal and antibacterial activity of some herbal remedies from Tanzania, Journal of Ethanopharmacology, 2005; 96:461-469. https://doi.org/10.1016/j.jep.2004.09.035