International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 300-304
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

AN INVESTIGATION OF *BACCHAROIDES ANTHELMINTICA* (L.) MOENCH SEED EXTRACT FOR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

Ved Prakash* and Anand Sagar

Department of Biosciences, Himachal Pradesh University, Shimla, (H.P.) 171005, India

***Corresponding Author Email: vedp685@gmail.com**

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.

KEY WORDS

Baccharoides anthelmintica, seed extract, agar-well diffusion, DPPH

INTRODUCTION

Baccharoides anthelmintica (Syn: *Vernonia anthelmintica* (L.) Willd.) is widely distributed in different parts of the world including India, Pakistan, Nepal, Sri Lanka, Zimbabwe, Zambia, Botswana, Malawi and Congo (Kinshasa). *B. anthelmintica* belonging to family Asteraceae is a large annual herb about 60-100 cm tall. Stem is robust, erect and leafy with velvety branches; alternatively arranged leaves, 5-8 cm long, usually obovate to lance-shaped. Flower heads are borne at the end of branches in 10-20 cm clusters; seed pods are 4-6 cm long, ribbed and oblong [1].

As the scientific name of the plant indicates, *B. anthelmintica* contains compounds that can be used as valuable anthelmintic medicine. This plant is also used for the treatment of asthma, hiccough, inflammatory

swellings, sores and itching of the eyes. The seeds of Ironweed are of great repute as a medicine for white leprosy (leucoderma) and other skin diseases [2]. Because of its above mentioned useful properties, we decided to investigate its seed extracts for their antibacterial and antioxidant activities in different solvents.

MATERIALS AND METHODS

Collection of plant material

Seeds of *B. anthelmintica* were plucked and collected from Singholi area of District Sirmaur, Himachal Pradesh, India. The plant material was then brought to the laboratory for further analysis.

Processing of plant material

Seeds of *B. anthelmintica* were washed thoroughly under tap water and then with 2% Mercuric chloride. Thereafter the leaves were cut into smaller pieces for quick drying and shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar and stored in an air tight container at room temperature.

Preparation of plant extracts in different solvents

5 g dried seeds of *B. anthelmintica* were taken in separate Erlenmeyer flasks to which 50 mL of required solvents i.e. methanol, acetone and water (aqueous) were added. The flasks were covered with aluminium foil and allowed to stand for about 3-5 days for extraction. The extracts were then filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected, weighed and finally stock solution of concentration 50 mg/mL was prepared.

Procurement of bacteria

Different strains of bacteria namely *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 912), *Listeria monocytogenes* (MTCC 657), *Pseudomonas aeruginosa* (MTCC 741), *Staphylococcus aureus* (MTCC 1144) and *Yersinia pestis* (MTCC 4912) have been procured from Indira Gandhi Medical College, Shimla and Department of Microbiology & Biotechnology, Himachal Pradesh, University Shimla (India) for screening antibacterial properties of plant extracts.

Maintenance and preservation of pure culture

The collected pathogens were revived in nutrient broth medium and stored in nutrient agar slants at 4°C. Pure cultures of all the bacteria were maintained on nutrient medium broth and preserved in refrigerator. Sub-culturing was done at regular intervals in order to maintain the cultures.

Evaluation of antibacterial activity by agar-well diffusion method

Seed extracts prepared in methanol, acetone and aqueous solvents were screened using agar well diffusion method used by Kannan et al. [3] and Hemashenpagam and Selvaraj [4] with slight modifications. Nutrient agar medium i.e. Beef extract 1 g, Yeast extract 2 g, Sodium Chloride 1 g, Peptone 5 g, Agar 20 g and Distilled Water 1000 mL was used throughout the investigation. The medium was autoclaved at 121.6°C for about 30 minutes and poured into Petri plates. A 100 µL of bacterial suspension was spread on each nutrient agar plate. 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 seeded with 25, 50, 75 and 100% concentration of prepared plant extracts. The Petri plate taken as a control contained pure solvent only. The Petri plates were incubated at 37±2°C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the zone of inhibition (ZOI) around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition zone diameter using positive control as standard.

Growth inhibition percentage (%) = $\left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \mathbf{x100}$

Where, 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 of *B. anthelmintica* was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as proposed by Blois [5] with some modifications. Briefly, to 1 mL of different concentrations (20, 40, 60, 80 and 100 µg/mL) of plant 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 taken as control. All the experiments were carried out in triplicate and the decrease in absorbance

was measured at 517 nm after 30 minutes in dark using UV-VIS spectrophotometer. The inhibition percentage was calculated using the following formula:

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

Graphs were plotted against inhibition percentage verses concentration of plant extract/standard ascorbic acid in order to find out the values of slope and y-intercepts. IC_{50} value (the amount of antioxidant required to decrease the initial DPPH concentration by 50%) for each extract and ascorbic acid was calculated using the following equation given below:

$$\mathbf{IC_{50}} = \frac{50 - Y - Intercept}{\text{Slope}}$$

RESULTS AND DISCUSSION

Antibacterial activity analysis

The antibacterial activity of *B. anthelmintica* seed extracts was determined by agar-well diffusion method. The results of the antibacterial assay are shown in Table 1. The screening revealed that the methanol seed extract of *B. anthelmintica* was quite effective against *L. monocytogenes* showing considerable diameters of zone of inhibition (20.40±0.68 mm at 100%, 16.20±0.05 mm at 75%, 13.70±2.22 mm at 50% and 12.00±1.20 mm at 25%) and showed minimum inhibition against *Y. pestis* (16.00±0.00 mm at 100%, 13.50±0.60 mm at 75%, 11.70±0.50 mm at 50% and 8.40±1.33 mm at 25%)**.** No inhibition was reported against *B. cereus.* The acetone seed extract was found to be most effective against *L. monocytogenes* (18.66±0.76 mm at 100%, 15.78±1.30 mm at 75%, 13.55±0.99 mm at 50% and 11.00±0.50 mm at 25%) and showed no inhibition against *E. coli*, *Y. pestis, P. aeruginosa, B. cereus* and *S. aureus*. The aqueous seed extract was found to be most effective against *Y. pestis* (15.88±2.08 mm at 100%, 14.00±0.00 mm at 75%, 12.60±0.45 mm at 50% and 10.40±0.34 mm at 25%) and showed no inhibition against *E. coli*, *P. aeruginosa, B. cereus* and *S. aureus*. It is evident from the results that plant extracts showed greater inhibition in gram-positive bacteria as compared to gram-negative bacteria.

Santosh *et al*. [6] carried out antimicrobial activity screening of ethanol extract of *V. anthelmintica* by using four bacterial strains *Staphylococus aureus, E. coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The seed extract showed good antibacterial activity against *B. subtilis* (20.21±0.8mm) and *P. aeruginosa* (19.25±0.6) whereas moderate activity was reported against *S. aureus* (15.34±0.5) and *E. coli* (14.56±0.8). Ravula *et al*. [7], Reddi [8] and Sobrinho *et al*. [9] also performed some experiments to check antimicrobial potential of *B. anthelmintica*.

Antioxidant activity analysis

In the present study, seed 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 2. The seed extracts were tested on a concentration range i.e. 20, 40, 60, 80 and 100 µg/mL and it was observed that the activity altogether increased with increase in concentration of plant extracts. The methanol seed extract showed highest DPPH scavenging activity in the range of 8.80-14.40%. Similarly, DPPH scavenging activity ranged from 5.32-13.20% in case of acetone seed extract whereas aqueous seed extract exhibited no free radical scavenging activity at all concentrations used.

Santosh *et al*. [6], Soni and Chauhan [10] and Dogra and Kumar [11] also evaluated seed extracts of *B. anthelmintica* in order to find their free radical scavenging ability. Nine highly oxygenated stigmastane-type steroids, vernoanthelcin A-I and two new stigmastane-type steroidal glycosides, vernoantheloside A and B have been reported from the aerial parts of *Baccharoides anthelmintica* by Lei *et al*. [2]. Several flavonoids including 2′,3,4,4′-tetrahydroxychalcone, 5,6,7,4′-tetrahydroxyflavone & butin and two novel elemanolide dimers, vernodalidimers A (1) and B (2) were separated from the seeds of *Baccharoides anthelmintica* by high-speed counter-current chromatography using a two-step operation [12,13].

Table 1: Zones of inhibition produced by seed extracts of *Baccharoides anthelmintica* at different concentrations

Plant Extract	Concentration %	Inhibition zone diameter (in mm± S.E.)					
		E. coli	***Y. pestis***	***P. aeruginosa***	***B. cereus***	***L. monocytogenes***	***S. aureus***
	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
Methanol	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
	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
Acetone	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
	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
Aqueous	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

Table 2: Antioxidant activity of *B. anthelmintica* seed extracts at different concentrations

	DPPH radical scavenging activity (%)			
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
IC_{50} (µg/mL)	609.52	475.10	-	41.44

CONCLUSIONS

As a conclusion, it could be speculated that the plant *B. anthelmintica* showed significant antibacterial activity at higher concentrations thereby confirming it as a good antibacterial agent. Methanol seed extract was found to be more effective followed by acetone and aqueous seed extracts. The higher inhibitory activity of methanol and acetone extracts can be attributed to the presence of higher amount of polyphenols as compared to aqueous/water extracts. This study further suggests that the *B. anthelmintica* seed extracts possess considerable antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases. Further investigations on the isolation and identification of antioxidant components of this plant may lead to chemical entities with potential for clinical use.

ACKNOWLEDGEMENT

Authors want to put on record their gratitude to the Chairman, Department of Bio-sciences, Himachal Pradesh University, Shimla (India) for providing Lab facilities. First author acknowledges the financial assistance provided by Indian Council of Medical Research (ICMR), Ansari Nagar, New Delhi to conduct this work.

REFERENCES

1. Ngeh J.T., Rob V., A review of the medicinal potentials of plants of the genus *Vernonia* (Asteraceae). Journal of Ethnopharmacology, 146: 681-723, (2013).
2. Lei H., Wei Y.Q., Syed H.H., Kun G., Mohammad A., Highly oxygenated stigmastane type steroids from the aerial parts of *Vernonia anthelmintica* Willd. Steroids, **77**: 811-818, (2012).
3. Kannan P., Ramadevi S.R., Hopper W., Antibacterial activity of *Terminalia chebula* fruit extract. African Journal of Microbiology, **3**: 180-184, (2009).
4. Hemashenpagam N., Selvaraj T., Antibacterial potential of different extracts of *Solanum xanthocarpum* Chard and Wendt. Plant Archives, 10: 387-390, (2010).
5. Blois M.S., Antioxidant determination by the use of stable free radicals. Nature, 26:1199-1200, (1958).
6. Santosh C.H., Attitalla I.H., Mohan M.M., Phytochemical analysis, antimicrobial and antioxidant activity of ethanolic extract of *Vernonia anthelmintica*. Int J Pharm Bio Sci, 4(1): 960-96, (2013).
7. Ravula G., Goluguri S.R., Konanki K., Evaluation of antibacterial effect of *Vernonia anthelmintica* seed extract and its synergistic effect with antibiotics on resistant bacterial strains. International Journal of Pharmacognosy and Phytochemical Research, 4(3); 79-81, (2012).
8. Reddi V.R.R., Phytochemical and antimicrobial activity of *Vernonia anthelmintica* (L.) Willd. Seed extracts. World Journal of Pharmacy and Pharmaceutical Sciences, 4(2):739-746, (2015).
9. Sobrinho A.C.N., de Souza E.B., Fontenelle R.O., A review on antimicrobial potential of species of the genus *Vernonia* (Asteraceae). J. Med. Plants Res, 9(31); 838-850, (2015).
10. Soni A.P., Chauhan G.N., Study of antioxidant and antimicrobial activity of medicinal plants utilized in cancer treatment. Research Journal of Recent Sciences, 4: 15-21, (2015).
11. Dogra N.K., Kumar S., Pharmacognostical and antioxidant activity investigations on *Vernonia anthelmintica* Wild fruits. International Journal of Pharmaceutical & Biological Archives, 8(6): 55-58, (2017).
12. Guilian T., Ubin Z., Tianyou Z., Fuquan Y., Yoichiro I., Separation of flavonoids from the seeds of *Vernonia anthelmintica* Willd by high-speed counter-current chromatography. Journal of Chromatography A, 1049: 219-22, (2004).
13. Yongqiang L., Alfarius E.N., Yusuke H., Vernodalidimers A and B, novel ortho esterelemanolide dimers from seeds of *Vernonia anthelmintica*. Tetrahedron Letters, 51: 6584-6587, (2010).

***Corresponding Author:**
Ved Prakash*
Email: **vedp685@gmail.com**