Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 3, 2012 ISSN - 0974-2441

Research Article

PHYTOCHEMICAL AND ANTIBACTERIAL EVALUATION OF *BARLERIA MONTANA NEES*. (MOUNTAIN BARLERIA)

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Received:17 February 2011, Revised and Accepted:30 April 2011

ABSTRACT

A total of ten biological compounds were isolated from the leaves of *Barleria montana* Nees. by using GC-MS analysis. Among them, the Benzaldehyde,2-hydroxy-6-methyl- and Urs-12-en-24-oicacid,3-oxo-,methyl ester,(+)- as major compounds. The aqueous and different solvent extracts (acetone, chloroform, di-chloromethane, ethanol and methanol) of the plant were tested for antibacterial activity against both gram positive and gram negative bacterial strains. The results highlighted that the acetone extracts exhibited remarkable antibacterial activity than other extracts. The aqueous extract showed significant activity in most of the organisms. The study encourages this plant may be used as alternative medicine for the treatment of diseases.

Keywords: *Barleria montana*, phytochemicals, antibacterial, leaves

INTRODUCTION

Human infections particularly those involving microorganisms *i.e.* bacteria, fungi, virus and nematode they cause serious infections in tropical & subtropical countries of the world. In recent years multiple drugs 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 all, the last three centuries intensive efforts have been made to discover clinically useful antimicrobial drugs [1-3]. Resistance to antimicrobial agents such as antibiotics is emerging in a wide variety of organisms & multiple drug resistance organisms pose serious threat to the treatment of infectious disease [4].

Barleria (Acanthaceae) is a large genus with over 300 species of herbs and shrubs, many of which are known for their ornamental and/or medicinal values. The biological reports of *Barleria* species showed potent anti-inflammatory, analgesic, antileukemic, antitumor, antihyperglycemic, anti-amoebic, virucidal and antibiotic activities [5-9]. The bioactivity of *B. montana* have been ignored by the researchers. The present investigation was deals with the identification of bioactive compounds and to screen the antibacterial assay of *B. montana* (mountain barleria), used as traditional medicine for sunstroke in the form of decoction or oral mode of administration [10].

MATERIALS AND METHODS

Plant material

The leaves of *Barleria montana* were collected from the higher altitudes (>1000m in MSL) of Kolli hills, Namakkal District, TamilNadu, South India and compared with the voucher specimen deposited in the National Herbarium, Botanical Survey of India (Southern Circle), Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The leaves were allowed to shade dry at room temperature for 10 days. The dried plant materials were made into a fine powder by mixer grinder.

Extraction

The air dried plant powder (10g) was extracted with 100ml of each solvent (acetone, chloroform, Di-chloromethane, ethanol, methanol and water) in 1:10 ratio. This mixture was kept in mechanical shaker up to 72 hours for separation of bioactive compounds. The extract was filtered through filter paper (Whatmann No 1) and allow to evaporation in a room temperature. Weigh the extract obtained with each solvent and calculate its percentage of the dried weight of the plant material. The obtained extracts were stored then subjected to further analysis.

Identification of phytochemicals

The identification of phytochemical constituents of the leaf samples were execute by Gas chromatography analysis (GC clarus 500 Perkin Elmer using Elite- 5MS column (5% Diphenyl/95% Dimethyl poly siloxane with 30×0.25mn×0.25µm thickness). Helium was used as carrier gas at a flow of 1ml per minute. The injection port was maintained at 250ºC and the split ratio was 10:1. Oven temperature programming was done from 5 ºC to 280 ºC at 10 ºC per minute and it was kept at 280ºC for 9 minutes. Interface temperature was kept at 250ºC. The ionization mode was electron impact ionization and the scanning range from 45 to 450 (m/z). Mass spectra were obtained at 0-2 minute's interval. The spectra of the compounds were matched with NIST version year 2005 library. The chemical structure of these compounds are drawn with the help of Chemdraw version 8.0.0 Cambridge Soft Corporation.

Screening for antibacterial assay

Bacteria Tested

The gram negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris)* and gram positive (*Bacillus subtilis, Streptococcus pneumoniae, Staphycoccus aureus, E.coli)* bacterial cultures were used for the antibacterial assay. All the cultures were obtained from clinical laboratory, Salem District, Tamil Nadu, India. The nutrient broth was used to prepare the bacterial inoculums (broth culture) of different organisms. The 16 hours old culture was used for the test.

Antibacterial activity

The antibacterial activity of the crude leaf extracts of *B. montana* were determined by the agar well diffusion method [11-12]. The inoculums (0.1ml) was poured over the top layer of the MHA medium and spread evenly with a sterile cotton swab. A well was made (5mm diameter) by using a sterile well cutter. The dried leaf extracts were dissolved in Dimethyl Sulfoxide (DMSO) solution prior to use. The each well was loaded with known quantity of each crude extract (70 µl) in a concentration of 0.2mg/ml. The sterile DMSO and standard antibiotic (streptomycin) was served as negative and positive control respectively. All the plates were incubated at 37ºC for 24-48 hours. After incubation, the diameter of the inhibition zone was measured. Each extract was tested in triplicate and the diameter of growth inhibition zone was calculated as standard deviation [13].

RESULTS AND DISCUSSION

GC-MS Analysis

The results of ethanolic extracts of *B. montana* showed a total of 10 compounds were identified with 99.99 %. The major compounds was identified as Benzaldehyde,2-hydroxy-6-methyl-and Urs-12-en-24-oicacid,3-oxo-,methyl ester,(+)- shows the peak area of 23.95% and 23.08% respectively. The remaining compounds are minor

components (table 1; figure 1&2). The similar type of investigation was performed by Luciane *et al.* [14] worked on the separation of dirhamnosyl flavonoid and other constituents from *Brillantaisia palisatii*. Another report focused on the chemical examination of *Barleria longiflora Linn.* for the extraction of stems and roots with solvents and column chromatography led to the isolation of four anthraquinones (tectoquinone and 1,3,5-trihydroxy-4- methoxy-2-methylanthraquinone and the new anthraquinones characterised as 3,8-dihydroxy-4- methoxy-2 methylanthraquinone and 1,3,4-trihydroxy-5 (or 8)-methoxy-2-methylanthraquinone) [15].

Table 1: Chemical compounds identified in the leaves of *Barleria montana* by GC-MS analysis

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	3.01	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	3.98
2	7.51	Benzaldehyde,2-hydroxy-6-methyl-	$C_8H_8O_2$	136	23.95
3	8.26	1,6-Anhydro-β-d-talopyranose	$C_6H_{10}O_5$	162	10.87
4	9.84	(1R,3R,4R,5R)-(-)-Quinic acid	$C_7H_{12}O_6$	192	15.24
5	13.50	1,2-Benzenedicarboxylic acid, butyl 8-methlinonyl ester	$C_{22}H_{34}O_4$	362	1.51
6	15.48	Phytol	$C_{20}H_{40}O$	296	6.71
7	19.61	β -Amyrin trimethylsilyl ether	$C_{33}H_{58}OSi$	498	4.89
8	23.61	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	23.08
9	24.13	Lup-20(29)-en-3-ol, acetate, (3 β)-	$C_{32}H_{52}O_2$	468	8.95
10	32.34	Stigmasterol	$C_{29}H_{48}O$	412	0.81

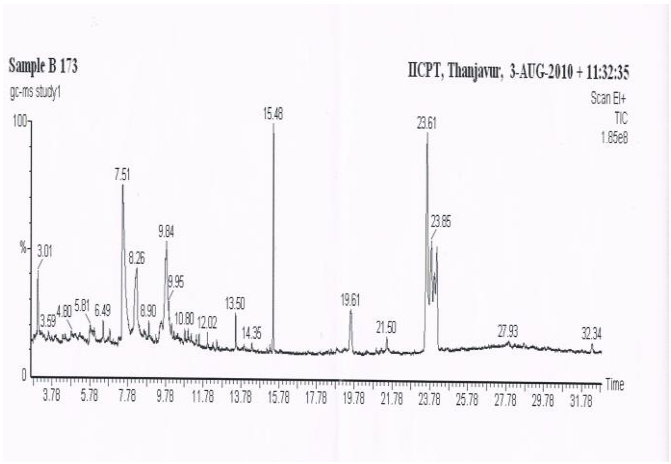
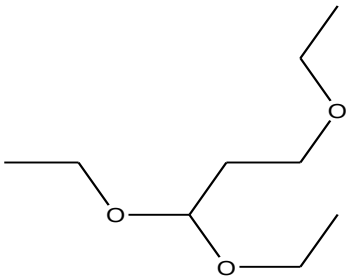
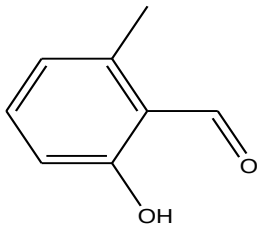


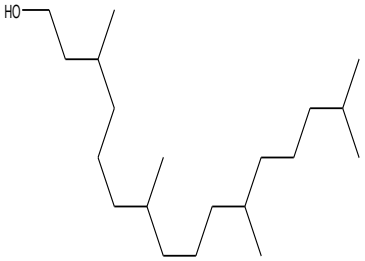
Figure 1: GC-MS analysis of leaf samples of *Barleria montana* Ness



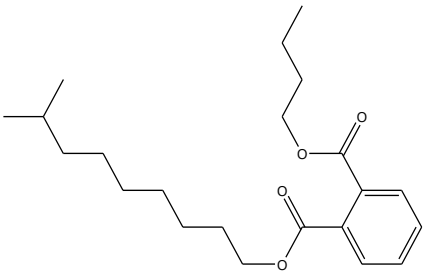
Propane, 1, 1, 3-triethoxy-



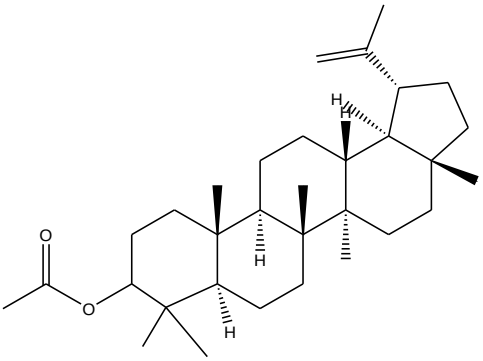
Benzaldehyde, 2-hydroxy-6-methyl-



phytol



1, 2-Benzenedicarboxylic acid,butyl 8-methylnonylester



Lup-20(29)-en-3-ol, acetate

Figure 2: Structural elucidation of some phyto-constituents from the ethanolic leaf extract of *B.montana Nees*

Antibacterial activity of *Barleria montana* Nees.

The results of aqueous and various solvents extracts from the leaves of *B.montana* were showed broad spectrum of antibacterial activity (table 2). The acetone extract exhibited highest inhibitory effect against *Pseudomanas aeruginosa*, *E. coli* followed by others. Similarly, the acetone extract of the lichen *Ramalina farinacea* and its (+)-usnic acid constituent showed better antimicrobial activity against several bacterial species [16]. The remaining solvents showed least to moderate activity. Overall, these studies are first time report in this plant. Another species from the genus reported to contain antimicrobial properties *i.e.,* various parts of *B. prionitis, B. greenii* and *B. albostellata* [17-18]. The present study was suggested that the plant may be used as alternative drugs against microbial infections. Further research is needed for isolation and structural elucidation of bioactive compounds and their clinical tests.

Table 2: Antibacterial activity of leaf extracts of *B. montana* Nees

Extracts	Name of the Organisms & Zone of Inhibition (diameter in mm)							
	B. subtilis	***P. aeruginosa***	***S. aureus***	***K. pneumoniae***	***S. typhi***	***S. pneumoniae***	***E. coli***	***P. vulgaris***
Methanol	12.6±1.63	19±1.41	10.6±0.82	7.3±0.81	7±1.41	11.6 ±0.82	10.6±0.82	8.3±1.63
Ethanol	15±0	9.6±2.16	7.6±0.82	12.3±0.81	12±1.41	12.3±1.63	11.3±0.81	10.3±0.81
Chloroform	9.3±2.16	11±5.7	14.3±2.16	10±2.4	9.3±3.26	13±1.41	12.6±1.64	6.2±0.41
Di-chloromethane	10±1.41	7.6±0.82	9.00±2.44	8.3±0.81	7.00±0.00	7.00±0.00	7.6±0.82	7.6±1.63
Acetone	12.3±1.86	22±3.87	14±1.41	7.6±0.82	17.6±2.16	13.6±2.16	21.3±4.46	9.3±0.81
Water	11.6±0.82	11.6±2.94	10.3±0.81	9.6±0.82	8.6±1.91	10.3±0.81	9.3±0.81	11.3±3.26
Streptomycin	15.0±0.00	20.00±0.82	15.33±0.81	13.0±0.00	15.0±0.00	17.0±0.81	20.0±0.00	15.0±0.81
DMSO	---	---	---	---	---	---	---	---

ACKNOWLEDGEMENTS

The authors thank to Department of Biotechnology, Periyar University, Salem for providing laboratory facilities to carry out this investigation. Thanks are also extended to Director & staff members, Indian Institute of Crop Processing Technology, Thanjavur for their technical help.

REFERENCES

1. Ahmad L, Mohammad Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol* 1998, 62,183-193
2. Perumal samy R, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. *J Ethnopharmacol* 2000, 69, 63-71.
3. Werner F, Okemo P, Ansorg R. Antibacterial activity of East African medicinal plants. *J Ethnopharmacol* 1999, 60, 63-71.
4. Tomin E, Tomasz A. β-lactum specific resistant mutants of *Staphylococcus aureus. Antimicro Agents Chemother* 1986, 30, 577-583.
5. Yosook C, Panpisutchai Y, Chaichana S, Santisuk T, Reutrakul V. Evaluation of anti-HSV-2 activities of *Barleria lupulina* and *Clinacanthus nutans. J Ethnopharmacol* 1999, 67,179–187.
6. Jassim SAA, Naji AM. Novel antiviral agents: amedicinal plant perspective. *J Appl Microbiol* 2003, 95, 412–427.
7. Suba V, Murugesan T, Arunachalam G, Mandal SC, Saha BP. Anti-diabetic potential of *Barleria lupulina* extract in rats. *Phytomedicine* 2004, 11, 202–205.
8. Suba V, Murugesan T, Kumaravelrajan R, Mandal SC, Sah BP. Antiinflammatory, analgesic and antiperoxidative efficacy of *Barleria lupulina* Lindl extract. *Phytother Res* 2005, 19, 695–699.
9. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *J Ethnopharmacol* 2005, 101,330–333.
10. Tayade SK, Patil DA. Hitherto untapped plantflore from Nandurbar district (Maharashtra). *Nat Prod Rad* 2005,4, 46-50.
11. Collins CH, Lyne A. In: microbiological methods. London, Butterworth, 1979; 416-424.
12. 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, 78,119-127.
13. Gupta, Statistical methods. New Delhi, S. Chand and Co, 1977.
14. Berrondo LF, Gabriel FT, de Oliveira Fernandes SB, de Sousa Menezes F. dirhamnosyl flavonoid and other constituents from *Brillantaisia palisatii. Quim. Nova* 2003,26, 922-923.
15. Venkata Rao E, Sridhar P, Ravi Kumar J, Vijaya Lakshmi T. Anthraquinones and arnidiol from *Barleria longiflora Linn F. Indian J Pharma Sci* 1999, 61,282-286.
16. Taya T, Türkb A.Ö, Yılmazb M, Türka H, Kıvanc M. Evaluation of the Antimicrobial activity of the acetone extract of the lichen *Ramalina farinacea* and its (+)-Usnic acid, Norstictic 384-388acid and Protocetraric acid constituents. *Z Naturforsch* 2004, 59c,
17. Amoo SO, Finnie JF, van Staden J. *In vitro* pharmacological evaluation of three *Barleria* species. J Ethnophrmacol, 2009; 121:274-277.
18. Aneja KR, Joshi R, Sharma C. Potency of *Barleria prionitis* & bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin. New York Sci J 2010; 3c: 5-12.