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# PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF HEPATOPROTECTIVE AND ANTIMICROBIAL ACTIVITIES ON THE AERIAL PARTS OF *BARLERIA MONTANA* (ACANTHACEAE)

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**ABSTRACT**

Phytochemical investigation of aerial parts of *Barleria montana* methanolic (BMM) extract afforded four bioactive metabolites namely *p*- hydroxy cinnamic acid, *p*- hydroxy benzoic acid, ursolic acid and barlerin. Their structures were confirmed on the basis of spectroscopic (IR, $^1$H NMR and MS) analysis and compared with the spectroscopic data reported in the literature. The BMM extract was evaluated for hepatoprotective activity against carbon tetrachloride ($CCl_4$) induced hepatotoxicity on rats and also tested for antimicrobial activity against various types of microorganisms like bacteria and fungi. The BMM extract showed good hepatoprotective and antimicrobial activities.

**Keywords:** BMM Extract, Barlerin, Hepatoprotective activity, Antimicrobial activity.



## INTRODUCTION

*Barleria montana* (Family: Acanthaceae) is well distributed common on hills of exposed slopes, plains among rocks and at higher elevations. Leaves are ovate or elliptic-lanceolate in shape, attenuate at base, ciliate at margins, acute at apex, glabrous. Stems shows terete and glabrous. Flowers are solitary, sessile or subseesile, axillary or in terminal spikes. Seeds are ovoid or subquadroid, 4.5 – 6 mm, clothed with appressed-silky hairs[1]. Ethnobotanical information of *barleria montana* stated that leaf is used in diabetes, wounds, and cuts. It can also be used as hepatoproctive drug[2,3].

Root paste when applied externally used for the treatment of rheumatism and joints pain[4]. The literature survey revealed that the plant was reported to have antioxidant activity and hepatoprotective activity against ethanol treated rats[5,6].

However, its effectiveness in protection against acute liver injury caused by carbon tetrachloride ($CCl_4$) had not been previously established. Hence, the aim of study was to investigate the bioactive metabolites and evaluate for hepatoprotective and antimicrobial activities of *Barleria montana* as the plant being used as a hepatoprotective drug by rural people.

## EXPERIMENTAL

### Collection of plant material

The plant, *Barleria montana* (2kg), was collected from Tirupati hills in the month of September. The plant was authenticated by Prof.M.Venkaiah, Department of Botany, Andhra University and the specimen was deposited in the herbarium (Voucher specimen number (BM/01)).

### Extraction

The aerial parts of *Barleria Montana* (1.5 kg) were dried in shade and then powdered in a Wiley mill. The powdered drug was extracted in a soxhlet apparatus for 6 hrs successively with petroleum ether (3 lit), chloroform (3 lit) and methanol (3 lit) and concentrated to dryness under vaccum which gave pet ether extract (20.32 gms), chloroform extract (32.14 gms) and methanolic extract (34.26 gms). All the extracts

were undergone for chemical reactions for the presence of compounds. The methanolic extract of the aerial parts of *Barleria montana* was then subjected to column chromatography over silica gel for the isolation of compounds.

**Hepatoprotective activity**

**Animals**

The experimental protocol was approved by the institutional animal ethics committee of Andhra University, Vishakhapatnam, which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA), Govt. of India (registration no.516/01/A/CPCSEA).Wistar albino rats of either sex (150-200 g) were maintained under controlled conditions for all sets of experiments The rats were allowed to take standard laboratory feed and water ad libitum.

**Design of experiment**

Each set of experiment was divided into groups consisting of 6 rats in each group towards control, toxicant, standard, and test. The methanolic extract obtained from the aerial parts of *Barleria montana* were suspended in 1% Sodium CMC and administered at a dose levels of 200, 400 and 800 mg/kg which were screened against $CCl_4$ induced toxicity by assessing them through biochemical parameters. The rats of control group I received three doses of 1% Sodium CMC (1 mL/kg p.o.) at 24 h intervals (0 h, 24 h and 48 h).

The animals in $CCl_4$ treated group II received vehicle at 0 h vehicle followed by $CCl_4$ diluted in liquid paraffin (1:1 i.p.) at a dose of 1.25 mL/kg, while at 48 h these animals received only vehicle. The test groups received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of $CCl_4$ and at 48 h the third dose of extracts. The group III received the first dose of silymarin (25 mg/kg) at 0 h, second dose of silymarin at 24 h followed by a dose of $CCl_4$ and at 48 h the third dose of silymarin.

Groups IV, V and VI received different doses of extracts viz 200 ,400 and 800 mg/kg . After 72 h blood was drawn from the retero-orbital plexus venous and allowed to clot for the separation of serum. The serum was used for the assay of the marker enzymes serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP). Total bilirubin(TBL) , cholesterol(CHL), total protein(TPTN) and albumin(ALB) contents were also estimated [7-12].

**Statistical analysis**

The values were expressed as mean±SEM. The data was subjected to the analysis of variance (one way ANOVA) to determine the significance of changes followed by students "t"-test[13-15]. The statistical significance of difference between two independent groups was calculated for the determination of levels of various biochemical parameters present in serum.

**Antimicrobial activity**

**Bacteria:** *Bacillus subtilis, Bacillus cereus, Bacillus pumilis* and *Staphylococcus aureus* (Gram +ve organisms) *Escherichia coli, Psuedomonas aeuriginosa, Psuedomonas vulgaris* and *Serratia marceseans* (Gram –ve organisms)

**Fungi:** *Aspergillus niger, Rhizopus stolonifer, Sacharomyces cerevisiae* and *Pencillium chrysogenum.*

The antimicrobial screening was estimated by cup plate method, where the antibacterial extracts are diffused from the cup through an agar layer in the petri dish[16,17]. The methanolic extract of the aerial parts of *Barleria montana* in two dose levels of 100mg/ml and 200mg/ml was screened for antimicrobial activity including antibacterial and antifungal activity against wide spectrum of microorganisms. Chloromphenical is used as standard for gram +ve and –ve organisms. Nystatin is used as standard for Fungi. The zone of inhibition was compared with that of the standard.

## RESULTS AND DISCUSSION

Phytochemical examination of methanolic extract of *Barleria Montana* (aerial parts) gave four compounds and was characterized by spectral analysis. The spectral data of the compounds are given below.

**Characterization of the compounds**

***p*- Hydroxy cinnamic acid**

White needle crystal powder, mp 214-217$^0$. IR (KBr) cm $^{-1}$ : 3388, 1678, 1600, 1509, 1444, 1242, 1165. $^1$H-NMR (400MHz, MeOD) : 7.58 (1H, d, H-7), 7.51 (2H,dd, H-2,6), 6.87 (2 H,dd, H-3,5), 6.30 (H, d, H-8). EI-MS m/z (rel.int.%) : 165[M]+(15%), 138(10%), 137(100%), 93(15%).

***p*- Hydroxy benzoic acid**

White solid, mp 209-211$^0$. IR (KBr ) : 3415, 2938, 1692, 1512, 1459, 1261, 1206, 1049, 762 cm-1. $^1$H NMR (DMSO, 400 MHz): 7.86 (2H, d, J = 8.8 Hz), 6.80 (2H, d, J = 8.8 Hz) . Mass spectra EI-MS m/z (rel.int %): 138(100%) [M]+,131(10%),89(55%).

**Ursolic acid**

White feathery needles, m.p 280-81°C , (C, 0.3 in pyridine) 59º. IR values in (cm-1): 3430, 2935, 1632,1463,1377,1022 and 963 cm-1. $^1$H-NMR (CDCl3, 400 MHz): 7.2 (1H, t, H-12), 2.98 (1H, dd, H-3α), 2.09 (1H, d, H- 18), 0.87, 1.02, 0.73 and 0.84 (each 3H, s, H-23, H-27, H-26 and H-24),0.89(3H,d,H-30), 0.79 (3H, d, H-29) and 0.66 (3H, s, H-25) . EI-MS, which gave M+ at 456(25%), 399(20%), 285(40%), 163 (100%), 70(15%).

**Barlerin**

Colorless needles, m.p 179$^{0,}$ (C, 0.1 in MeOH) -79º. IR (KBr) : 3346 (OH), 2926 (C-H), 1713 (C=O), 1602 (C=C), 1037 (C-O-C) cm-1. $^1$H NMR (400 MHz, CD3OD): 5.91 d (2.4), 7.44 d (1.2), 3.06 dd (9.0, 3.0), 4.33 m, 2.02 dd (14.8, 5.2), 3.00 dd (9.0, 2.4), 1.51 s, 3.72 s, 2.01 s, 4.63 d (8.0), 3.17 dd (8.0, 9.0), 3.36 dd "t" (9.0), 3.26 dd "t" (9.0), 3.32 m, 3.89 dd (12.0, 2.0), 3.66 dd (12,0, 6.4) . EI-MS: m/z 449 [M+] (30%), 173(50%), 107(100%), 74(5%).

Results from the Table-1 revealed that Silymarin (standard drug) at the dose of 25 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL (100.4 ± 1.71, 101.2 ± 0.80, 207.5 ± 1.68, 1.28 ± 0.05, and 111.1± 0.42) and increased the levels of TPTN and ALB (6.76 ± 0.17 and 3.61± 0.18) respectively. The methanolic extract of *Barleria montana* at 400 mg/kg significantly reduced the increased levels of SGOT, SGPT, ALKP, TBL and CHL (124.0 ± 2.03, 128.0 ± 1.91, 267.4 ± 3.66, 2.50± 0.21 and 167.6 ± 2.02) and increased the levels of TPTN and ALB (5.55 ± 0.20 and 3.57 ± 0.18, where as methanolic extract of *Barleria montana* at 800mg/kg produced SGOT, SGPT, ALKP, TBL and CHL levels 109.5 ± 2.05, 105.6 ± 2.01, 244.1 ± 3.18, 1.69± 0.17 and 154.8± 3.20 respectively and increased the levels of TPTN and ALB in a manner like 5.46 ± 0.18 and 3.64 ± 0.17.

Table-1: Effect of methanolic extracts of aerial parts of *Barleria montana* on $CCl_4$ induced hepatotoxicity in rats

| Group | SGOT (IU/L) | SGPT (IU/L) | ALKP (IU/L) | TBL (mg/dL) | CHL (mg/dL) | TPTN (g/dL) | ALB (g/dL) |
|---|---|---|---|---|---|---|---|
| Group-I (Control) | 104.3±2.31 | 93.90±3.32 | 213.50±1.86 | 1.19±0.10 | 103.6±2.21 | 5.70±0.21 | 3.06±0.49 |
| Group-II ($CCl_4$) | 304.2± 11.79 | 236.6± 4.04 | 423.1 ± 22.47 | 3.11± 0.34 | 263.7± 10.19 | 2.85± 0.28 | 1.62 ±0.12 |
| Group-III (Silymarin) | 100.4 ±1.71* | 101.2± 0.8* | 207.5 ± 1.68* | 1.28 ±0.05* | 111.1± 0.42* | 6.76 ±0.17** | 3.61±0.18** |
| Group-IV (BMM 200 mg/kg) | 283.9 ± 5.13 | 245.5±2.8 | 398.7±2.86 | 2.48±0.18 | 270.3±4.54 | 3.85±0.23 | 2.32±0.24 |
| Group-V | 124.0±2.03* | 128.0±1.91* | 267.4±3.66* | 2.5±0.21* | 167.6±2.02* | 5.55±0.20** | 3.57±0.18** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (BMM 400 mg/kg) | | | | | | | |
| Group-VI (BMM 800 mg/kg) | 109.5±2.05* | 105.6±2.01* | 244.1±3.18* | 1.69±0.17* | 154.8±3.20* | 5.46±0.18* | 3.64±0.17* |

Data expressed in mean ± s.e.m, n=6 * Significant reduction compared to hepatotoxic group (P<0.05),** Significant increase compared to hepatotoxic group (P<0.05)

The rats treated with the methanolic extracts of *Barleria montana* and silymarin showed a significant (P<0.05) decrease in all the elevated SGOT, SGPT, ALKP, TBL, CHL and significant increase (P<0.05) in TPTN and ALB levels at 400 and 800 mg/kg.

Table-2: Antibacterial activity of methanolic extract of *Barleria montana* aerial parts

| plant material | Concentration (mg/ml) | zone of inhibition (diameter in mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gram (+)ve | | | | Gram (–)ve | | | |
| | | *B.s.* | *B.c* | *B.p* | *S.a* | *E.c* | *P.a* | *P.v* | *S.m* |
| *Schrebera swietenoides* | | | | | | | | | |
| Methanolic extract | 100 | 23 | 27 | 27 | 19 | 17 | 24 | 17 | 20 |
| | 200 | 26 | 30 | 25 | 19 | 16 | 26 | 15 | 18 |
| Standard: | | | | | | | | | |
| Chloramphenicol (µg/ml) | 10 | 25 | 32 | 22 | 18 | 26 | 19 | 18 | 18 |
| Vehicle: | | | | | | | | | |
| DMSO | - | - | - | - | - | - | - | - | - |

Zone of inhibition in millimeters, cup diameter: 6mm
*B.s* (*Bacillus subtilis*), *B.c* (*Bacillus cereus*), *B.p* (*Bacillus pumilis*), *S.a*(*Staphylococcus aureus*), *E.c* (*Escherichia coli*), *P.a* (*Psuedomonas aeuriginosa*), *P.v* (*Psuedomonas vulgaris*), *S.m* (*Serratia marceseans*)

Table-3: Antifungal activity of methanolic extract of *Barleria montana* aerial parts

| Plant Material | Concentration (mg/ml) | Zone of inhibition* (diameter in mm) | | | |
|---|---|---|---|---|---|
| | | *A.n* | *R.s* | *S.c* | *P.c* |
| *Schrebera swietenoides* | | | | | |
| Methanolic extract | 100 | 22 | 23 | 20 | 21 |
| | 200 | 23 | 22 | 19 | 19 |
| Standard Nystatin(µg/ml) | 10 | 25 | 21 | 20 | 22 |
| Vehicle: DMSO | - | - | - | - | - |

*Zone of inhibition in millimeters, cup diameter: 6mm *A.n* (*Aspergillus niger*), R.s (*Rhizopus stolonifer*), *S.c* (*Sacharomyces cerevisiae*) *P.c* (*Pencillium chrysogenum*)

The methanolic extract showed moderate activity against gram positive and gram negative organisms (*Bacillus subtilis, Bacillus cereus, Bacillus pumilis, Staphylococcus aureus*, *Escherichia coli, Psuedomonas aeuriginosa, Psuedomonas vulgaris, Serratia marceseans*) and showed moderate activity against fungi like *Aspergillus niger, Rhizopus stolonifer, Sacharomyces cerevisiae* and *Pencillium chrysogenum* at a dose levels of 100mg/ml and 200mg/ml and was represented in Table 2 and 3

## CONCLUSIONS

The chemical examination of aerial parts of *Barleria montana* resulted four compounds on column chromatography and repeated crystallizations , named as *p*-Hydroxy cinnamic acid, *p*-Hydroxy benzoic acid, ursolic acid and Barlerin. All these compounds were characterized by conventional chemical tests, physical properties and spectroscopic methods like IR, NMR and Mass spectra. Out of these compounds

Ursolic acid is triterpenoid, *p*-Hydroxy cinnamic acid and *p*-Hydroxy benzoic acid are carboxylic acids and barlerin is irridoid glycoside. All these compounds have been reported first time from this species . The barlerin is also frequent in the genus barleria and thus serving as a chemotaxanomic marker of this genus. The study on the above plant revealed that the methanolic extract of aerial parts of *barleria montana* decreased the increase in the levels of biochemical parameters SGOT, SGPT, ALKP, TBL and CHL and increased the TPTN and ALB levels in a dose dependent manner. Hence it showed significant hepatoprotective activity against $CCl_4$ induced hepatotoxic model. BMM extract at different dose levels also showed antimicrobial activity. It is inferred that the presence of ursolic acid and iridoid glycosides might be responsible for showing hepatoprotective and antimicrobial activities[18,19].

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