PHYTOCHEMICAL AND PHARMACOGNOSTICAL SCREENING LEAVES OF BARLERIA GIBSONII

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Abstract:

Plants play a key role in integrative and associative approaches in the field of drug innovation. Phytomics is a unique practice in which the different types of metabolites from flora origin, moss, algae, fungi, and ferns will be investigated.

To present succinctly, it can be stated that the present investigation, pharmacognostic, phytochemical and pharmacological properties were carried out to ascertain the ethnobotanical claims of pharmacological potentials of the leaves of *Barleria Gibsonii*.

The leaves of *Barleria Gibsonii* were collected and authenticated by its morphological and histological characters. The percentage of macronutrients like sodium, potassium, and magnesium were within the stipulated limits. Toxic and heavy metals resembling palladium, arsenic, lead and mercury were within the limit and ensure the safety of the study.

Preliminary phytochemical studies established the presence of tannins, steroids, triterpenoids, carbohydrates, flavonoids, and proteins in EEBG as well as steroids, triterpenoids, flavonoids, tannins, triterpenoids, phenolics, carbohydrates and proteins in CEBG.

The results of the acute toxicity studies of CEBG and EEBG as per OECD guidelines 425 did not show any sign and symptoms of toxicity or mortality upto 5000 mg/kg, hence considered as category 5.

The efficacy of any hepatoprotective drug is dependent essentially on its capability of reducing the harmful effects of hepatotoxin and in maintaining the normal hepatic physiological mechanism.

Hepatoprotective activity of EEBG and CEBG were studied in CCl_4 and thioacetamide induced liver injury in rats using Silymarin as standard drugs respectively. The curative efficacy of administered extracts was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be through promotional activation of antioxidative enzymes and regeneration of hepatocytes that restore the structural and functional integrity of liver. The protective effect observed on treatment with the tested extracts strongly indicates that the extract may have the ability to mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to hasten regeneration of parenchymal cells, and preserve the integrity of the plasma membranes.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal places, and central veins. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty erosion were observed in thioacetamide and CCl_4 intoxicated animals.

In the Oral glucose tolerance test the tested extracts were found to be equally effective with the standard drug Glibenclamide. Further, the tested extracts have shown a dose dependent decrease in the serum glucose levels on 7^{th}, 14^{th} and 21^{st} day on streptozotocinized rats. The plausible mechanism behind the antidiabetic potential of tested extracts could be due to the presence of tannins, flavanoids and phytosterols which would have augmented the activity of enzymes responsible for utilization of glucose by insulin-dependent pathway and due to extra-pancreatic effects or regeneration of β-cells in pancreatic islets.

Keywords: *Barleria*, phytochemical, heptotoxic, diabetes,

INTRODUCTION

Recent years have seen a significant increase in interest in herbal medicines as complementary therapies for illnesses linked to modern lifestyles. Early humans have long used plants as remedies. Traditional herbal medicine practitioners have detailed the medicinal usefulness of several indigenous plants for a variety of ailments [1-5]. According to estimates from the World Health Organization (WHO), traditional medicine serves as the major source of care for roughly 80% of the populace in underdeveloped nations.

Plants play a key role in integrative and associative approaches in the field of drug innovation. Phytomics is a unique practice in which the different types of metabolites from flora origin, moss, algae, fungi, and ferns will be investigated [6-9].

Plant description:

Barleria (*Acanthaceae*) is a large genus with about 230 species of herbs and shrubs distributed chiefly in the tropical and subtropical parts of the world. About thirty species occur in India, many of which are known for their ornamental and/or medicinal value. Some of the important species of this genus are *Barleria prionitis, Barleria greenii, Barleria albostellata, Barleria cristata, Barleria strigosa, Barleria Tomentosa*, etc., In some *Barleria* species, biological activities such as anti-inflammatory, analgesic, antileukemic, and hypoglycemic have been reported [10-15].

BARLERIA GIBSONII

Synonym	:	Barleria glauca, Barleria gibsonioides
Family	:	Acanthaceae
Common Name	:	Gibson's Barleria

Scientific Classification

Kingdom	:	Plantae
Clade	:	Asterdis
Order	:	Lamiales
Family	:	Acanthaceae
Sub family	:	Acanthoideae
Tribe	:	Barleria
Genus	:	Barleria L

Morphology

Gibson's Barleria is a perennial herb or undershrub, erect, woody, up to 1 m tall. Stems are round, angular, hairless. Leaves are ovate-elliptic, 7-17 x 5-9 cm, rounded, wedge-shaped or shortly winged at base, scabrous at margins, pointed to tapering at tip, lineolate above, hairless, glaucous beneath; lateral veins 5-8 pairs; leafstalks 1-2.5 cm long. Flowers are borne solitary, in leaf-axils and in branch-end spikes; bracts linear, 1-2 x 0.4-0.8 cm, pointed at tip. Outer sepals elliptic-oblong or ovate-oblong, nearly equal, 2.5-3.5 x 1.8-2.3 cm; inner sepals linear-lanceshaped, 1-1.5 cm long. Flower are 6-8 cm long, hairless, pink-purple; tube 3-4 cm long; petals obovate, nearly equal, 2.5-3.5 x 2-2.8 cm, rounded at tip, with or without mauve blotches on two lateral lobes. Stamnes are protruding. Capsules are oblong- lanceoloid, 2-2.5 x 0.5-1 cm, hairless, turgid. Gibson's Barleria is found in Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Odisha, and Tamil Nadu. Flowering: September-March [16-18].

Fig 2.1. *Barlaeria gibsonii* flowers

Fig 2.2. External morphology of *Barlaeria gibsonii*

2.2.1. Reported biological activities of antiulcer and Antioxidant Activity

Oral administration of ethanol extract of leaves at doses of 250, 500 mg/kg p.o. reduced significant gastric lesions induced by pylorus ligation-induced ulcer as compared to standard omeprazole (20 mg/kg p.o.). The IC_{50} values were found to be 150 µg/mL in leaf extract. The ethanol extracts showed good antioxidant capacity in the DPPH radical scavenging assay and NO radical scavenging activity when compared to standard. The total phenolic content using Folin–Ciocalteu reagent estimated in 1 mg of leaves extracts was 368 µg and 481 µg with gallic acid equivalent and the total flavonoid content was found to be 240 and 410 µg, respectively, with quercetin equivalence [19-22].

Experimental Investigations

4.1. PLANT MATERIAL

Fresh leaves of *Barleria gibsonii* were collected from Kadapa district of Andhra Pradesh, India, in the month of October and authenticated by Prof. Dr. K. Madhava Chetty, Taxonomist, S.V. University, Tirupati. A.P. Specimen vouchers (Ref No: SCIENT/SVU/2011- 107) were deposited at Department of Pharmacognosy for further reference.

4.2. STANDARDIZATION AND PHYTOCHEMICAL APPROACH [23-26]

4.2.1. Microscopy

At first, plant parts had been retained in the field by solution containing formalin, acetic acid, and seventy percent alcohol with volumes 5, 5 and 90ml respectively. Couple slicing namely free hand slicing and paraffin wax slicing and maceration had been implemented whenever required. Images were drawn using camera lucida of prism type. Drying up was done to plant materials with NBA and TBA [16].

Table 4.1. N-butyl alcohol (NBA) dehydration

S. No	Ethanol (95 % V/V)	NBA	Distilled water	Time (h)
1	20	10	70	1
2	25	15	60	1
3	30	25	45	1
4	30	40	30	1
5	25	55	20	2
6	20	70	10	2
7	15	85	-	2
8	-	100	-	2

Table 4.2 List of Preparations of tertiary butyl alcohol (TBA) dehydration:

S. No	Distilled water	TBA	Ethyl alcohol 95%(v/v)	Ethyl alcohol 100%	Time (h)
1	50	10	40	-	2
2	30	20	50	-	12
3	15	35	50	-	1
4	-	55	45	-	1
5	-	75	-	25	1
6	-	100	-	-	1
7	-	100	-	-	12
8	-	100	-	-	1

Dessicated components had been strained at fifty-five to sixty degrees and integrated in paraffin wax. Subsequently, with the use of microtone slices were made transversely at 15 microns dense. The paraffin segment on the slide was made by adhesive agent with four percent formalin.

Staining:

The schedules followed were,

a) Toluidine blue 'O'

b) Safranin 'O' and Fast green 'FCF'

a) Toluidine blue 'O'

Devoid of removing the paraffin wax, stained segments can be separated with the help of 0.05% toludine blue in water. The sections had been dehydrated; de waxed in xylene and set up in DPX.

b) Safranin 'O' and Fast green 'FCF'

A quantity of 225ml of ninety five percent of ethanol had been mixed with 2.25 grams of safranin. Clove oil and ethanol mixed in a ratio of 75:25 is blended in 1gram of fast green. Until the accumulated color had been extracted that de waxed slides are tainted in saffron to adequate period. Further the slides are differentiated off clove oil and spirits by spotting using fast green. Kept under xylene they are mounted in DPX.

4.2.2. Ash value

A variety of ash values like total, water- soluble, acid -insoluble and sulphated ash values have been established for both leaves of *Cleome gynandra* and whole plant of *Pulicaria wightiana* according to the method of Ayurvedic Pharmacoepia. At first each shadow dried up components are transferred via sieve sixty then utilized for analysis of ash contents [17-20].

4.2.2.1. Evaluation of total ash value

In the beginning the crucible was ignited, then three grams of extract was kept in crucible and covered with rake. In the crucible the powder was consistently laid and then the powder was made colorless by sparking with progressively improving the heat to 500 degrees centigrade. Under denotation towards each dehydrated content that percentage of total ash value was determined.

4.2.2.2. Estimation of water-soluble ash content

Twenty-five ml of water is introduced towards crucible featuring total ash and warmed for five-minute period and negated via ash less filter paper. The indissoluble filtrate is transmitted to a crucible, warmed and that strategy is duplicated until a consistent mass is attained.

After that, water soluble ash value is acquired by deducting this obtained weight from total ash weight. Percentage of ash value is calculated in terms of air-dried material.

4.2.2.3. Acid insoluble ash content

The acquired ash material through that before stated procedure had been heated with two molar HCL for five minutes. Accumulating the insoluble ash content on ash less filter paper, it had been cleaned till that filter is normal. The indissoluble product had been directed towards crucible and heated to a consistent mass. Based on air-dried drug the percentage of acid insoluble ash was determined.

4.2.2.4. Sulphated ash content

Silica crucible with three grams of fine-grained material had been burnt till that material is carbonated and then chilled. The acquired deposit is warmed by incorporating one ml of H_2SO_4 till white vapors won't be developed along with burning at five hundred to six hundred degrees till most carbon molecules vanished. The crucible is again contained with couple of falls with H_2SO_4 and burned like before and additionally allowed for cooling and then accessed. Unless the consistent mass maintains the process should be duplicated. Based on air-dried drug the percentage of sulphated ash value was determined.

4.3. Analysis of elements

With the help of ICP-OES many elements like Mn, As, K, Pb, Pd and Hg were analyzed in the powdered drug [21-22].

4.3.1. Processing of sample for elemental analysis

Using a crucible, a precise amount of three grams of dehydrated fine-grained sampling is attained and enkindled through progressively maximizing heat around five hundred to six hundred degrees unless without color of ash is attained. The procured ash (one gram) got once again enkindled in 10 ml of HNO_3 unless its amount is lowered to 50 percent. Yet again HNO_3 of 10 ml is incorporated to the prior solution to enkindle with 250 degrees until the quantity of HNO_3 lowered to 5ml. This solution sustaining beneath the normal temprature is warmed by pretreating through 3 ml of $HClO_4$ being change over to thick smoke, that could be further transferred into ICP-OES.

4.3.2. Analysis of elements using AAS

The processing trials had been examined to get elements utilizing AAS. Elements like Mn, As, K, Pb, Pd and Hg were analyzed in the powdered drug. Following exposure of a quantity of homogeneous fluid sample through the flame, free atoms will be revealed as a consequence of thermal and chemical reactions and such atoms are suitable of absorbing, emitting and fluorescing in the distinctive wavelength.

4.4. EXTRACT PREPARATION

The extract procedure used in this study is soxhlation using methanol as solvent. An amount of five hundred grams was taken and the soxhlation was proceeded till the infusion get colorless. The acquired infusion was evaporated and dried out, kept under dessicator, and used for research.

4.5 QUALITATIVE PHYTOCHEMICAL STUDIES:

The qualitative phytochemical screenings of extracts were carried out to detect the presence of various plant constituents [23, 26-30].

4.5.1 Detection of alkaloids:

Small portions of the solvent free extract were treated separately with few drops of dilute hydrochloric acid and filtered. Filtrates were tested with alkaloid reagents.

4.5.1.1 Mayer's test:

Filtrates were treated with Mayer's reagent (Potassium mercuric iodide). Formation of a cream-colored precipitate indicates the presence of alkaloids.

4.5.1.2 Dragendorff's test:

Filtrates were treated with Dragendorff's reagent (solution of Potassium bismuth iodide). Development of orange precipitate specifies the presence of alkaloids.

4.5.1.3 Wagner's test:

Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Development of brown/reddish precipitate specifies the presence of alkaloids.

4.5.1.4 Hager's test:

Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow precipitate indicates the presence of alkaloids.

4.5.2 Detection of carbohydrates:

Small quantity of extract was dissolved in 5 ml distilled water and filtered. The filtrate was tested for the presence of carbohydrates.

4.5.2.1 Molisch's test:

Filtrates were treated with 2 drops of alcoholic α-naphthol solution and to add con. H_2SO_4 through sides of test tube. Development of the violet ring at the junction shows the presence of Carbohydrates.

4.5.2.2 Fehling's test:

Filtrates were hydrolysed with dil. Hcl, neutralized with alkali, then heated with Fehling's A & B solutions. Development of red precipitate shows the presence of reducing sugars.

4.5.2.3 Benedict's test:

Filtrates were treated with Benedict's reagent and heated gently. Red precipitate shows the presence of reducing sugars.

4.5.3 Detection of Anthraquinone Glycosides:

4.5.3.1 Modified Borntrager's Test:

Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was taken out and reacted with ammonia solution. Development of pink colour in the ammonical layer shows the presence of Anthraquinone-C-glycosides (eg: Barbaloin).

4.5.4 Detection of Saponins:

4.5.4.1 Froth test:

Extracts were diluted with distilled water to 20ml, and this was shaken for 15 min. Development of 1 cm layer of foam shows the presence of saponins.

4.5.5 Detection of Flavonoids:

4.5.5.1 Ferric chloride test:

Extracts were treated with 3-4 drops of ferric chloride solution. Development of bluish black colour shows the presence of phenols.

4.5.5.2 Shinoda test (magnesium – hydrochloric acid):

Extracts were treated with few magnesium turnings and concentrated hydrochloric acid. Flavanoids produce magenta, crimson red colour or occasionally green to blue colour.

4.5.5.3 Lead acetate test:

Extracts were treated with few drops of lead acetate solution. Development of yellow colour precipitate shows the presence of flavonoids.

4.5.5.4 Alkaline reagent test:

Extracts were treated with few drops of sodium hydroxide solution. Development of intense yellow colour, which becomes colorless on addition of dilute acid, shows the presence of flavonoids.

4.5.6 Detection of Phenolics and Tannins:

4.5.6.1 Gelatin test:

To the extract, 1% gelatin solution containing sodium chloride was added. Development of white precipitate indicates the presence of tannins.

4.5.6.2 Vanillin hydrochloric acid test:

Extracts were treated with a few drops of vanillin hydrochloric acid reagent. The formation of pinkish red colour indicates the presence of phenolic compounds.

4.5.7 Detection of proteins and amino acids:

4.5.7.1 Ninhydrin Test:

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Development of blue colour shows the presence of amino acids.

4.5.7.2 Xanthoproteic test:

The extracts were treated with few drops of conc. HNO_3. Formation of yellow colour indicates the presence of proteins.

4.5.8 Detection of fixed oils and fats:

4.5.8.1 Stain test:

The extracts were constrained in between two filter papers. The stain on filter paper shows the presence of fixed oils.

4.5.8.2 Saponification test:

The extracts were treated with a few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein and heated on the water bath for 1-2 h. The soap formation or partial neutralization of alkali indicates the presence of fixed oils and fats.

4.5.9 Detection of Phytosterols and triterpenoids:

4.5.9.1 Libermann-Burchard's test:

Extracts were treated with chloroform and filtered. Then few drops of acetic anhydride was added to the filtrate, boiled and cooled, and then conc. H_2SO_4 was added, shaken and allowed to stand for 10 min. Formation of brown ring at the junction indicates the presence of phytosterols.

4.5.9.2 Salkowski test:

Extracts were treated with chloroform and filtered. Filtrate was boiled and cooled, and then conc. H_2SO_4 was added, shaken and allowed to stand for 10 min. Appearance of golden yellow colour indicates the presence of triterpenes.

EXPERIMENTAL RESULTS

5.1. PHYSICOCHEMICAL, HISTOLOGICAL AND PHYTOCHEMICAL ANALYSIS

Histology of powdery plant product is used to differentiate real and unique products with their adulterate samples [39].

Figure 5.1-5.4 elicited the microscopic view of leaves of *Barleria gibsoni* includes outstanding jutting midrib. Midrib looks thin, penetrating as well as appearance towards the side of leaves. The stomata are predominantly paracytic type on both sides of the lamina. They are oblong and lie parallel to the guard cells. The guard cells are 15x20 µm is size. They occur in singles in a mesophyll cell or quite uniquely, the druses are associated with the minor veins of the lamina.

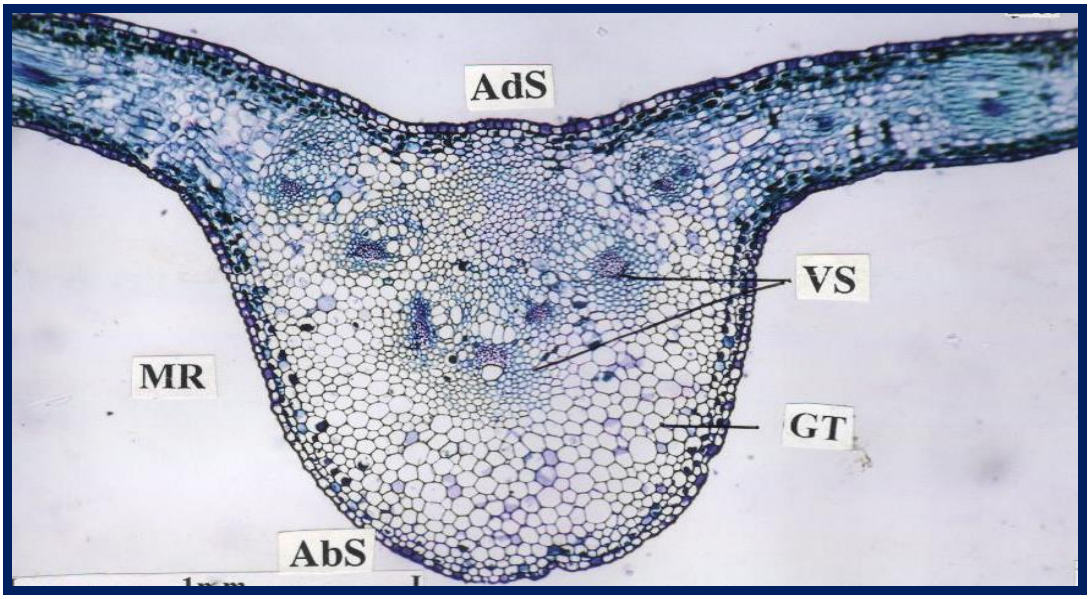

Figure. 5.1 TS of *Barleria gibsoni* leaf through midrib and lamina

AdS: Adaxial Surface, VS: Vessels, AbS: Abaxial Surface, GT: Glandular Trichome, MR: Midrib

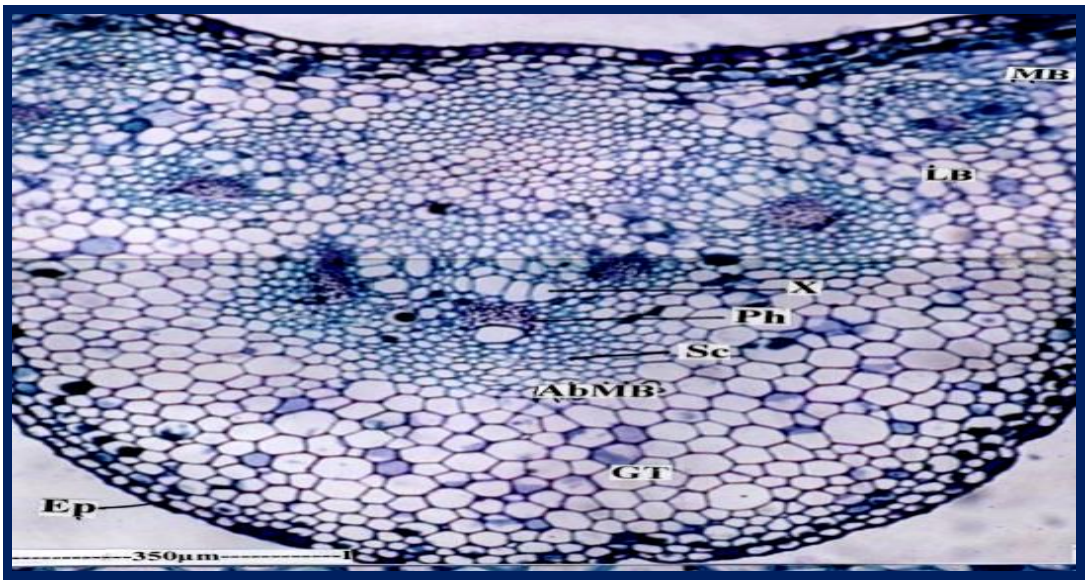

Figure. 5.2 TS of *Barleria gibsoni* leaf midrib-magnified

Ph: Phloem, X: Xylem, AbMB: Abaxial Midrib, GT: Ground Tissue, Ep: Epidermis, LB:Lateral Bundle, Sc: Sclerenchyma

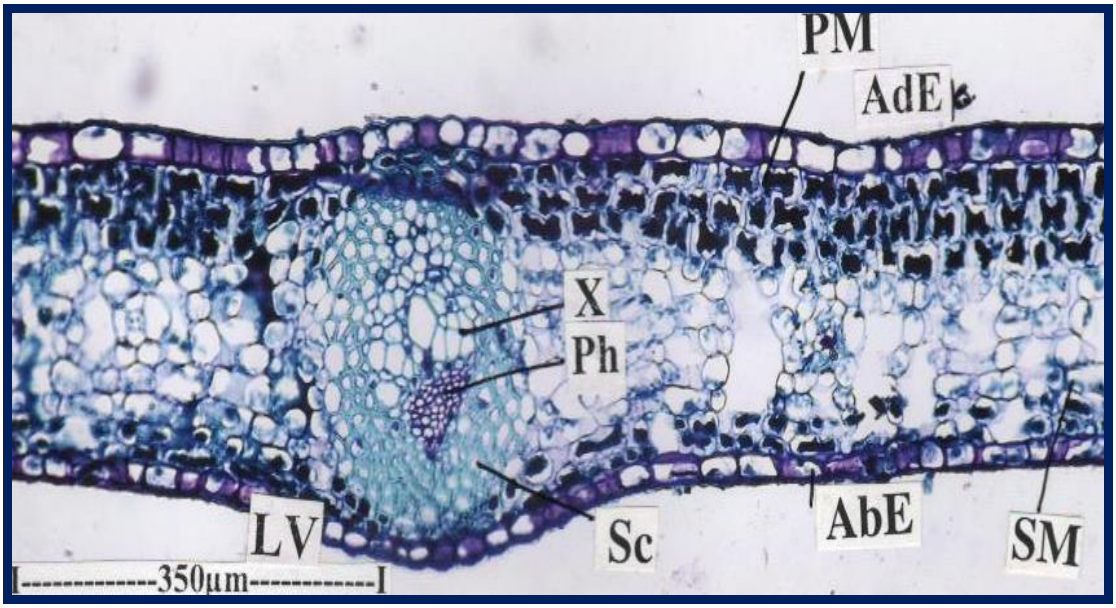

Figure 5.3. TS of *Barleria gibsoni* leaf via lateral vein and lamina part

Ph: Phloem, X: Xylem, Ab E: Abaxial Epidermis, Ad E: Adaxial Epidermis, GT: Ground Tissue, EP: Epidermis, LV: Lateral Vein, Sc: Sclerenchyma, PM: Pallisade Mesophyll, SM: Spongy Mesophyll

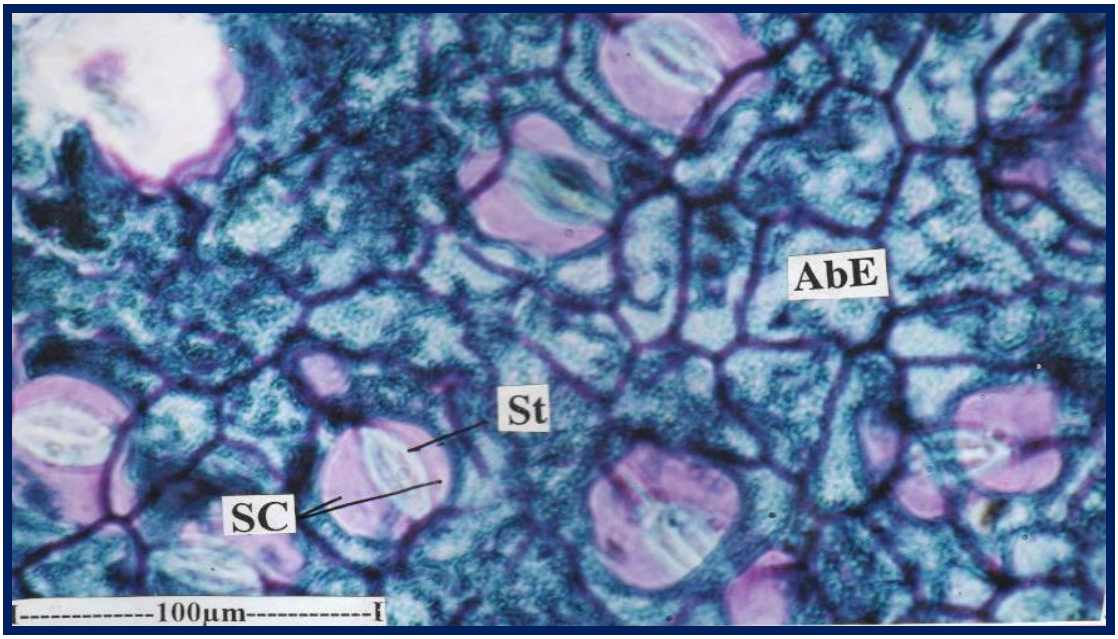

Figure 5.4. TS of *Barleria gibsoni* leaf stomata through paradermal sectional view
AbE: Abaxial Epidermis, St: Stomata, SC: Sclerenchyma

5.2. HEAVY METAL ANALYSIS

In the universal premise, heavy metals had become a current issue [40] as many of the herbal formulations have the content of heavy metal beyond their allowable restrict according to the regulations of world health organization and food and agricultural organization of United States of America [3]. Macronutrients percent was discovered to be inside of their standard controls as depicted in table 5.1. Harmful and heavy metals are even discovered to be within specific standard restricts then ascertained towards the condition of research [41] as illustrated in table 5.2.

Table: 5.1 Macronutrient content in of *Barleria gibsoni*

Metals	**BG**	
	Ethanolic Extract	**Chloroform Extract**
Sodium	41473.7	18972.5
Magnesium	32157.2	31248.9
Potassium	65548.2	3784.1
Calcium	45892.1	37485.1

Each value represented in ppm.

Table: 5.2 Toxic heavy metals content in *Barleria gibsoni*

Metals	BG	
	Ethanolic Extract	**Chloroform Extract**
Arsenic	0.147	0.039
Lead	0.235	0.127
Mercury	0.231	0.195
Palladium	0.412	0.039

Each value represented in ppm.

5.3. EXTRACTIVE YIELD

After extraction by soxhlation the yield of extracts of *Barleria gibsonii* are resulted in table 5.3.

Table: 5.3 Extractive values of EEBG & CEBG

S. No	Plant Name	Solvents used	Extractive yield (%)
1	*Barleria gibsoni*	Ethanol: water (80:20)	17.9
2	*Barleria gibsoni*	Chloroform: water (80:20)	20.1

5.4. PHYTOCHEMICAL EVALUATION

Chemical constituents present in the extracts of EEBG and CEBG were evaluated and illustrated in table 5.4. Alkaloids and glycosides are absent and carbohydrates, flavonoids, saponins and tannins are elicited in both the extracts.

Table 5.4 Preliminary phytochemical evaluation of EEBG & CEBG

Chemical constituent	EEBG	CEBG
Alkaloids	A	A
Carbohydrates	P (***)	P (***)
Glycosides	A	A
Saponins	P (***)	P (***)
Flavonoids	P (**)	P (***)
Tannins	P (**)	P (***)
Proteins & Amino acids	P (*)	P (*)
Fixed oils	P (**)	P (***)
Steroids & Terpenoids	P (**)	P (***)

P: Present; A: Absent *- Less; **-Moderate; ***- High

SUMMARY

To present succinctly, it can be stated that the present investigation, pharmacognostic, phytochemical and pharmacological properties were carried out to ascertain the ethnobotanical claims of pharmacological potentials of the leaves of *Barleria Gibsonii*

The leaves of *Barleria Gibsonii* were collected and authenticated by its morphological and histological characters. The percentage of macronutrients like sodium, potassium, and magnesium were within the stipulated limits. Toxic and heavy metals resembling palladium, arsenic, lead and mercury were within the limit and ensure the safety of the study.

Preliminary phytochemical studies established the presence of tannins, steroids, triterpenoids, carbohydrates, flavonoids, and proteins in EEBG as well as steroids, triterpenoids, flavonoids, tannins, triterpenoids, phenolics, carbohydrates and proteins in CEBG.

7.2 Conclusion:

In the light of the above consideration, it can be concluded that the ethanolic and chloroform extracts of *Barleria Gibsonii* were found to be rich source of phytoconstituents. The extracts were found to possess anti-diabetic, and hepatoprotective activities.

Acknowledgements: None

Conflict of interest: None

REFERENCES

[1]. Ramawat, K. G.; Goyal, S. The Indian herbal drugs scenario in global perspectives, Springer, Berlin Heidelberg, **2008**, 323.

[2]. Gupta, R.; Chadha, K. L. Medicinal and aromatic plants research in India. Malhotra Publication, New Delhi, 11, **1995**, 429.

[3]. Ramawat, K. G.; Merillon, J. M. Biotechnology: Secondary Metabolites: Plants and Microbes, 2nd edition, Science Publishers, Enfield, CT,**2007**, 131.

[4]. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D.; Mazur, M.; Telser, J. Int. J. Biochem. & Cell Biol, 39, **2007**, 44–84.

[5]. Rahimi, R.;Nikfar, S.;Larijani, B.;Abdollahi, M. A.Biomed Pharmacother, 59, **2005**, 365–373.

[6]. Holm, G.; Herbst, V.; and Teil,B.;Planta Medica.67,**2001,** 263-269.

[7]. Kokwaro, J.O.;"Medicinal plants of east africa".Kenya Lit. Bureau. Nairobi. **1993.**

[8]. Iwu, M.M.; Duncan, A.P.; and Okuni, CO.; "New antimicrobial of plant origin". Alexandria, VA. Egypt, **1999.**

[9]. "ICMRh". Quality Standards of Indian Medicinal Plants. 1, **2003.**

[10]. Rahman, A.u.; Iqbal, C.M.I.; and Thomson. W.J.; "Mannual of bioassay techniques for Natural product Chemist". HA Publishers.**20001,** 542.

[11]. Alagar Yadav S, Jabamalai Raj A, Sathishkumar R. *In vitro* antioxidant activity of *Barleria noctiflora* L. f. Asian Pac J Trop Biomed. 2012;2:S 716–22.
[12]. Friedman, Scott E.; Grendell, James H.; McQuaid, Kenneth R. (2003). Current diagnosis & treatment in gastroenterology. New York: Lang Medical Books/McGraw-Hill. pp. 664–679.
[13]. Greenhough S, Hay DC (2012). "Stem Cell-Based Toxicity Screening: Recent Advances in Hepatocyte Generation". Pharm Med. 26 (2): 85–89.
[14]. McNally, Peter F. (2006). GI/Liver Secrets: with STUDENT CONSULT Access. Saint Louis: C.V. Mosby.
[15]. Ostapowicz G, Fontana RJ, Schiødt FV, et al. (2002). "Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States". Ann. Intern. Med. 137 (12): 947–54.
[16]. Anonymous.; “Pharmacopoeia of India”. Ministry of Health, Govt. of India Publication, New Delhi.1966.
[17]. Anonymous.; “Pharmacopoeia of India”. Ministry of Health, Govt. of India Publication, New Delhi. 1985.
[18]. Anonymous.; “Pharmacopoeia of India”. Ministry of Health, Govt. of India Publication, New Delhi. 1996.
[19]. Anonymous.; “The Ayurvedic Pharmacopoeia of India”. Ministry of Health, Govt. of India Publication, New Delhi. 1(I-VI), 2008, 242-243.
[20]. Ewing, G.W.; “Instrumental Methods of Chemical Analysis”. McGraw-Hill International Editions. **1985,** 109.
[21]. Braun, R.D.; “Introduction to Instrumental Analysis”. Pharmamed Press. Hyderabad. 1987, 175.
[22]. Kokate,C.K.; Khandelwal, K.R.; Pawar, A.P.; and Gokhale, S.B.; Practical Pharmacognosy, Nirali Prakashan, Pune. **1995,** 137.
[23]. Ghosh, M.N.; Fundamental of experimental Pharmacology 2nd ed. Scientific book agency: Calcutta: India. **1984,** 53.

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