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Research Article

Pharmacognostical, Preliminary Phytochemical Screening and Antimicrobial Studies of Leaves of *Barleria prionitis* Linn.

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ABSTRACT

Besides the various uses of use of traditional plant *Barleria prionitis* linn for the treatment of infectious diseases caused by micro-organisms we have to focus on antimicrobial activity of this plant in this paper. The whole plant was subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethanol and with distilled water. The various extracts, were subjected to preliminary phytochemical screening for different classes of phytoconstituents. Phytochemical study showed that all the four extracts gave positive tests for phenolic compounds & tannins, alkaloids, glycosides, saponins, carbohydrates, protein and amino acids, flavonoids and phytosterols. The three different concentrations range from 2.5, 5 & 10 mg/ml of the extracts were tested for antibacterial activity using agar disc diffusion assay method against *Salmonella typhi, Bacilus subtilis, Staphylococcus aureus, Vibrio cholera and E. coli*. Streptomycin was used as a reference drug. The ethanolic extract exhibit highest zone of inhibition against *Staphylococcus aureus* is 12 mm with 10 mg/ml concentration. Slurry of ethanolic extract is processed with different solvents by increasing polarity to obtain various fractions. All the extracts and column fractions were used for the determination of the minimum inhibitory concentration. The *S. typhi* [Chloroform extract, Column fraction]; *V. cholera* [Column fraction]; *E. coli* [Chloroform extract, Column fraction] were inhibited at 5 mg/ml level. *Staphylococcus aureus* [Pet. ether extract] inhibited at 3.33 mg/ml level.

Keywords: *Barleria prionitis* linn**,** Antimicrobial activity, Minimum inhibitory concentration, Secondary metabolites, Extracts, Zone of inhibition.

INTRODUCTION

Antibiotics are used from 20th century for the treatment of microbial disease. But its chemotherapy brought dramatic changes in microbial infections dealing with mankind. Various synthetic antimicrobial drugs like methicillin and vancomycin have prominent therapeutic effects in evading microbial pathogens. But due to re-emerging of microbial infection its chemotherapy needs more attention. *S. aureus* become resistant to methicillin by producing an enzyme which inhibits binding of drug to its target site. *E. coli* become resistant to quinolone by mutation in gyr A & par C target site[1]. Therefore there is a need of advancement due to their adverse effects like Vancomycin adverse effects are ototoxicity, tachycardia, nephrotoxicity and hypersensitivity. Cefixime adverse effects are superinfections, nephrotoxicity and anaphylaxis[2]. Trimithoprim causes rashes and hypersensitivity reactions especially in AIDS patients[3].

With this regard the arrival of new effective and safe antimicrobial therapeutic agent is necessary. The naturally occurring medicinal plants having active constituents which show the antimicrobial activity may provide the wide area of research. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body[4].

Barleria prionitis L. commonly called 'Porcupine flower' **is** especially well known for treating bleeding gums and toothache. Because of its anti dontalgic property it is known as 'Vajradanti'[5]. *B. prionitis* has been the centre of interest to pharmacologists as it exhibits a variety of pharmacological activities *viz.* antibacterial activity[6,7,8], antifungal activity[8,9], anthelmintic activity[10], antifertility activity[11,12], antidiabetic activity[13], anti-diarrhoeal activity[14], enzyme inhibitory effects[7,9,15,16], anti-inflammatory activity[17,18,19,20], anti-arthritic activity[20], cytoprotective activity[20,21], hepatoprotective activity[22], diuretic effect[23], anti-nociceptive activity[24] etc. Keeping in mind the traditional/ alternative and complementary medicinal uses and diverse activity potential, *B. prionitis* seems to hold a great potential for in depth investigation for various biological activities, especially antibacterial[6]

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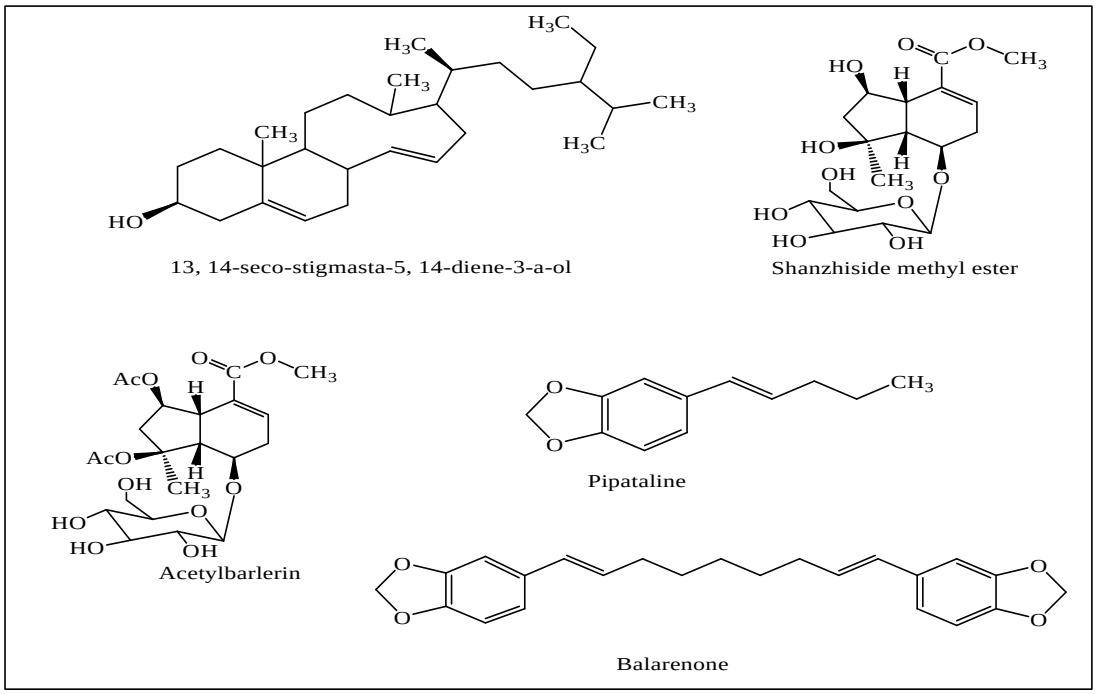

Fig. 1

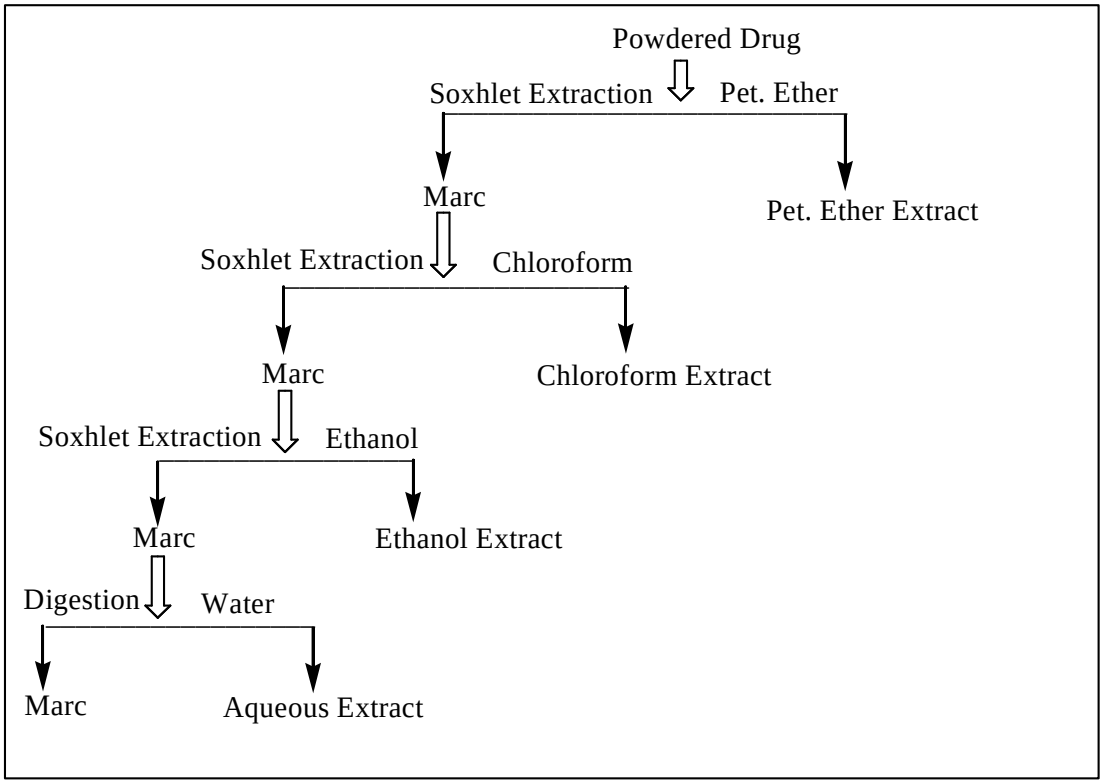

Fig. 2

and antifungal activity[8,9].

The antimicrobial activity of *B. prionitis* may be due to the presence of acetylbarlerin, barlerin, shanzhiside methyl ester, verbascoside, balarenone, pipataline, 13, 14-seco-stigmasta-5, 14-diene-3-a-ol and 6-*O*-acetyl shanzhiside methyl ester[7,8].

Plan of Work

- Subjecting *B. prionitis* to detailed pharmacognostic studies.
- Phytochemical screening.
- Subjecting *B. prionitis* leaves for anti-bacterial activities.
- Determination of Minimum Inhibitory Concentration (MIC).

Plant Material: The whole plant of *B. prionitis* were collected from HARI OM HERBS of Santinagar Chhutmalpur in july 2012. The plant was authenticated by Dr. K. MADHAVA CHETTY, Sri Venkateswara University, TIRUPATI and the plant specimen is kept at the Herbarium of Lala Lajpat Rai College of Pharmacy,

Table 1: Angle of repose

Drug	Height of pile (cm)	Diameter of the base of the pile (cm)	Radius of the base of the pile (cm)	Angle of repose (θ)
Barleria prionitis L.	4.8	13	6.5	36.12

Table 2: Bulk Density (g/cm3)

Drug	I	II	III	Average
Barleria prionitis L.	0.32	0.33	0.33	0.33

Table 3: Tapped Density (g/cm^3)

Drug	I	II	III	Average
Barleria prionitis L.	0.35	0.34	0.35	0.35

Moga.

Standard Drug: Streptomycin was used as a standard drug for antibacterial activity. The drugs obtained were free sample from Cilvil Hospital Jagraon, district- Ludhiana, Punjab, India.

Micromeretic Parameters[25]

Angle of repose: Angle of repose is the maximum angle possible between the surface of a pile of the powder and the horizontal plane. (Table: 1)

Procedure: A glass funnel is held in place with a clamp on a ring support over a glass plate. Approximately 50 gm of powder is transferred in to the funnel by keeping the orifice of the funnel blocked by the thumb. As the thumb is removed, the lab-jack is adjusted so as to lower the plate and maintain about a 3 mm gap between the bottom of the funnel stem and the top of the powder pile. When the powder is emptied from the funnel, the angle of the heap to the horizontal plane is measured with the protractor and calculated by following formula.

$\theta = \tan^{-1} h/r$

Where, h = height of pile, θ = angle of repose & r = radius of the base of the pile

Bulk Density: Bulk density is the mass of powder divided by its bulk volume. A Powder (about 60 gm) was passed through a standard sieve no. 20. A weighed amount (approx. 50 gm) was introduced in to the bulk density apparatus, (aim is that to fill the measuring cylinder up to 75 ml) and the timer knob was set for 100 tapings. The volume occupied by the powder was noted. This final volume was the bulk volume. Then bulk density was calculated by using this equation. (Table: 2)

Bulk density (ñ) = Mass of powder / Bulk volume

Tapped Density: Tapped density was achieved mechanically by tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder was mechanically tapped, and volume readings were taken until little further volume change was observed. (Table: 3)

Tapped density (ñ) = Mass of powder/ Tapped volume

Physical Evaluation[26]

Determination of solvent extractive value[27]

Determination of water soluble extractive value: 5 gm of powdered drug was macerated with 100 ml of water closed flask for 2 hrs and was occasionally shaked with 6 hrs time period and was allowed to stand for 18 hrs. After filtration, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. Percentage of water soluble extractive value was calculated with reference to the air dried drug. (Table: 4)

Determination of alcohol soluble extractive value: Alcohol is an ideal solvent for extraction of various chemicals like tannins, alkaloids, resins etc. Ethyl alcohol (95% v/v) was used for determination of alcohol soluble extractive. 5 gm of powdered drug was macerated with 100 ml of ethanol closed flask for 24 hrs and was occasionally shaken for 6 hrs time period and was allowed to stand for 18 hrs. After filtration, 25 ml of the filtrate evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to the air dried drug. (Table: 4)

Determination of foreign matter: About 10 gm of the sample was weighed and spread on a white tile uniformly without overlapping. Then the sample was inspected by means of 5x lens and the foreign organic matter was separated. After complete separation the matter was weighed and percentage w/w was determined. (Table: 5)

Determination of Moisture Content[26]: The percentage of active constituents in crude drug is mentioned on air dried bases. Hence, the moisture content of the crude drugs should be determined and should also be controlled. The moisture content should be minimized in order to prevent decomposition of crude drugs either due to chemical changes or microbial contamination.

Procedure: The powdered sample of leaves of *B. prionitis* weighed 5 gm accurately and kept in IR moisture balance. The loss in wt. was recorded as percentage (%) moisture with respect to air-dried sample of crude drug. (Table: 5)

Determination of Ash value[27,28]

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of adulteration. Many a time the crude drugs are admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic content. Ash value is a creation to judge the purity of crude drugs. Generally either total ash value or acid-insoluble ash value or both is determined. Total ash usually consists of phosphates, silicates and silica. On the other hand, acid-insoluble ash, which is a part of total ash insoluble in dilute hydrochloric acid, contains adhering dirt and sand.

Physical Evaluation

Table 4: Solvent Extractive Value

Name of the drug	Water soluble Extractive value (%w/w)	Alcohol soluble Extractive value (%w/w)	Chloroform soluble Extractive value (%w/w)	Moisture Content (%w)
Barleria prionitis L.	27.18	12.40	5.68	2.2

Table 5: Physico-chemical Characteristics

Name of the drug	Foreign Organic Matter (%)	Total Ash Value (%)	Acidic Insoluble Ash Value (%)	Water Soluble Ash Value (%)
Barleria prionitis L.	0.7	6.3	0.90	0.92

Table 6: Preliminary phytochemical screening observed for *B. prionitis* whole plant extracts

S. no	Phytochemical screening	Pet ether extract	Chloroform extract	Ethanol extract	Water extract
1.	Alkaloids	+	-	+	-
	1. Mayer's reagent	+	-	-	-
	2. Hager's reagent	+	-	+	-
	3. Wagner's reagent	+	-	+	-
	4. Dragendorff's reagent				
2.	Phenolic compounds and Tanins				
	1. $Fecl_3$	-	-	+	+
	2. Lead acetate test	-	-	+	+
	3. Bromine water test	-	-	+	+
3.	Saponin				
	1. Frothing test	-	-	+	+
4.	Carbohydrates				
	1. Molisch test	-	-	+	-
	2. Fehling's solution A	-	-	+	-
	2. Fehling's solution B	-	-	+	-
	3. Benedict's test	-	-	+	-
5.	Protein and Amino acids				
	1. Millon's test	-	-	-	-
	2. Biuret test	-	-	+	-
	3. Ninhydrin test	-	-	+	-
6.	Glycosides test				
	1. Borntrager's test	-	-	+	-
	2. Legal's test	-	-	+	-
7.	Flavonoids test				
	1. Alkaline reagent test	-	+	+	+
	2. Shinoda test	-	+	+	+
8.	Phytosterols test				
	1. Liebermann's test	-	-	+	+
	2. Libermann Burchard test	-	-	+	+

+ = present , - = absent

Determination of total ash: Total ash was determined by weighing 2 gm of the air dried crude drug in the tared platinum or silica dish and incinerated at a temperature not exceeding 450°C until free from carbon and then was cooled and weighed. (Table: 5)

Determination of acid insoluble ash: The ash obtained from the previous process was boiled with 25 ml of 2M HCI for 5 min. and the insoluble matter was collected on ash-less filter paper and was washed with hot water, ignited, cooled in a dessicator and weighed. Percentage of acid insoluble ash was calculated with reference to the air dried drug. (Table: 5)

Determination of water soluble ash: The ash was boiled with 25 ml of water for 5 min. and the insoluble matter was collected on ash less filter paper and was washed with hot water, ignited for 15 min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash and this represents the water soluble ash. Percentage of water soluble ash was calculated with reference to the air dried drug. (Table: 5)

Preparation of Extracts: The whole plant of *B. prionitis* were dried in shade and coarsely powdered. 500 gm powder material was subjected to successive Soxhlet extraction using different solvents in an increasing order of their polarity *viz* starting from petroleum ether (60-80°C), chloroform, ethanol and then distilled water for not less than 48 hrs. After each extraction the powdered material

Table 7: Antibacterial activity of whole plant extracts of *B. prionitis*

Microorganism	Zone of inhibition (mm) Pet ether (mg/ml)			Chloroform (mg/ml)			Ethanol (mg/ml)			Aqueous (mg/ml)			Streptomycin (µg/ml)
	2.5	5	10	2.5	5	10	2.5	5	10	2.5	5	10	10
Salmonella typhi	8	9	10	7	9	11	-	8	11	-	7	10	11
Bacilus subtilis	-	7	10	-	-	-	-	-	-	-	-	-	10
Staphylococcus aureus	7	9	10	-	7	10	7	10	12	-	7	9	12
Vibrio cholerae	-	7	9	7	8	10	7	10	11	7	9	10	10
E. coli	-	8	9	-	9	11	7	9	11	7	8	9	16

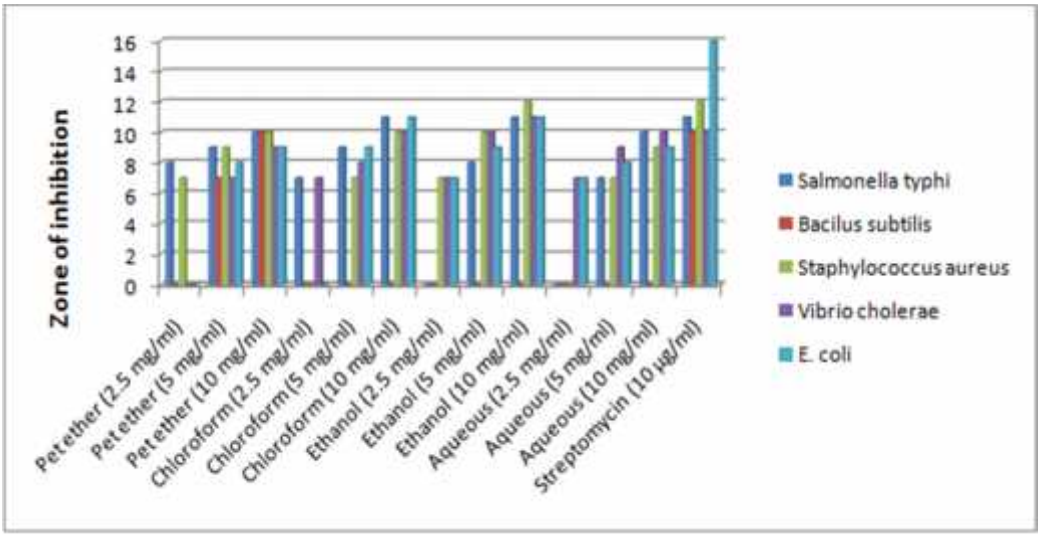

Fig. 3

Determination of Minimum Inhibitory Concentration of Extracts:

Table 8: Effect of *B. prionitis* Petroleum ether whole plant extracts on different group of bacteria

Name of Strains	Concentration (mg/ml)			
	3.33	6.66	16.66	33.33
Salmonella typhi	+	-	-	-
Bacillus subtilis	-	-	-	-
Staphylococcus aureus	-	-	-	-
Vibrio cholerae	+	-	-	-
E. coli	+	+	-	-

Table 9: Effect of chloroform extract of *B. prionitis* on different group of bacteria

Name of Strains	Concentration (mg/ml)			
	5	10	25	50
Salmonella typhi	-	-	-	-
Bacillus subtilis	+	+	+	+
Staphylococcus aureus	-	-	-	-
Vibrio cholerae	+	+	-	-
E. coli	-	-	-	-

was dried in air at room temperature. Finally, marc was digested with distilled water for 24 hrs or more to obtain aqueous extract. Each extract was concentrated in vacuum using Rotatory evaporator (Laborota 4001-efficent, Heidolph Instruments, Germany). Extracts were weighed subsequently and the percentage yields were calculated of each extract obtained individually in terms of the air dried weigh of plant material.

Table 10: Effect of ehanolic extract of *B. prionitis* on different group of bacteria

Name of Strains	Concentration (mg/ml)			
	10	20	50	100
Salmonella typhi	+	-	-	-
Bacilus subtilis	+	+	+	+
Staphylococcus aureus	-	-	-	-
Vibrio cholera	+	+	+	-
E. coli	-	-	-	-

Table 11: Effect of aqueous extract of *B. prionitis* on different group of bacteria

Name of Strains	Concentration (mg/ml)			
	10	20	50	100
Salmonella typhi	+	+	-	-
Bacillus subtilis	+	+	+	+
Staphylococcus aureus	+	+	-	-
Vibrio cholera	+	+	+	-
E. coli	-	-	-	-

Phytochemical Screening: Weighed quantity of the various extracts, were subjected to preliminary phytochemical screening using standard methods[29]. All the extracts of *B. prionitis* were screened for different classes of phytoconstituents *viz*. alkaloids, steroids, terpenoids, anthraquinone, glycosides, flavonoids, tannins and phenolic compounds, saponins, carbohydrates, proteins and amino acids using specific standard reagents[30] and standard methods[31].

Anti-Bacterial Activity

Microorganisms (bacterial strains): Clinically important microbial strains were obtained from Sanjay Biologicals Museum, Amritsar, Punjab, India.

Media Used For Antibacterial Activity

Dehydrated Nutrient agar media[32] (HI MEDIA Laboratories, Mumbai)

Ingredient----------gm/litre
Sodium chloride----------05
Beef extract----------03
Peptone----------05
Agar----------28
Yeast extract----------05

Preparation of Media: Dehydrated nutrient agar medium (28 gm) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

Media Used For Minimum Inhibitory Concentration

- Solid Media (HI MEDIA Laboratories, Mumbai)

Bacteriological Peptone (Oxoid) --1% w/v
Beef Extract (Oxoid) ----------2% w/v
Sodium Chloride (Analar) ----------0.5% w/v
Agar (Oxoid) ----------1.5% w/v
Distilled Water ---------- q.s. to 100ml
pH----------7.2 - 7.4

- Liquid media (HI MEDIA Laboratories, Mumbai)

Bacteriological Peptone (Oxoid) -----1% w/v
Beef extract (Oxoid) ---------- 0.5% w/v
Sodium Chloride (Analar) ----------0.5% w/v
Distilled Water ----------q.s. to 100ml
pH----------7.2 - 7.4

- Peptone water (HI MEDIA Laboratories, Mumbai)

Bacteriological peptone (Oxoid) ------1% w/v
Sodium Chloride (Analar) ----------0.5% w/v
Distilled Water ----------q.s. to 100ml
pH----------7.2 - 7.4

Preparation of extract samples from dried residues: A suitable chemical which is able to dissolve plant extracts without its own activity is chosen. Good solvent for experimental purposes is DMSO (Merck, 2001), so it was used for dissolving various extracts obtained as a result of soxhlet extraction for antibacterial testing. Variable concentrations of extracts were prepared by dissolving dried residues in DMSO for testing inhibitory efficacy against selected bacterial strains. Stock solutions of extracts were diluted in DMSO to produce concentrations sterilized by filtration by using sintered glass filter and stored at 4°C.

Anti Bacterial Assay: The three different concentrations of the extracts were tested for antibacterial activity using agar disc diffusion assay method[33]. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 370°C for 24 hrs and were referred to as seeded broth. Prepared media was poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 370°C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10µg/disc) was used as standards. All data on antibacterial activity were average of triplicate. The solvent loaded disc without extracts in it served as control in the study. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

Fractionation of most active extract: Among four extracts of plant *B. prionitis*, the extract having potent antibacterial activity is further fractionated by column chromatography. The ethanolic extract exhibit most effective antibacterial activity than other extracts. Slurry of this extract is

Table 12: Effect of column fractions of *B. prionitis* on different group of bacteria

Name of Strains	Concentration (mg/ml)			
	5	10	25	50
Salmonella typhi	-	-	-	-
Bacillus subtilis	-	-	-	-
Staphylococcus aureus	+	+	-	-
Vibrio cholera	-	-	-	-
E. coli	-	-	-	-

Table 13: Comparitive Study of MIC

Name of Strains	Minimum Inhibitory Concentration (mg/ml)				
	Pet ether extract	Chloroform extract	Ethanol extract	Aqueousextract	Column fraction
Salmonella typhi	6.66	5	20	50	5
Bacillus subtilis	3.33	Ni	Ni	Ni	5
Staphylococcus aureus	3.33	5	10	50	25
Vibrio cholera	6.66	25	100	100	5
E. coli	16.16	5	10	10	5

Ni: Not inhibited

processed with different solvents by increasing polarity to obtain various fractions.

Column chromatography of ethanol extract: The ethanol extract (5 gm) of *B. prionitis* was loaded on to a column packed with silica gel (60-120 mesh), and eluted using chloroform and chloroform - ethanol as mobile phases. A total of 155 fractions, each of 250 ml were collected. These were pooled based on similar thin layer chromatography to get 4 fractions (F_1 to F_4), which were evaluated for antimicrobial activity at a concentration of 5, 10, 25 and 50 mg/ml. Determination of Minimum Inhibitory Concentration (MIC): MIC is defined as the lowest conc. of a extract that completely inhibits the growth of the microorganism in 24 hours[34].

Petroleum ether extract was added to molten nutrient agar in the following concentration (mg/ml): 3.33; 6.66; 16.66; 33.33. Chloroform extract and column fraction was then added to molten nutrient agar in the following concentration (mg/ml): 5; 10; 25; 50. Ethanol (70%) extract added to 9 ml molten nutrient agar in the following concentration (mg/ml): 10; 20; 50; 100 and poured into sterile petri dish. The pH of the media was maintained at 7.2-7.4. The inoculums consisted of an overnight grown broth culture of a bacterium diluted in such a manner that a 2 mm (internal diameter) loopful of that culture contain 10^5 colony forming unit (CFU). These were then spot inoculated at the sterilized laminar air flow on nutrient agar plates containing increasing amount of a compound, inoculated at 370°C up to 72 hrs for determination of the minimum inhibitory concentration (MIC)[35].

RESULTS AND DISCUSSION

Micromeretic Parameters

Physiochemical study showed that total ash value was 6.30 %, acid insoluble ash was 0.90 % water insoluble ash was 0.92 %, foreign organic matter 0.7 %, alcohol soluble extractive value was 12.40 %w/w, water soluble extractive value was 27.18 %w/w, chloroform soluble extractive value 5.68 %w/w and loss on drying was 5.20 %.

Phytochemical study showed that pet ether, chloroform, methanol and water extracts gave positive tests for phenolic compounds and tannins, alkaloids, glycosides, saponins, carbohydrates, protein and amino acids, flavonoids and phytosterols.

Plant is further subjected to antimicrobial study. Among the tested bacteria it showed the highest zone of inhibition against *Staphylococcus aureus* is 12 mm with 10 mg/ml ethanolic extract. The results obtained showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract where as significant activity was observed with both chloroformic and ethanolic extracts. Chloroform and ethanol extracts of *B. prionitis* at a concentration of 10 mg/ml were equally inhibit *Salmonella typhi* and *E.coli* with zone of inhibition is 11mm. Pet ether and aqueous extracts of *B. prionitis* at a concentration of 10 mg/ml were equally inhibit *Salmonella typhi* and *E.coli* with zone of inhibition are 10 mm & 9 mm respectively. It was observed that with increasing concentration from 2.5 mg/ml to 10 mg/ml of pet ether, methanol, chloroform and water extracts increases the zone of inhibition respectively.

Result given in table 8 indicates that *Bacillus subtilis* and *Staphylococcus aureus* were found to be highly sensitive to the drug as they were inhibited at 3.33 mg/ml. *Salmonella typhi* and *Vibrio cholerae* were inhibited at 6.66 mg/ml level. A MIC of 16.66 mg/ml was observed against *E. coli.*

Result given in table 9 indicates that *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* were found to be highly sensitive to the drug as they were inhibited at 5 mg/ml level. *Vibrio cholerae* was inhibited at 25 mg/ml level. The remaining *Bacilus subtilis* was resistant to the drug.

Result given in table 10 indicates that *Staphylococcus aureus and E.coli* were found to be highly sensitive to the drug as they were inhibited at 10 mg/ml level. *Salmonella typhi* was inhibited at 20 mg/ml level. An MIC of 100 mg/ml was observed against *Vibrio cholerae*. The *Bacilus subtilis* was resistant to the drug.

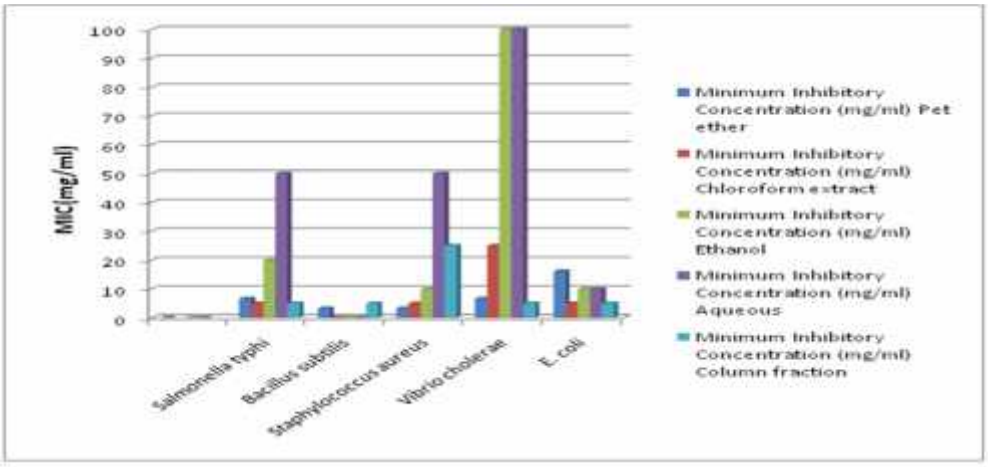

Fig. 4

Result given in table 11 indicates that *E. coli* was found to be highly sensitive to the drug as they were inhibited at 10 mg/ml level. *Salmonella typhi* and *staphylococcus aureus* were inhibited at 50 mg/ml level. The *Bacilus subtilis* was resistant to the drug.

Result given in table 12 indicates that *Salmonella typhi*, *Bacillus subtilis*, *Vibrio cholerae* and *E. coli* were found to be highly sensitive to the drug as they were inhibited at 5 mg/ml level. A MIC of 25 mg/ml was observed against *Staphylococcus aureus*.

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids[36]. The antimicrobial potency of *B. prionitis* may be due to the presence of five Iridoid glucoside esters, acetylbarlerin (6, 8-di-*O*-acetyl shanzhiside methyl ester), barlerin (8-*O*-acetyl shanzhiside methyl ester), shanzhiside methyl ester and 6-*O*-acetyl shanzhiside methyl ester, verbascoside (6-*O*-trans-p-coumaroyl-8-*O*-acetylshanzhiside methyl ester).[37,38]

From the fig. no. 4 it can be concluded that the *S. typhi* [Chloroform extract, Column fraction]; *V. cholera* [Column fraction]; *E. coli* [Chloroform extract, Column fraction] were inhibited at 5 mg/ml level. *Staphylococcus aureus* [Pet. ether extract] inhibited at 3.33 mg/ml level.

CONCLUSION

The present study was designed to study the pharmacognostic characters of *B. prionitis* and evaluation of its antimicrobial activity. The physiochemical parameters like that total Ash value, acid insoluble ash, water insoluble ash, foreign organic matter, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value, and loss on drying were following WHO guidelines and their results were noted. The soxhlet extraction of powdered plant was carried out for preparation of various extracts with the solvents in increasing order of polarity *viz* pet ether, chloroform, methanol and water. The physical appearance and percentage yield of various extracts were noted. The phytochemical screening of extracts was carried out and it revealed the presence of alkaloids and phenolic compounds & tannins in pet ether and ethanol extracts, saponins in ethanol and aqueous extracts, carbohydrates, protein & amino acids and glycosides are in ethanol extract, flavonoids in chloroform, methanol and aqueous extracts, phytosterol compounds in ethanol and aqueous extract.

The antimicrobial activity of pet ether, chloroform, methanol and aqueous extracts was evaluated by disc diffusion method. All the extracts were evaluated in concentration range from 2.5 mg/ml to 10 mg/ml. The chloroform and methanol extracts showed significant antibacterial activity than the other extracts.

So, it is concluded that *B. prionitis* possesses good antibacterial activity. The antimicrobial activity of *B. prionitis* may be due to the presence of acetylbarlerin, barlerin, shanzhiside methyl ester, verbascoside, balarenone, pipataline, 13,14-seco-stigmasta-5,14-diene-3-*α*-ol and 6-*O*-acetyl shanzhiside methyl ester[7,8].

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REFERENCE

1. Saga T and Yamaguchi K. History of antimicrobial agents and resistant bacteria. *Japan Medical Association Journal.* 2009; **52(2)**: 103-08.
2. http://www.flashcardmachine.com/antimicrobial-drugs.html
3. Skold O. Sulfonamide resistance: mechanisms and trends. *Drug Resistance Updates.* 2000; 3: 155-60.
4. Agrawal P. et al. Randomized, placebo-controlled, single-blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. *International Journal of Clinical Pharmacology and Therapeutics.* 1996; 34: 406-409.
5. Banerjee AK, Maji S, Mahapatra S and Banjeri P. *Barleria prionitis* linn.: A review of its traditional uses, phytochemistry, pharmacology and toxicity. *Research journal of phytochemistry*. 2012; 6: 31-41.
6. Shukla P, Singh A, Gawri S, Alexande A and Sonwane S. In vitro propagation of *Barleria prionitis* Linn and its antibacterial activity. *Int. J. Pharma Prof. Res.* 2011; 2: 198-200.
7. Kosmulalage KS, Zahid S, Udenigwe CC, Akhtar S, Ata A and Samarasekera R. Glutathione S-transferase,

acetylcholinesterase inhibitory and antibacterial activities of chemical constituents of *Barleria prionitis*. *Z. Naturforsch*. 2007; 62b: 580-586.
8. Aneja KR, Joshi R and Sharma C. Potency of *Barleria prionitis* L. bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin. *New York Sci. J.* 2010; 3(11): 5-12.
9. Amoo SO, Ndhlala AR, Finnie JF and Staden JV. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three *Barleria* species. *S. Afr. J. Bot.* 2011; 77: 435-445.
10. Chavan CB, Hogade MG, Bhinge SD, Kumbhar M and Tamboli A. *In vitro* anthelmintic activity of fruit extract of *Barleria prionitis* Linn. Against Pheretima posthuma. *Int. J. Pharm. Pharm. Sci.* 2010; 2: 49-50.
11. Gupta R, Thakur B, Singh B, Singh HB, Sharm VD, Katoch, VM and Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Indian J Med Res.* 2010; 131(6): 809-813.
12. Verma PK, Sharma A, Joshi SC, Gupta RS and Dixit VP. Effect of isolated fractions of *Barleria prionitis* root methanolic extract on reproductive function of male rats: Preliminary study. *Fitoterapia*. 2005; 76: 428-432.
13. Dheer R and Bhatnagar P A. study of the antidiabetic activity of *Barleria prionitis* Linn. *Ind. J. Pharm.* 2010; 42(2): 70-73.
14. Jaiswal SK, Dubey MK, Verma AK, Das S, Vijaykumar M and Rao CV. Evaluation of iridoid glycosides from leave of *Barleria prionitis* as an anti-diarrhoeal activity: An Ethnopharmacological study. *Int. J. Pharm. Sci*. 2010; 2: 680-686.
15. Ata A, van den Bosch SA, Harwanik DJ and Pidwinski, GE. Glutathione S-transferase and acetylcholinesterase-inhibiting natural products from medicinally important plants. *Pure Appl. Chem*. 2007; 79: 2269-2276.
16. Ata A, Kalhari KS and Samarasekera R. Chemical constituents of *Barleria prionitis* and their enzyme inhibitory and free radical scavenging activities. *Phyto chem. Lett.* 2009; 2: 37-40.
17. Amoo SO, Finnie JF and Staden JV. In vitro pharmacological evaluation of three *Barleria* species. *Journal of Ethanopharmacology*. 2009; 121(2): 274-277.
18. Singh B, Bani S, Gupta DK, Chandan BK, and Kaul A. Anti-inflammatory activity of TAF an active fraction from the plant *Barleria prionitis* Linn. *J. Ethanopharmacol.* 2003; 85: 187-193.
19. Khadse CD and Kakde RB. Anti-inflammatory activity of aqueous extract fractions of *Barleria prionitis* L. roots. *Asian J. Plant Sci. Res.* 2011; 1: 63-68.
20. Manek RA, Sheth NR, Vaghasiya JD, Malaviya SV, Jivani NP and Chavda JR. Study on herb-herb interaction potential of *Glycyrrhiza glabra* with *Solanum xanthocarpum* and *Adhatoda vasica* on mast cell stabilizing activity. *Int. J. Pharmacol.* 2011; 7: 589-598.
21. Maji AK, Bhadra S, Mahapatra S, Banerji P and Banerjee D. Mast cell stabilization and membrane protection activity of *Barleria prionitis* Linn, *Pharmacog*. J. 2011; 3: 67-71.
22. Singh B, Chandan BK, Prabhakar A, Taneja SC, Singh J, Qazi GN. Chemistry and hepatoprotective activity of an active fraction from *Barleria prionitis* Linn. In experimental animals. *Phytother Res*. 2005; 19(5): 391-404.
23. Musale SB, Jagtap VA, Patil MS, Chittam KP and Wagh KP. Diuretic activity of *Barleria prionitis* Linn Flower extract. *Int. J. Drug Discovery Herbal Res*. 2011; 1: 20-21.
24. Jaiswal SK, Dubey MK, Das S, Verma AR, Vijayakumar M and Rao CV. Evaluation of flower of *Barleria prionitis* for anti-inflammatory and anti-nociceptive activity. *Int. J. Pharm. Biol. Sci.* 2010; 1: 1-10.
25. Martin AJ, Physical Pharmacy, fourth edition, BI Waverly Pvt. Ltd., New Delhi, 1994, 423-452.
26. Harborne JB. Phytochemical Methods, chapman and hall, Newyork. 1984.
27. Mukherjee PK, Quality Control of Herbal Drugs, 1st Edition, Business Horizons Pharmaceutical Publishers, 2002, 398-99, 677.
28. Indian Pharmacopoeia, IP, Vol 2. Published by the Controller of Publications, Ministry of Health and Family Welfare, Government of India, New Delhi, 1996. A48, A50-A54, A96, A99.
29. Ambi AA, Abdurrahman EM, Sule MI, Pateh UU, Abdurrahman YR and Ibrahim NDG. Phytochemical Screening and Histopathological Studies on the Seeds of *Colocynthis citrullus* in Albino Rats. *Nigerian Journal of Pharmaceutical Sciences.* 2007; 6(2): 7-13.
30. Khandelwal KR. *Preliminary Phytochemical Screening in Practical Pharmacognosy*. Niralli Parkashan, Pune, 2004, 149-56.
31. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical Screening and Extraction: A Review, *Internationale Pharmaceutica Sciencia.*2011; 1(1): 98-106.
32. Chavan CB, Shinde UV, Hogade M and Bhinge S. Screening of *in vitro* antibacterial assay of *Barleria prionitis* linn. *Journal of Herbal Medicine and Toxicology.* 2010; 4 (2): 197-200
33. Bauer A N, Kirby WMM, Sherries JC and Truck M. *American Journal of Clinical Pathology.* 1996; 45: 493
34. Aneja KR and Joshi R. Antimicrobial activity of *Amomum subulatum* and *Elettaria cardamomum* against dental caries causing microorganisms. *Ethnobotanical Leaflets*. 2009; 13: 840-849.
35. Dastidar SG, Chaudhury A, Annaduria S, Ray S, Mookherjee A and Chakrabarty AN. *In vitro* and *in vivo* antimicrobial action of fluphenazine. *J Chemother*. 1995; **7**: 201-206
36. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews.* 1999; 12(4): 564-82.
37. Chen JL, Blance P, Stoddart CA, Bogan M, Rozhon EJ, Parkinson N, Ye Z, Coopre R, Balick M, Nanakorn W

and Kernan MR. New iridoids from the medicinal plant *Barleria prionitis* with potent activity against respiratory syncytial virus. *Journal of Natural Products*. 1998; 61: 1295-1297.

38. Suri JL, Banerjee SK, Taneja SC, Chandra S, Anand AS, Prabhakar A, Jaggi A, Sing B, Saxena AK, Chandan BK, Krishan B and Swami S. United States Patent Application Publication, 2003;20030181397.