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IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF SAPONIN FRACTION FROM THE LEAVES OF *BARLERIA CRISTATA* L.

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ABSTRACT

A simple HPTLC method was used to determine the saponin profile of *Barleria cristata* L. crude leaf extract. The antimicrobial activity of saponin fraction from the leaves of *Barleria cristata* L. was studied *in-vitro* against four bacterial species and four fungal species by agar disc diffusion method. *Klebsiella Pneumonia*, *Staphylococcus aureus, E. coli, Aspergillus parasites* were the most inhibited microorganism. The present study suggests that the saponin fraction possess significant antimicrobial activity and can be used to develop a potential antimicrobial agent.

INTRODUCTION: Medicinal plants are widely used for the treatment of human diseases all over the world because they contain components with therapeutic value [1]. According to World Health Organisation (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

The medicinal value of plants lies in some chemical substance that produces a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, saponins, flavonoids, tannins and phenolic compounds [2]. Diseases that have been managed traditionally using medicinal plants include malaria, diarrhoea, dysentery, fungal and bacterial infections, respiratory infection and skin diseases [3, 4]. In the last few years, a number of studies have been conducted to prove that plant has antimicrobial activity. Most of the studies reported were using crude extracts [5, 6]. But studies with purified plant compounds are very scanty.

Barleria cristata L. is a shrub found widely in subtropical Himalaya, Sikkim, Central and South India. It has various medicinal and therapeutic uses. Different parts of *Barleria cristata* L. have been used in the treatment of various diseases like anemia, toothache and cough. Root and leaves are used in the treatment of swelling and inflammation [7].

Phytoconstituent saponins are steroidal glycosides generally associated with plant defence but also have wide range of biological properties [8]. These include deterrence to insects [9], antifungal properties [10, 11], anti – inflammatory [12] and anti – cancer [13, 14] properties.

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Although synthetic and semi synthetic antimicrobial drugs available in various markets today there is a need for new one to cope with the increased evolution of multiple antimicrobial resistant strains of organisms [15]. Numerous methods have been previously reported for the determination of saponins in the plant extract. In the present study a simple HPTLC method was used for the qualitative identification of saponins and antimicrobial potential of pure saponin fraction from the leaves of *Barleria cristata* L.

MATERIALS AND METHODS:

Collection of plant sample: The plant *Barleria cristata* L. commonly knowns as KodilKannu (in south india) was collected from the rural areas around Erode District, Tamilnadu in the month of December. It was then authenticated by Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (N BSI/SC/5/23/08-09). Fresh plant leaves were washed, shade dried and were powdered using mixer grinder.

Extraction of sample for HPTLC analysis: The dried *Barleria cristata* L. leaf powder (5 gm) was extracted with 100 ml of methanol in soxlet apparatus for 6 hours and filtered the extract through what man No.1 filter paper. The crude extract was condensed by using vacuum evaporator. The residues were dissolved in methanol and made up to 20 ml. This solution was used as test solution for analysis.

HPTLC analysis of leaf sample for saponin profile: The following chromatographic conditions were used to determine the saponin profile of *Barleria cristata* L. leaf extract.

Stationary phase: Silica gel 60F254 precoated TLC plates

Mobile phase: Chloroform: glacial acetic acid: Mehanol: water (6.4: 3.2: 1.2: 0.8)

Derivatization reagent: Anisaldehyde – sulphuric acid reagent.

Sample volume: 8 µl

Temperature: Ambient temperature.

Migration distance: 8 cm.

Procedure: Before spotting the HPTLC plate was prewashed with methanol. The test solution was applied as 10 mm sharp band by means of Linomat 5 sample applicator. The spot was air dried. The mobile phase solvent was (25 ml) poured into a twin trough glass chamber and was left to equilibrate for 15 minutes. Then the plate was placed in the chamber. The plate was developed until the solvent front had travelled at a distance of 8 cm above the base of the plate. The plate was removed and air dried to evaporate solvents from the plate[16].

Then, the plate was kept in photodocumentation chamber and viewed under white light, UV – 254 nm and UV - 366 nm wavelength light from Reprostar – 3. Then the plate was sprayed with respective spraying reagent for saponins (for derivatization) and kept in hot air oven at 120^0C for 5 minutes. The plate was cooled and scanned at 500 nm for saponins using TLC scanner-3. The peak areas were observed from the peak table.

Preparation of pure saponin fraction for antimicrobial activity study: The powdered leaf sample was defatted by petroleum ether for 3 hours at 40^oC. After filtering the petroleum ether, the sample was extracted with methanol for 3 hours with mild heating [17]. The combined methanol extract was concentrated and methanol extract of sample was obtained. To get crude saponin extract the sample was dissolved in methanol and acetone (1: 5 v/v) to precipitate the saponins [18]. The precipitate was dried under vacuum to get whitish amorphous powder named as crude saponin extract (CSE). To get pure saponin fraction (PSF), certain amount of CSE was fractioned by applying to Merck silica gel60 (230-400 mesh) column chromatograph and eluted with chloroform – methanol – water (70: 30: 10) [19]. Five fractions were collected and were evaporated under reduced temperature. Fraction 1was chosen based on the level of total saponin concentration [20].

Test microorganism: Four bacterial strains viz., *Staphylococcus aureus, Salmonella para typhi, E. coli, Klebsiella pneumonia* and four fungal strains such as *Aspergillus niger, Aspergillus fumigates, Aspergillus parasites* and *Candida albicans* were used for testing antimicrobial ativity.

Antibacterial and Antifungal activity: The antibacterial and antifungal activity analyses were carried out by disc diffusion technique. The bacterial strains were inoculated into nutrient broth and incubated. The turbidity of culture was standardized to that of Mc Farland standards. Muller Hinton agar solid medium was used for culturing bacteria. The standardized inocula were spread on the petriplates and the discs impregnated in pure saponin fraction and standard Ciprofloxacin (5 µg/disc) discs were placed with the help of sterile forceps. The plates were incubated at 37^0C for 24 hours.

Each fungal culture inocula was evenly spread on sabourand Dextrose agar using a sterile swab. The Clotrimazole (10 µg/disc) standard disc and pure saponin fraction soaked discs were placed. The plates were incubated at 37^oC for 48 hours. At the end of incubation, the inhibition zones formed were measured using a vernier calliper. Average diameter of two of each zones were calculated. All the experiments were done in 3 replicates.

RESULTS:

HPTLC analysis of *Barleria cristata* L. leaf extract for saponin profile: The coarse leaf powder of *Barleria cristata* L. was analysed for saponin profile opting simple HPTLC method. The HPTLC chromatographic plates were derivatized with Anisaldehyde – Sulphuric acid reagent, dried and scanned at 500 nm for saponins.

The green, violet, yellow, brown coloured zones at Rf value 0.28, 0.32, 0.4, 0.46 and 0.64 present in the chromatogram confirm the presence of saponins in the sample. The total saponin content of crude leaf extract is 3.48 ± 0.527 mg% and the table 1 and figure 1 confirms the occurrence of 5 different saponin compounds in the leaf extract.

TABLE 1: HPTLC CHROMATOGRAPHIC PEAK TABLE FOR SAPONIN PROFILE

Track	Peak	Rf	Height	Area	Assigned substance
A	1	0.02	448.9	9473.6	unknown *
A	2	0.1	384.3	16572.7	unknown *
A	3	0.2	29.7	543.4	unknown *
A	4	0.22	30	539.3	unknown *
A	5	0.28	70.1	2390.2	Saponin 1
A	6	0.32	51	1520.1	Saponin 2
A	7	0.4	51.1	1514.9	Saponin 3
A	8	0.46	196.5	6660.8	Saponin 4
A	9	0.53	19.9	473.6	unknown *
A	10	0.64	66.9	3114.4	Saponin 5
A	11	0.78	46.9	2600.1	unknown *

* Compounds not related to Saponins

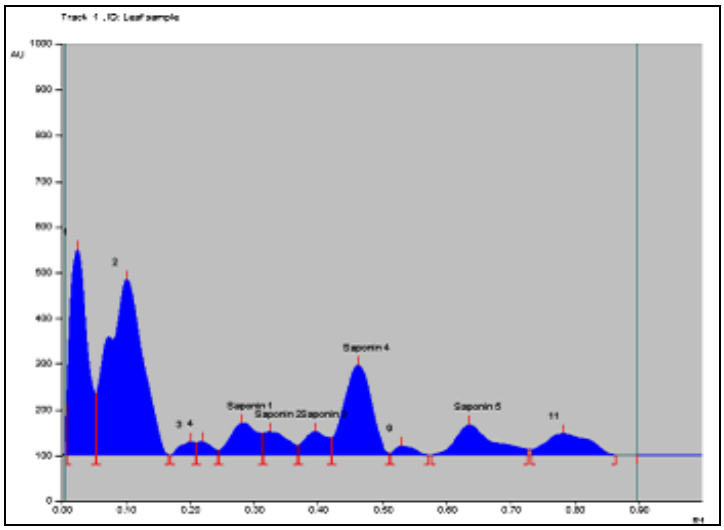

FIGURE 1: PEAK DENSITOGRAM OF *BARLERIA CRISTATA* L. LEAF EXTRACT FOR SAPONIN PROFILE

Antimicrobial activity of Pure Saponin Fraction: *In vitro* antimicrobial activities of pure saponins from the leaves of *Barleria cristata* L. is shown in figure 2 and **table 2**.

The total saponin concentration of fraction 1 was 15 mg/ml. The pure saponin fraction of the plant studied showed various antimicrobial activities against the microorganisms tested. Among the four bacterial pathogens tested, the saponin fraction extract was found to be effective against *Klebsiella pneumonia* (10 mm), *S.aureus* (9 mm), *E. coli* (9 mm) followed by *Salmonella paratyphi* (8 mm). The zone of inhibition by the pure saponin was comparable with the standard Ciprofloxacin (5 µg/disc) antibiotics.

It is interesting to note that pure saponin fraction from the leaves of *Barleria cristata* L. showed significant antifungal activity when compared to standard fungicide, Clotrimazole (**table 3**). The extent of inhibition was greater against fungal pathogens than bacterial species. The pure saponin fraction showed maximum activity against *Aspergillus parasites* (12 mm) followed by *Aspergillus niger* (9 mm), *Aspergillus fumigates* (9 mm) and *Candida albicans* (9 mm).

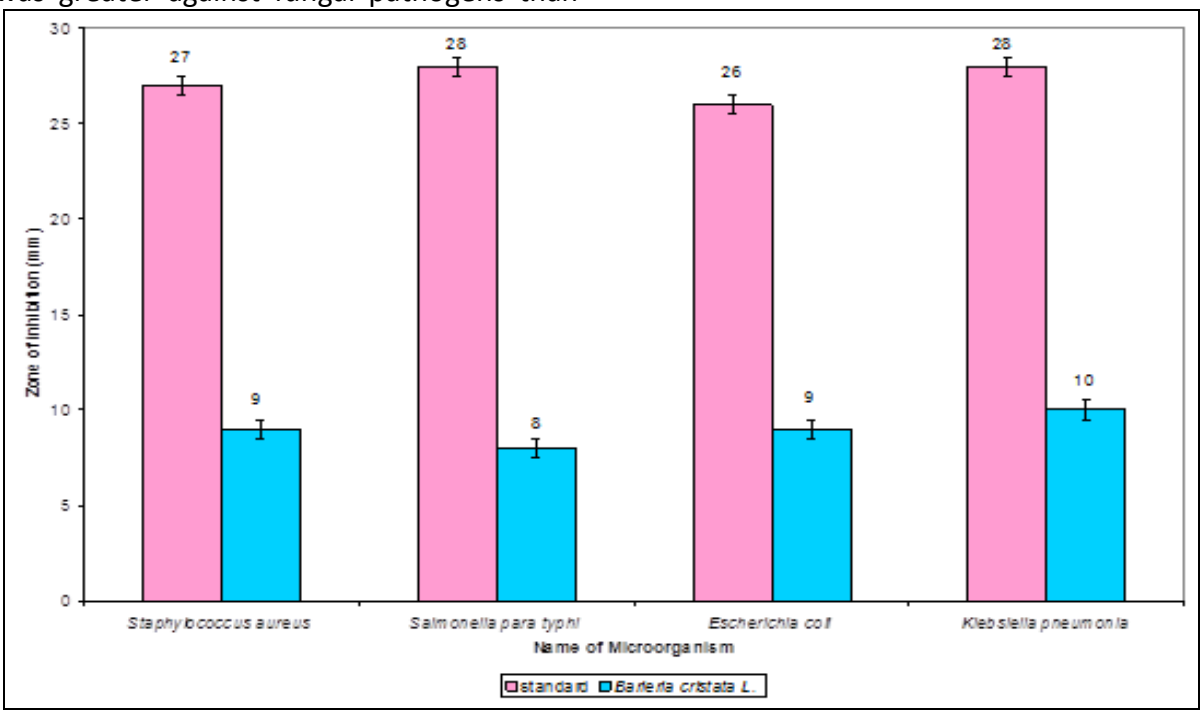

FIGURE 2: ANTIBACTERIAL ACTIVITY OF PURE SAPONIN FRACTION FROM THE LEAVES OF *ARLERIA CRISTATA* L. Standard - Ciprofloxacin (5 µg/disc).

TABLE 2: ANTIFUNGAL ACTIVITY OF PURE SAPONIN FRACTION FROM LEAVES OF *BARLERIA CRISTATA* L.

S. No.	Name of Microorganism	Standard [A] (10µg/disc)	*Barleria cristata L.*
		Zone of inhibition (mm)	
1	*Aspergillus niger*	10	9
2	*Aspergillus fumigates*	10	9
3	*Aspergillus parasites*	11	12
4	*Candida albicans*	11	9

A – Clotrimazole (10 µg/disc).

DISCUSSION: The results obtained from this study revealed that the pure saponin fraction of the plant contained saponins connected with antimicrobial properties. Many scientists [21, 22, 23] have been screening the antifungal activity of medicinal plants against dermatophytes but in this study first attempt was made to invegtigate the antimicrobial activity of pure saponin fraction of medicinal plant *Barleria cristata* L. against dermatophytic fungi like *Aspergillus niger*.

CONCLUSION: The results of this study suggest that the plant saponins can be used as therapeutic agents in the development of new drugs for the treatment of infectious diseases.

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