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Sudhir Singh Chandel
Research Scholar, Department of Botany, S.G.S. Govt. P.G. College, Sidhi, Madhya Pradesh, India

M Salim
Retired Professor and Head, Department of Botany, S.G.S. Govt. P.G. College, Sidhi, Madhya Pradesh, India

IP Kumhar
Professor and Head, Department of Botany, S.G.S. Govt. P.G. College, Sidhi, (Madhya Pradesh, India

Corresponding Author:
Sudhir Singh Chandel
Research Scholar, Department of Botany, S.G.S. Govt. P.G. College, Sidhi, Madhya Pradesh, India

Investigation of phytochemical and antimicrobial activity of different extracts of leaves of *Barleria cristata* L. family acanthaceae

Sudhir Singh Chandel, M Salim and IP Kumhar

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Abstract
The antibacterial activity of the methanol and aqueous extracts of the leaves of *Barleria cristata* was investigated against Gram positive organism *Streptococcus pyogenes* and Gram negative organism *Escherichia coli* NCTC 10418 using well diffusion technique. Results showed that the methanolic extracts of *Barleria cristata* were effective against the test microorganisms. The percentage of zone of inhibition on *E. coli* by methanolic extract and aqueous extract is 78.13 and 66.45 respectively and the percentage of zone of inhibition on Streptococcus by methanolic extract and aqueous extract is 78.84 and 69.11 respectively. The results of the study provide scientific basis for the use of the plant extract in the treatment of wounds and skin diseases.

Keywords: *Barleria cristata*, *Streptococcus pyogenes*, *Escherichia coli*, well diffusion technique and antibacterial activity

Introductions
Ayurveda is traditional Hindu system of medicine which is incorporated in Atharva veda, the last of four vedas. It is based on the idea of balance in body systems through use of proper diet, yogic breathing and herbal treatment. Ayurveda considers three elemental substances as doshas which are called as vata, pita and kapha. According to ayurveda balance of these doshas results in health while imbalance causes disease. In India, use of medicinal plants has been in practice from ancient times. The system of medicine is prevalent in many other countries like Korea, China, Singapore and west Asia. The use of plants in treatment is not only confined to doctors but is also known to households. Now at present the tendency to shift from synthetic to natural based products and medicinal plants is growing all over the world but only one third of the infectious diseases known have been treated from these synthetic products (Sharma, 2001) [1]. Herbal medicines are defined as branch of science in which plant based formulations are used to alleviate the diseases (Joy *et al.* 2008) [2]. The other names for herbal medicines are botanical medicines or phyto medicines. Herbal medicines are plant derivatives which are given in various types of formulations some of which may have antibacterial activity (Ahmad and Beg, 2001) [3].
The antibacterial activity of plant extracts and phytochemicals can be evaluated by using antibiotic susceptible and microorganisms which are resistant. A microorganism is a microscopic organism which may be single celled or multicellular (Enne *et al.* 2001) [4]. These are diverse and they include bacteria and most protozoa. This group may also contain algae, fungi and some micro animals such as Rotifers. Microorganisms belong to prokaryotic members with no nucleus and organelles. Bacteria has two classes namely, Gram positive bacteria e.g. Streptococcus, staphylococcus and Gram negative bacteria Eg. *E. coli* and Pseudomonas. Antibiotics are also called as antibacterials (Madigan and Martinko, 2006) [5]. These are one type of antimicrobials used in treatment and prevention of bacterial infections. These antibiotics may be bactericidal or bacteriostatic in nature. The term antibiotic can also be referred as substance used against microbes.
Barleria cristata L. grows as a shrub. It belongs to family Acanthaceae which is a dicotyledonous flowering plant. It contains about 250 genera and 2500 species. In India 508 species are present. Southern China, India and Myanmar are the native places where wide range of these species is found.

These are cultivated as ornamental plants and also grown as ruderal species along road sides and area from sea level to about 100 meters (Factsheet for experts, 2014) [6]. Leaves are elliptical to ovate in shapes which are dark green on upper side and pale green on lower side. Funnel shaped flowers in violet, pink and white colour. Fruits are ellipsoid capsules which become glossy and smooth at the stage of maturity. Seeds are attached to hooked stalk that ejects them from the capsule (Calderon & Sabundayo, 2007) [7]. The calyx has four lobes and corolla with five lobes. Stamens are arranged in pairs on corolla. Ovary is superior which bicarpellate with axile placentation is. This contains many phytochemical constituents. The present study emphasized phytochemical constituents and antibacterial activity of methanolic and aqueous extracts against two species from the aerial parts of *Barleria cristata*.

Materials and Methods

Collection of leaves and authentication

The fresh leaves of *Barleria cristata* leaves were collected from Majhauli Distt. Sidhi (M.P.), India, in the month of January 2021. The plant was authenticated by Dr. A.A. Khan, Retd. Prof. of Botany, Govt. Girls P.G., Rewa, Madhya Pradesh, India. The leaves were shade dried at room temperature and the dried leaves of *Barleria cristata* were powdered to 40 mesh size.

Extraction of leaves of *Barleria cristata*

The powdered material of plant was passed through 40 mesh size. The dried powder (50 g) was extracted with methanol and distilled water using soxhlet apparatus for about 72 hrs. After extraction with solvent, the marc was dried in hot air oven below 40 °c and was concentrated by distilling off the solvent and evaporating to dryness. The dried extract was subjected to preliminary phytochemical screening for detection of various phytoconstituents.

Microorganisms used

Gram positive organism *Streptococcus pyogenes* and Gram negative organism *Escherichia coli* NCTC 10418 were used as test organisms to determine antibacterial activity of plant extracts.

Media

Nutrient agar media and blood agar media were prepared. Nutrient agar media consists of composition of beef extract, peptone, sodium chloride, agar and distilled water, which is used for cultivation of *Escherichia coli* and blood agar media consists composition of peptone, yeast extract, agar, sodium chloride, sheep blood and distilled water, which is used for cultivation of *Streptococcus pyogenes*.

Preparation of Nutrient agar media

Dissolve the dehydrated medium in the appropriate volume of distilled water i.e., 23 gm dehydrated nutrient agar in 1000 ml distilled water (Nateshan, 2012) [8]. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Sterilize the medium by autoclaving (121 °C for 15 min). Dispense the medium into plates. The agar medium is left to solidify and store. Determine the pH of the medium (pH 6.8 +/- 0.2) with a pH meter and adjust if necessary.

Preparation of Blood agar media

Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Heat this mixture while stirring to fully dissolve all components. The dissolved mixture was subjected for sterilization at temperature of 1210 C for 15 minutes by using autoclave. Once the nutrient agar has been autoclaved, allow it to cool but not solidify (Purushot, 1971) [9]. When the agar has cooled to 45-50 °C, Add 5% (v/v) sterile defibrinated blood that has been warmed to room temperature and mix gently. Avoid Air bubbles. Dispense into sterile plates while liquid.

Sterilization of media

Sterilization is a process of removal of microorganisms or pathogens by treating with chemicals or subjecting to high heat or radiation. The conical flasks containing nutrient agar medium and blood agar medium were plugged with non-absorbent cotton and covered with aluminium foil. Then the mediums were sterilised by autoclaving at 15lbs pressure for 15 minutes.

Well diffusion method Assay

The antimicrobial activity was evaluated by agar well diffusion method (Murray, 1995) [10] and it is modified by Olurinola (1996) [11] and Akinyemi *et al.* (2005) [12]. The inoculum suspension was swabbed uniformly to solidified 20 mL Nutrient Agar for bacteria, and then the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the agar using Glass Pasteur pipettes (P.K.S. and Das, 1991) [13]. Aliquot of 10µl from each plant crude extract, stock solution (1mg/ml) at different concentrations 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml were added into each seeded medium and allowed to stand for 1hr for proper diffusion and thereafter incubated at 37 °C for 24 hrs. The resulting inhibition zones were measured in millimeters (mm). While doing the assay Prepare 5 dilutions (S1-S5) representing 5 test levels of sample and increasing the concentrations in the following manner such as 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml. % of zone of inhibition was calculated by using the following equation 1.

Equation 1

$$\% \text{ of zone of inhibition} = \frac{\text{Zone of inhibition by test (plant extract)}}{\text{Zone of inhibition of standard drug}} \text{ x } 100$$

Results

Zone of inhibition by different concentrations of plant extracts on both *E.coli* and Streptococcus is as follows.

a) Zone of inhibition by the concentration of 100 µg
   E.coli: Methanol - 12.7 mm
   Aqueous- 10.8 mm
   Streptococcus: Methanol - 16.6 mm
   Aqueous - 14.7 mm
b) Zone of inhibition by the concentration of 200 µg
   E.coli: Methanol - 14.8 mm
   Aqueous - 12.9 mm
   Streptococcus: Methanol - 18.8 mm
   Aqueous - 16.4 mm
c) Zone of inhibition by the concentration of 300 µg
   E.coli: Methanol - 20.2mm
   Aqueous - 17.9 mm

Streptococcus: Methanol - 24.5 mm
Aqueous - 22.5 mm

d) Zone of inhibition by the concentration of 400 µg
E.coli: Methanol - 17.5 mm
Aqueous - 15.8mm
Streptococcus: Methanol - 20.4 mm
Aqueous - 18.7 mm

e) Zone of inhibition by the concentration of 500 µg
E.coli: Methanol -18.4 mm
Aqueous - 13.7 mm
Streptococcus: Methanol - 21.8 mm
Aqueous - 17.2 mm

The diameter of zone of inhibition of the extracts was tabulated in Table 1 and it is graphically shown in Figure 1. The percentage of zone of inhibition on *E.coli* by methanolic extract and aqueous extract is 78.13 and 66.45 respectively and the percentage of zone of inhibition on Streptococcus by methanolic extract and aqueous extract is 78.84 and 69.11 respectively and it is tabulated in table 2 and it is graphically shown in Figure 2. So when compared to the aqueous extract, methanolic extract has well antibacterial activity. Effect of standard drug streptomycin on *E.coli* and Streptococcus microorganism was tabulated in table 2 and it is graphically shown in Figure 1.

Table 1: Zone of inhibition of methanolic and Aqueous extract of *Barleria cristata* on *E.coli* and Streptococus.

Tested Microorganisms	Diameter of zone of inhibition												
	Methanolic extract (µg/ml)						Aqueous extract µg/ml						streptomycin
	100	200	300	400	500	Avg.	100	200	300	400	500	Avg.	30 µg/ml
E.coli	12.7	14.8	20.2	17.5	18.4	16.7	10.8	12.9	17.9	15.8	13.7	14.2	21.4
Streptococcus	16.6	18.8	24.5	20.4	21.8	20.4	14.7	16.4	22.5	18.7	17.2	17.9	25.9

Table 2: Percentage Zone of Inhibition by of methanolic and Aqueous extract of *Barleria cristata*

Test Drug	Test Organism	Average Inhibition Zone	Zone of Inhibition by Standard Drug	Calculation	% of Zone of Inhibition
Methanolic Extract	*E.coli*	16.7	21.4	16.7/21.4×100	78.13
	Streptococcus	20.4	25.9	20.4/25.9×100	78.84
Aqueous Extract	*E.coli*	14.2	21.4	14.2/21.4×100	66.45
	Streptococcus	17.9	25.9	17.9/25.4×100	69.11

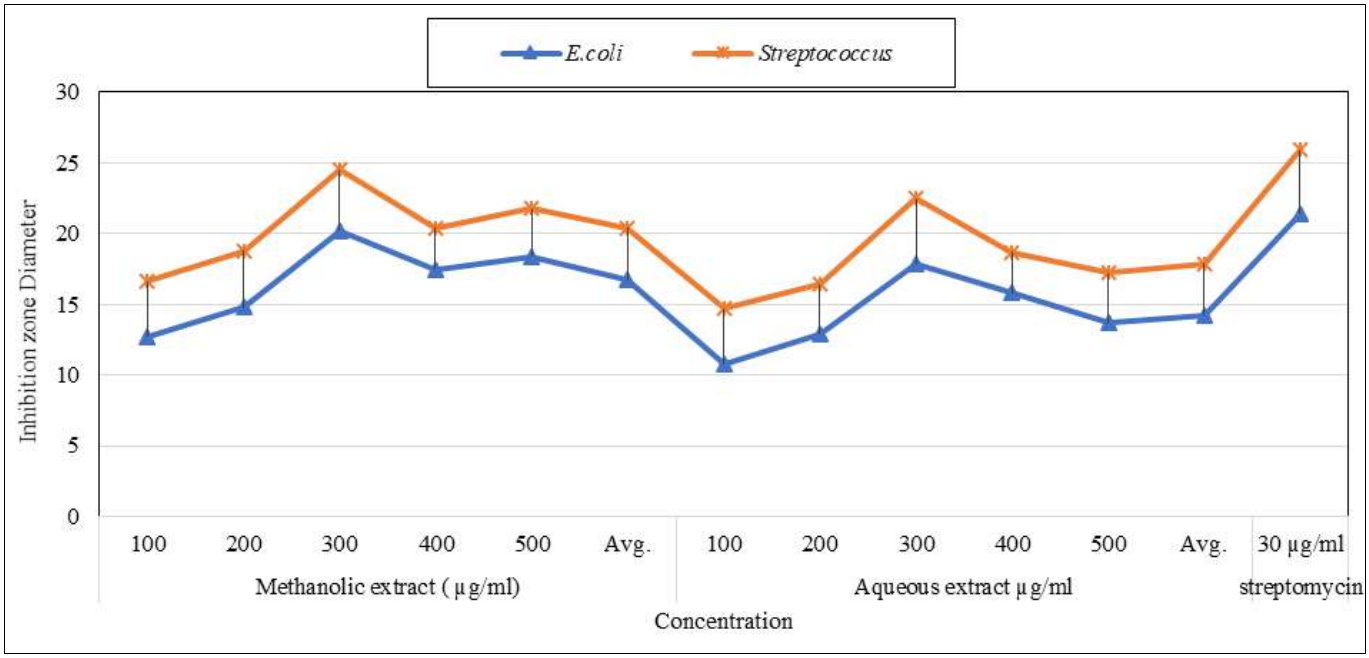

Fig 1: Graph analysis of methanolic and Aqueous extract of *Barleria cristata* on *E.coli* and Streptococus.

Discussion

Many compounds which are naturally occurring in plants were shown to have antibacterial functions and act as source of antibacterial agents against pathogens (Kelmanson *et al.* 2000) [14]. In worldwide the main cause of mortality and morbidity are bacterial infectious diseases. Therefore, the interest of developing new antibacterial agents is increasing. In present study main objective was to evaluate the ability of plant extract in inhibiting pathogenic bacterial growth. When the zone of inhibition is greater than 5 mm antibacterial activity was recorded. In our present experiment as many researchers, well diffusion method was used to assess the activity of plant extracts as it shows highest activity against *E.coli* and Streptococcus (Arora and Kaur, 2007) [15-16].

Streptomycin is used as standard drug which shows bactericidal action. It irreversibly combines with the 30S subunit of the70S ribosome's which are found typically in prokaryotes and inhibits protein synthesis. As normal sequence of translation is disrupted, the bacteria are unable to synthesize proteins that are vital for its cell growth.

It was also noted that alcoholic extract has greater effect in

the inhibition from aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts. The difference in antibacterial activity of a plant extract might be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process.

Agar well diffusion method was used to determine the lowest plant extracts concentration that inhibiting the growth of the bacteria and found effective in the evaluation of MIC. The MIC value of *Barleria cristata* was found as the 20.5 mm at lowest 300 µg/ml against *S.pyogenes* and the MIC value of *Barleria cristata* against *E.coli* was 24.6mm at lowest 300 µg/ml. The MIC for *Barleria cristata* extracts against *S.pyogenes* particularly was found to be significantly active exhibiting the potency with solvent methanol used (50 mg/ml).

Conclusion

This study investigated the antibacterial activity of extract of leaves of *Barleria cristata*. This study demonstrated that the aqueous and methanolic extract of *Barleria cristata* were active against the test organisms. They exhibited antibacterial activity against gram +ve Streptococcus and gram -ve *E.coli*. The values of MIC indicating that very small amount of the extracts are required to inhibit the growth of the bacteria thus the leaf extract of *Barleria cristata* (methanol and aquatic extract) had very potent activity against *Streptococcus pyogenes* when compared to *E.coli*.

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