

Research Article

Evaluation of the Leaves of the *Gynocardia odorata* Plant for Antibacterial Activity

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Abstract: The present investigation was undertaken to appraise the antibacterial activity of the leaves of the *Gynocardia odorata* plant. The study covered the various macroscopic and microscopic behaviors of the proposed leaves. The phytochemical analysis gave valuable information about the different valuable phytoconstituent present in the various extract. The results of the pharmacological activity show that the methanolic extract of the leaves having good antibacterial activity.

Keywords: *Gynocardia odorata*, antibacterial activity, *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholerae*, *Escherichia coli*, methanolic extract, standard antibiotic.

INTRODUCTION

“A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs” [1]. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. Medicinal and aromatic plants of high altitude region are an invaluable resource not only to local communities and the nation, but also to the global community at large. They have high ecological values as well as poor rural communities are highly dependent on them for their health and economic benefit derived from harvesting for trade. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. The resistance of antibacterial is a worldwide public health problem. This is responsible for number of infections that are becoming untreatable in both hospital and community settings. Use of herbal basis antibacterial may contribute the solutions of the worldwide public health problem. So, the exploration of medical properties of crude medicinal plants is needed.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [2]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used

include root, stem, flower, fruit, twigs exudates and modified plant organs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [3].

There are several well-known antibacterial plants exists in the nature which are used traditionally to serve the mankind in various ways in their day-to-day life. Among these all the *Gynocardia odorata* is also an antibacterial plant which is not much familiar between the human beings. Therefore, we have chosen the leaves of the *Gynocardia odorata* plant for our research work and try to evaluate its antibacterial activities.

Gynocardia odorata is a plant under the family Flacourtiaceae, the kingdom is plantae, class is angiospermae, order is Malpighiales and genus is *Gynocardia* [4].

In Sanskrit it is known as *Alasakapaha*, in Nepali it is known as *Gantey*, in Hindi it is known as *Chhalmugra* and in English it is known as *Gynocardia Odorata*. *Gynocardia odorata* is a native of India, flourishing wild in sub-Himalayan region along with North East India. It occurs in dense tropical and temperate forest, also found in secondary forest margin. And the climatic zone is tropical and subtropical.

Gynocardia odorata is a tree with thickly-spreading branches that grows from 10 to 20 meters

high [5]. Its leaves are dark green, coriaceous, and oblong - poisonous to cattle. Gante's flowers are yellow or yellowish green, appearing in April until late May. The tree can be easily recognized by its hard, round, dark-gray, rough-textured fruit, growing on its stems and main branches [5].

The fruit juice of *Gynocardia odorata* can be taken one time daily for 2 week as antipyretic agent [6], the fruits of *Gynocardia odorata* are crushed & the seeds are extracted & used as lotion in leprosy & other skin diseases [7], the isolated oil from the seeds is employed internally and externally in the treatment of skin diseases, scrofula, rheumatism, eczema, also in leprosy, as a counterirritant for bruises, sprains, etc., and sometimes applied to open wounds and sores [8]. The leaves extract is used in the treatment of tooth decay [9]. The Nyishi (Daffla) tribes of Arunachal Pradesh use the fruit of *Gynocardia odorata* and mixed it with water after pounding, they applied the mixture in the extraction of teeth, Chaulmugra oil is used both internally and externally in *leprosy*, *secondary syphilis*, *rheumatism*, *scrofula*, and in *phthisis* [10]. By expression, or by boiling the crushed seeds in water, fixed oil is isolated which is of a pale or golden amber color, and a faint and somewhat unpleasant smell, the oil has long been applied for stiff joints and sprains, rheumatism and neuralgia. It has come into professional use in eczema, psoriasis and other inflammatory skin diseases. The young shoots were grounded and the juice was drunk to combat jaundice [11].

Method and Methodology

Materials and methods

Leaves of *Gynocardia odorata* were obtained from Natural Remedies, Rangpo and Singtham area of Sikkim and authenticated by the pharmacognosy department of Himalayan Pharmacy Institute, Majhitar and also from the BSI (Botanical survey of India, Gangtok) and were used for the present study.

Extraction of *Gynocardia odorata*

The leaves of the plant were collected and washed thoroughly with water to remove any unwanted material and dried in shade. After drying it was powdered and passed through sieve no. 60 and stored in an air tight container. Accurately weighed 500 gm of powdered leaves were taken and was extracted in a round bottom flask (cold maceration) with the solvent of increasing polarity as follows, Chloroform, Methanol, Ethanol, Water. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven, percentage yield of each extract was calculated and the dried extract was stored in air tight containers for further studies [12].

Determination of zone of inhibition

Preparation of extract: 200 mg of chloroform, methanol, ethanol and water extract of *Gynocardia odorata* leaves were screened for their antibacterial

activity. All the extracts were dissolved in 10 ml of DMSO to get the concentration of 20mg/ml. Evaluation of activity were carried out by using disc diffusion method by using nutrient agar medium and the antibacterial activity was measured in term of zone of inhibition.

Micro-organisms used

Gram- Positive: *Staphylococcus aureus* (SA28530), *Bacillus subtilis* (B5 8241)

Gram-Negative: *Vibrio cholerae* (64) and *Escherichia coli* (E78241)

Preparation of Inoculums

Suspension of organism was prepared as per McFarland nephelometer standard (Ellen & Sydney 1990). A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted.

Procedure: Nutrient ager media (500 ml)

The standard agar dilution method

Peptone	– 5g
Sodium chloride	– 2.5g
Beef extract	– 5g
Agar	– 10g
Distilled water	– q.s
Adjust PH	– 7.2 - 7.4

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121°C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160°C for 1 ½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40°C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. The test solution may be placed in sterile paper discs (6mm diameter) were prepared from standard Whatman No.1 filter paper. And these discs impregnated with different concentrations of test compounds (0.01ml/disc). Test compound impregnated discs were gently placed on pre inoculated agar plates. The agar plates were then kept in refrigerator for about 30 minutes to arrest the growth of an organism during the time required for the test compounds to diffuse into the agar medium. 0.1ml of the standard Streptomycin at a concentration of 100 µg / ml was taken as standard. The Petri plates seeded with organisms, containing extracts and the standard were kept in refrigerator at 40°C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion the Petri plates were incubated at 37 ± 10°C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale. The results of the antibacterial activity of *Gynocardia odorata* leaves are tabulated in Table 1.

RESULTS AND DISCUSSIONS

The powdered leaves of *Gynocardia odorata* is green in colour, mild bitter in taste and has characteristic odour. The powdered leaves of the plant was mounted with chloralhydrate, phloroglucinol, weak Iodine and HCl and stained with saffranin; it showed Flatted Starch grains, Fragment of vessels, Fibres, Calcium oxalate crystal, Trichome, Xylem and Phloem.

The physicochemical constants like ash value such as total ash, acid insoluble ash, water soluble ash, moisture content; extractive values such as water soluble extractive value and alcohol soluble extractive value were determined. These helped in formulating Pharmacopoeial standards for the drug.

Fluorescence analysis of the powder material was also carried out. Powdered drug showed the brown colour in visible rays with different solvents / reagents whereas it was found to be, light green, dark green colour in short UV rays (254 nm) and blue and dark green under long UV rays (365 nm). Behaviors of

powder of *Gynocardia odorata* with different reagents / solvents were also observed. This study helped in distinguishing the drug in powder form.

The extracts of the plant material were obtained by successive solvent extraction which was done by using Chloroform, Methanol, Ethanol and Water. Methanol and Ethanol gave reddish brown extract; Chloroform and Water extract gave brown colour extract. Natures of all the extracts were sticky in nature. The quantity of extract was found to be maximum in case of Water extract and minimum in case of Ethanol extract. The extracts obtained by successive solvent extraction were subjected to preliminary phytochemical analysis, which revealed the presence of Flavonoid, Glycosides, Tannins, Fixed oils and fats, Saponins, Steroids and Triterpinoids.

The TLC studies showed in naked eyes and under different wavelength. The presence of some compound like, Flavonoid, Triterpinoids and their significant Rf values were found.

Table 1: Antibacterial activity of different extracts of *Gynocardia odorata*

Bacterial strain	Chloroform	Ethanol	Methanol	Water	Standard
<i>S. aureus</i>	7± 0.29**	8± 0.25**	10.85 ± 0.27*	8.47±0.17**	8 ± 0.25
<i>B. substilis</i>	6.77 ± 0.11**	8.07 ± 0.39**	12.25 ± 0.21*	7.12 ± 0.42**	9.65 ± 0.32
<i>E. coli</i>	6.5 ± 0.26**	7.12 ± 0.14**	10.65 ± 0.48***	6.45 ± 0.27**	12 ± 0.49
<i>V. cholerae</i>	6.77 ± 0.16**	6.45 ± 0.21**	9 ± 0.33**	8.22 ± 0.22**	10 ± 0.25

Each value represents Mean ± SEM; * Activity significantly higher than standard antibiotic. (P<0.01).** Activity significantly lower than standard antibiotic (P<0.01).*** Activity significantly lower than standard antibiotic (P<0.05)

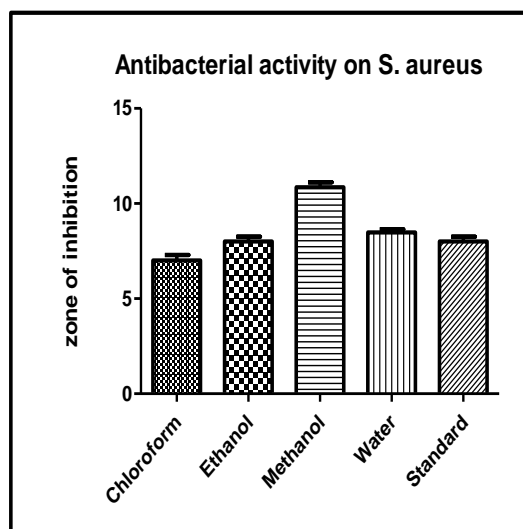


Fig-1: Antibacterial activity on *S. aureus*

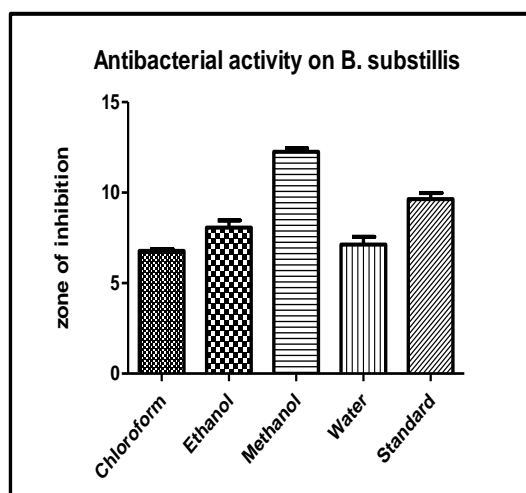


Fig-2: Antibacterial activity on *B. subtilis*

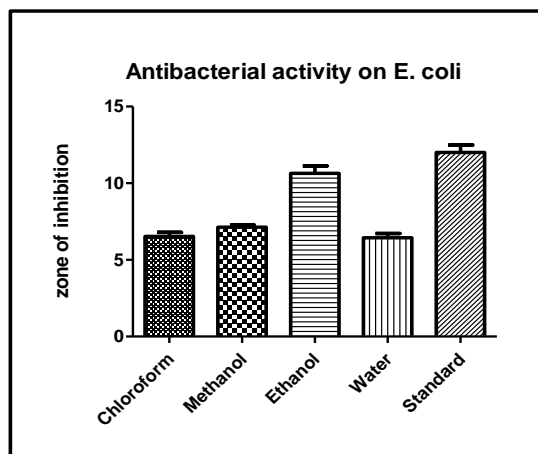


Fig-3: Antibacterial activity on *E. coli*

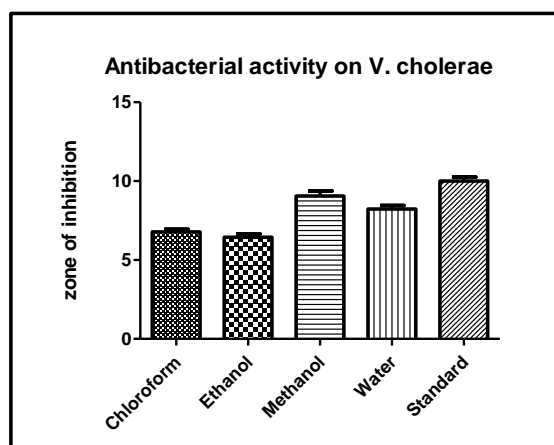


Fig-4: Antibacterial activity on *V. cholera*

The antibacterial activity of all extract of *Gynocardia odorata* against four bacterial strains was evaluated by disk diffusion method. It has been observed that methanolic extract at dose level of 6 μ g/ml showed significant antibacterial activity against most of the tested bacteria (*Staphylococcus aureus*, *Vibrio*

cholerae, *Bacillus subtilis*, and *Escherichia coli*). Among the various extracts assayed the methanolic extract exhibit good activity against *Staphylococcus aureus* (10.88 \pm 0.26), *Bacillus subtilis* (12.22 \pm 0.4) and *Vibrio cholerae* (9 \pm 0.33). Inhibition against *Escherichia coli* (10.66 \pm 0.33) was moderate.

The methanolic extract had MICs 4µg/ml against *S. aureus*, 5µg/ml against *B. subtilis*, 6µg/ml against *E. coli* and 5µg/ml against *V. cholerae*. Out of them MIC against *S. aureus* is lowest.

CONCLUSION

The antibacterial activity of methanolic extract of *Gynocardia odorata* has shown better results in comparison with the other extracts of the same plant.

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