Antibacterial Activity of 50% Ethanolic Leaf Extract of *Gymnema hirsutum* Wight. & Arn.

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ABSTRACT

India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. Antibacterial activity of the 50% ethanolic leaf extract of *Gymnema hirsutum* Wight. & Arn. was screened against four human pathogenic bacteria, *Eschericha coli, Pseudomonas aeruginosa, Bacillus subitlis* and *Klebsiella pneumonia.* The maximum zone of inhibition at 3mg/ml concentration was found to be for *B subtilis, K. Pneumonia, P. aurginosa* and *E.coli.* Gentamycin was used as standard antibiotic in this study. The phytochemical analysis of the 50% ethanolic leaf extract of *Gymnema hirsutum* Wight. & Arn. showed the presence of alkaloids, flavonoids, saponins, phenolics, terpenoids, and tannins. Flavonoids, Tannins, Alkaloids, Saponins, Triterpenoids and Phenolic compounds of the plant extract may be the response for the antibacterial properties. The findings provide support for the use of this plant in traditional medicine

***Key words*:** Antimicrobial and antibacterial activity, *G. hirsutum*, 50% ethanolic extract.

INTRODUCTION

The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while [1]. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of

human ailments. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future [2]. Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current widespread belief that "Green Medicine" is safe and more dependable than the costly synthetic drugs [3].

Salmonella is responsible for 3 billion human infections each year worldwide. The WHO has estimated that typhoid fever accounts for 21.7 million illnesses and paratyphoid fever accounts for 5.4 million of these cases annually [4].

Since the last decade, the rise in the failure of the chemotherapeutics and antibiotics resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Many plants contain substances that are useful to control the growth of microorganisms and plants are the possible source of antimicrobial agents [5].

The antimicrobial abilities of essential oils among which citrus oils are also shown to be a particularly interesting field for applications within the food and cosmetic industries [6] with some modifications [7]. It has also been used as an anti-diabetic, carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, anti-yeast and antimutagenic agent[8].

Acetone, ethanol and petroleum ether extracts of the leaves, stem and root of *Aristolochia bracteolata* Lam. exhibit potential antibacterial effect against four human bacterial species, *E.coli, P. aerugnosa, K. pneumonae* and *B subtilis* [9].

Gymnema hirsutum Wight. & Arn., a member of *Asclepiadaceae* family is distributed in Southern part of Uttar Pradesh adjoining Madhya Pradesh, Western Ghats of Nilgris in Tamil Nadu and Bihar. Folklore based medicobotanical investigations in Nilgiris and village herbal healers were interviewed to know plant or plant product that are used for curing various diseases. Leaves of *G. hirsutum* Wight. & Arn. (local name is kadu gidu) which is one among the plants is said to be used to treat diabetes, infertility, inflammation and jaundice by both external and internal administrations [10].

MATERIALS AND METHODS

Collection and identification of plant materials

Gymnema hirsutum Wight. & Arn. leaves were collected from Western Ghats of The Nilgiris, Tamil Nadu with the help of

Badaga community people. It was identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant Identification No. - BSI/SC/5/23/09-10/Tech – 369).

Preparation of plant extracts

The leaves of *Gymnema hirsutum* Wight. & Arn. were shade dried and ground to coarse powder. The coarse powder was kept in air tight container and their extracts were prepared using different solvents viz., benzene, acetone, ethanol, 50% ethanol and water. The selected extracts were condensed to dryness using rotary evaporator and then used for the qualitative analysis of phytochemicals. The 50% ethanolic extract of *Gymnema hirsutum* Wight. & Arn. was tested for antibacterial activity by agar well diffusion method [11]

Test microorganisms

Human pathogenic bacteria such as *Eschericha coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Klebsiella pneumonia* were collected from Microbiology Lab, Department of Microbiology, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India. All the test bacterial species were maintained on nutrient agar media.

Antibacterial Activity

Peptone Agar medium (38 g) was mixed with 1000 ml of distilled water and sterilized by autoclaving at 120°C for 20 minutes. The test stain has an inoculums size (108 cells/ ml) when the temperature reached 37^0C. Under aseptic conditions, in the laminar flow hood 15 ml of agar medium was dispensed into pre-sterilized petridishes to yield a uniform depth of 4 mm. After solidification of the medium, the bacterial cultures were inoculated by spread plating technique. In this study, standard strains of *E. coli, P. aeruginosa, K. pneumoniae* and *B subtilis* were used as the test strains.

Agar well diffusion method

Wells of 3 mm in diameter were prepared by well puncture method. 20 mg/ml, 40 mg/ml and 60 mg/ml concentration of *Gymnema hirsutum* Wight. & Arn. leaf extracts were prepared using sterile water. 50 µL from each were poured into all the wells. The wells were spaced far enough to avoid being closer to the edges of the petridishes and to prevent overlapping rings of inhibition as well. Finally, the petridishes were incubated for 24 hrs at 37°C. After 24 hours, the diameter (mm) of the inhibition zone around each well was

measured. Antibacterial activities were indicated by clear zone of growth inhibition.

RESULTS AND DISCUSSION

Contents of plant leaf extract

Phytochemicals present in the various solvent extracts are given in the Table 1.

Antibacterial Effect

The present study describes the antibacterial activity of the 50% ethanol extract of the leaves of *G. hirsutum* Wight. & Arn. using agar diffusion method against human pathogenic bacteria such as *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and a standard *Escherichia coli*.

The antibacterial activity of extract of *G. hirsutum* leaves was expressed as zone of inhibition (Table - 2 and Plates 1- A, B, C & D). Numerous plants have been used as medicine because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be examined to better understand their activities and efficiency [12]. Antimicrobial compounds of plant origin may be found in plant roots, stem leaves, flowers or fruits and bark [13]. The investigation was carried out to check antibacterial activity of 50% ethanolic extract of *G. hirsutum* leaves at the concentrations of 1mg/ml, 2mg/ml and 3mg/ml. The maximum zone of inhibition (mm in diameter) at 3mg/ml concentration was found to be 16 ± 2mm, 19 ± 1mm, 11 ± 2mm and 19 ± 1mm for *B subtilis, K. Pneumonia, P. aurginosa* and *E.coli* respectively. Negative control DMSO (5%) did not show any activity against the selected bacteria. This result showed potential antibacterial activity against the bacterial strains tested. The zone of inhibition by Gentamycin was larger than those produced by the plant extract.

The antimicrobial compounds found in the plant extracts may be Flavonoids, Tannins, Alkaloids, Saponins, Triterpenoids and Phenolic compounds and they may be the response for the antibacterial properties of plants [14]. Benefits derived from plants with antimicrobial activity were preferred as consequences of problem associated with use of synthetic antibiotics [15].

Table 1: Phytochemical constituents of *G. hirsutum* leaf extract.

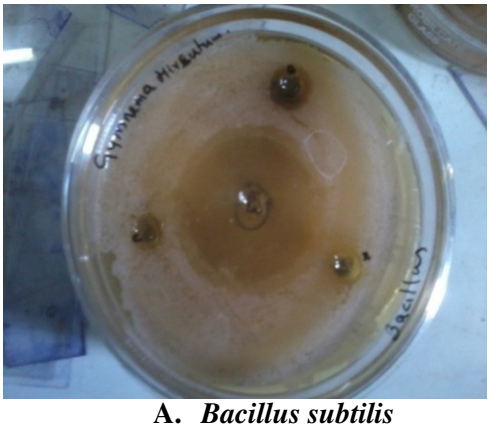
S. No.	Plant Constituents	Solvent Extracts			
		Water	50% ethanol	Benzene	Chloroform
1	Alkaloid	+	++	-	+
2	Flavanoid	-	+	+	-
3	Saponin	+	+	-	-
4	Phenols	+	+	-	-
5	Tannins	++	++	-	-
6	Carbohydrates	+	+	-	-
7	Glycosides	++	++	-	-
8	Proteins	+	+	-	-
9	Steroids	-	-	+	+

+ = Presence - = Absence

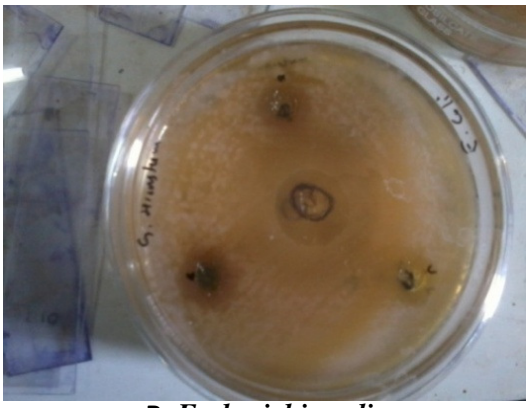
Table 2: Antibacterial activity of 50% ethanolic extract of *G.hirsutum* Wight. & Arn. leaves

S. No.	Bacterial species	Zone of inhibition in mm diameter		
		1 mg/ml	2 mg/ml	3 mg/ml
1.	*B subtilis*	8 ± 1 mm	11 ± 1 mm	16 ± 2 mm
2.	*K. pneumoniae*	12 ± 2 mm	16 ± 1 mm	19 ± 1 mm
3.	*P. aurginosa*	7 ± 1 mm	9 ± 2 mm	11 ± 2 mm
4.	*E. coli*	11 ± 1 mm	15 ± 1 mm	19 ± 1 mm

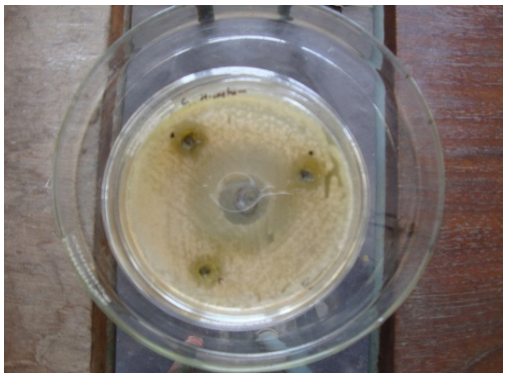
Antibacterial activity of *G.hirsutum* Wight. & Arn. leaf extract, zone of inhibition in mm diameter



A. *Bacillus subtilis*



B. *Escherichia coli*



C. *Pseudomonas aeruginosa*

D. *Klebsiella pneumoniae*

Plate 1:

Antibacterial activity of *G.hirsutum* Wight. & Arn. leaf extract, zone of inhibition in mm diameter

C- *Control – Gentamycin* **1 - *1 mg/ml*** **2 – *2 mg/ml*** **3 - *3 mg/ml***

CONCLUSION

In conclusion, 50% ethanolic extract of the leaves of *G. hirsutum* Wight. & Arn. possess critical inhibitory result against the tested microorganisms. The results of the study support the traditional declare of this plant.

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