



Original Research Article

Antimicrobial activity of Gymnemic acid on pathogens- *Gymnema sylvestre*

R.Br.Pratibha Gupta^{1*} and Pratibha Singh²

¹Department of Botany, Govt. Motilal Vigyan Mahavidyalaya, Nr
Old Vidhansabha, Bhopal, India

²Department of Botany, Sarojini Naidu Govt. Girls P.G. (Autonomous) college Bhopal,
Barkatullah University Bhopal (M.P.), India

*Corresponding author

ABSTRACT

The petroleum benzene, ethanol, and aqueous leaf extract and isolated gymnemic acid from aqueous extract of *Gymnema sylvestre* was assayed *in vitro* searching for antimicrobial activity against human pathogenic microorganism (*Escherichia coli*, *Vibrio cholera*, *Streptococcus mutans*, *Staphylococcus aureus* *Candida albicans* and *Aspergillus niger*). The *in vivo* leaf extract were extracted from Soxhlet extractor and Gymnemic acid was isolated from TLC method. Different solvents Extract of *Gymnema* leaf and isolated Gymnemic acid was assayed by *in vitro* screening for antimicrobial activity on human pathogen by using the disk diffusion method. Standardized micro-organisms obtained from Microbiology Department Barkatullah University Bhopal. Organisms were growing individually and homogeneously grow in NAM and PDA plate and extract dipped disk were parsed. Inhibition was evaluated and controlled by the use of the fluoroquinolone ciprofloxacin. The aqueous extract and isolated gymnemic acid of such medicinal plant showed the best zone of inhibition against the organisms. A maximum zone of inhibition was obtained *Staphylococcus aureus* (9.25mm) on gymnemic acid and aqueous extract showed (8.50mm) in comparison to others but aqueous extract present large inhibition of *Escherichia coli* (9.00mm) and *Candida albicans* (8.76mm). The petroleum benzene extracts that showed minimum zone of inhibition or negative result against microorganisms. On the other hand ethanol extract had performed comparatively the best inhibition on organisms then petroleum extract The botanical extracts showed activity against microorganisms had at least 70% of antimicrobial activity when compared to disk diffusion by the commercial antibiotic fluoroquinolone ciprofloxacin utilized as a control. Of plants extracts in all solvent and gymnemic acid assayed, the *Staphylococcus aureus* had the best performance, sometimes exhibiting higher activity than ciprofloxacin. A tiny amount of data is presented, as the preliminary antimicrobial properties of the medicinal plant, under the urgent necessity of new antibiotics in the market and in the face of the increased resistance of infectious microorganisms to antimicrobials.

Keywords

Antimicrobials;
Candida albicans;
Aspergillus niger;
Escherichia coli;
Vibrio cholera;
Streptococcus mutans;
Staphylococcus aureus; petroleum benzene; ethanol; *Gymnema sylvestre*;
Gymnemic acid and aqueous.

Introduction

Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds (Hancock et al. 2012). There

was a necessity to developed new drug require varied strategies, among them, the secondary metabolites produced by medicinal plants. According to World

Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. The use of photochemical for pharmaceutical purpose has gradually increased in many countries; these plants are still widely used in ethno-medicine around the world. (Ellof. J.N., 1998) Medicinal plants represent a rich source of antimicrobial agents and powerful drugs. (Srivastava, J., 1996) The crude extracts of plant leaf and isolated active phytochemical gymnemic acid, of known antimicrobial properties, can be of great significance in the therapeutic treatments, which are due to the secondary metabolites synthesized by the plant. These products are known by their active substances like, alkaloids (saponins), phenolic compounds, as well as in tanning, which are used either directly as precursors in the pharmaceutical industry. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. (Anjana. S., 2009) In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects, need to search new infection-fighting strategies to control microbial infections (Sieradzki et al, 1999). *Gymnema sylvestre* R. Br. (Asclepiadaceae), use of plants as medicine is as old as human civilization. Its use in the treatment of asthma, eye complaints, inflammations, family planning and snake bite (Anonymous, 1956; Uniyal, 1993; Selvanayagam et al., 1995). It is a potent antidiabetic plant and used in folk, Ayurvedic and Homeopathic system of medicine, studied by Kapoor, (1977), Ravi and Wahi, (1995), Mitra et al, (1995). The leaves of this plant, to

appreciate the taste of sugar has been tested by Mr. D. Hooper in (1887). The present study was to evaluate the antibacterial and antifungal activity of *Gymnema sylvestre* R.Br. used in Ayurveda and traditional medicinal system for treatment of diabetes and manifestations caused by microorganisms. The leaf extracts and gymnemic acid of the following solvents were tested for their potential activity against fungal pathogens like, *Candida albicans* and *Aspergillus niger* and bacterial stain like, *Escherichia coli*, *Vibrio cholera*, *Streptococcus mutans*, *Staphylococcus aureus*. We have to investigate the antimicrobial activity of medicinal plant- *Gymnema sylvestre* R.Br. currently used by native plant to treat their ailments. The very short work has been studied on the antimicrobial activity of this endangered medicinal plant; it needs further study to verify the activity against pathogenic microorganisms.

Materials and Methods

Plant Materials

The leaves of *Gymnema sylvestre* used in Ayurveda and traditional systems of medicine were collected from its natural habitat from Kasturi Herbal farm Misrod, Bhopal in the month of July. The plant was authenticated from Laghu Vanupaj Prasannskarn & Anusandhan Kendra Barkheda Pathani, Bhopal (MP). The collected plant materials were washed thrice in tap water and twice with distilled water to remove the adhesive contaminants and dust particles, then plant material were dried in shade.

Preparation of Plant Extracts

The powdered plant materials were extracted successively with different solvent non-polar to polar (petroleum

benzene, ethanol and distilled water to afford Soxhlet extractor (Hoopers, s method). Solvents were evaporated under reduced pressure and stored at °C for use.

Extraction with petroleum ether

200gm of dry leaf powder was packed into a clean Soxhlet extraction unit and 1 liters of petroleum ether (30-40°C) was added and extracted for 24-36 hours till all the components are soluble in petroleum. Petroleum is extracted is collected and distilled in a distillation unit. Then a net weight of 15 gm of petroleum ether extracts was obtained. Petroleum ether extraction was used for defatting dried leaf powder.

Extraction with ethanol-

The plant material is then extracted with ethanol (50-60°C). Ethanol was added and the extraction was carried out in 24-36 hours till the total ethanol soluble extract was obtained. The ethanol soluble extract was distilled and finally 30gm of the thick paste were obtained.

Extraction with distilled water –

The dry leaves part is also extracted with distilled water (80-90°C). Water was poured and the extraction was carried out approx 24-36 hours till the total water soluble extract was obtained, leaves become colorless. The aqueous extract was distilled and finally 65gm of the dry extract were obtained.

Isolation of Gymnemic acid from aqueous extract of *Gymnema sylvestre*

TLC technique is reported to be effective in generating metabolite profiles of various plant compounds and their purification. In the present studies thin

layer chromatographic screening of the metabolites, indicated the separation at R_f values compared with standard gymnemic acid R_f values.

Micro-organisms culture and maintenance

Clinical isolates of the microorganisms were used along with the standard strains. Quality control strains of *Escherichia coli*, *Vibrio cholera*, *Streptococcus mutans*, *Staphylococcus aureus* *Aspergillus niger* and *Candida albicans* obtained from, Department of microbiology Barkatullah University Bhopal M.P. Nutrient agar medium and Potato Dextrose Agar was used as the media for the culturing of strains by using pour plate method of all the microbial cultures were inoculated in the nutrient broth (NA) at 37°C for 72 h and fungus were maintained on Potato dextrose broth at 28°C.

Antimicrobial Screening

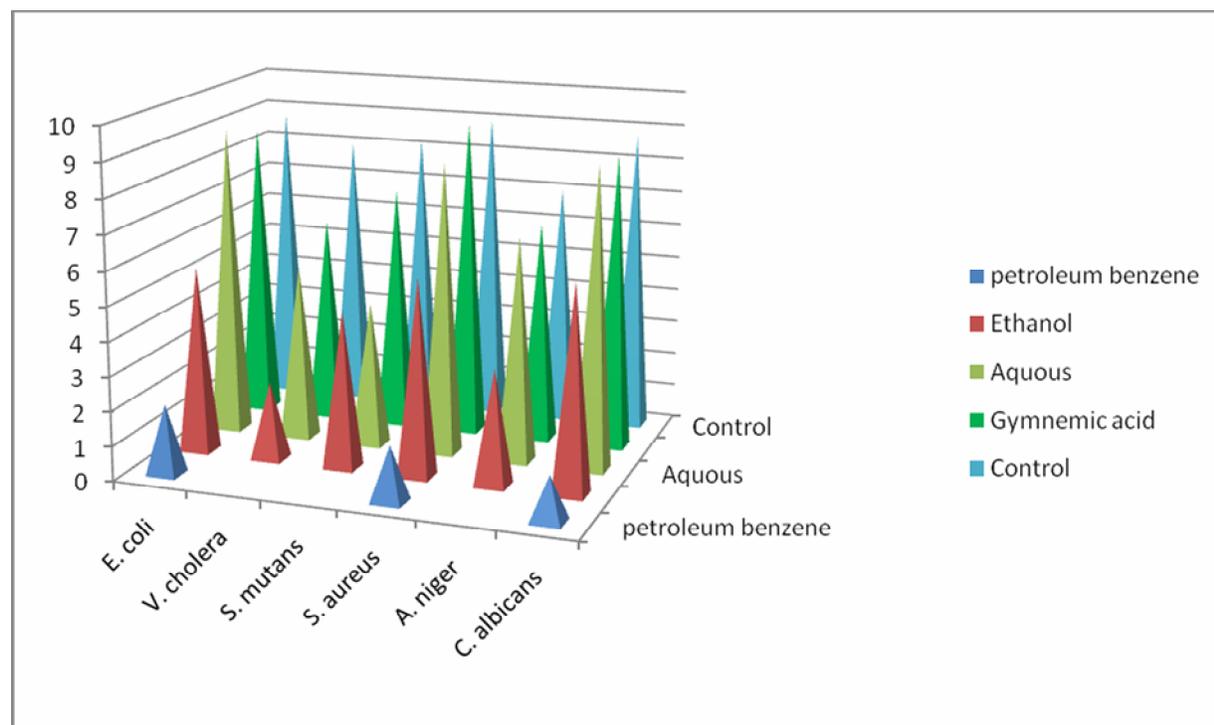
Antimicrobial activities of the extracts were determined by the disk diffusion method as described by Taylor, R.S.L., 1995. Each purified extract and gymnemic acid were dissolved in DMSO with (100µg/ml), sterilized by filtration using a sintered glass filter, and stored at 4°C. PDA and NAM plates were inoculated with each fungal and bacterial culture by pouring plate method.

The filter paper discs (5mm in diameter) impregnated with concentrations of the extracts was placed on test organism-seeded plates. DMSO was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. For the determination of zone of inhibition microbial strains were taken as a standard

Table.1 Antifungal activity of *Gymnema sylvestre* leaf extract in various solvents, Gymnemic acid and standard control against microorganism species tested by disc diffusion method after 24hours

Zone of inhibition (mm) on various solvent leaf extract, gymnemic acid and control in 100µg/ml					
Microbial culture	Petroleum benzene	Ethanol	Aquous	Gymnemic acid	Control
<i>Escherichia coli</i>	2.00	5.43	9.00	8.65	8.75
<i>Vibrio cholera</i>	—	2.24	5.13	6.00	8.00
<i>Streptococcus mutans</i>	—	4.56	4.21	7.12	8.25
<i>Staphylococcus aureus</i>	1.60	5.72	8.50	9.25	9.00
<i>Aspergillus niger</i>	—	3.35	6.54	6.43	7.00
<i>Candida albicans</i>	1.31	6.00	8.76	8.60	8.75

Chart.1 Antifungal activity of *Gymnema sylvestre* leaf extract in various solvents, Gymnemic acid and standard control against microorganism species tested by disc diffusion method after 24hours – zone of inhibition in mm



antibiotic for comparison of the results. The proper control experiment was carried out under similar condition by using fluoroquinolone ciprofloxacin for antimicrobial activity as standard drugs. The activity was determined after 24h of incubation at 27-31⁰C. The diameters of the inhibition zones were measured in mm.

Results and Discussion

The results obtained in the present study relieved that the tested *Gymnema Sylvestre* leaf extract from different solvent and gymnemic acid posses' potent antimicrobial activity against *Escherichia coli*, *Vibrio cholera*, *Streptococcus mutans*, *Staphylococcus aureus*, *Aspergillus negier* and *Candida albicans* on different solvent (non polar – polar) extract dissolve in DMSO. (Table. 1, Fig.1 and Plate A-L) Antimicrobial activity was observed by filter Paper Disc Diffusion method and compare with fluoroquinolone ciprofloxacin as a Standard Drug as a control sample. Remarkable antimicrobial activities were recorded with *S. aureus* (9.25mm) (fig.1, plate. A) on gymnemic acid and on aqueous extract(8.50mm) (Plate. G), its show good result compares to standard drug. On the other side aqueous extract present maximum zone of inhibition on *E. coli* (9.00mm) (Plate. H) and *C. albicans* (8.76mm) (Plate. I) showed comparatively best inhibition then standard drug and gymnemic acid (8.65mm and 8.60mm))(Plate B and C). According to that work petroleum benzene extract was shown significantly negative result on all microorganisms. (table. 1) (Fig.1) (Minal Wani, et. al., 2012) On the other hand ethanol leaf extract shows positive result in comparing to petroleum benzene extract against organisms. (Table and Fig. 1) Comparatively the aqueous leaf extract was shown significantly

maximum zone of inhibition result against both types of pathogen fungus species then gymnemic acid and some time control drug but gymnemic acid present large zone of inhibition on bacterial strains. (Plate. A-L, Table- 1, Fig.1) (Beverly C David, et al., 2013) Same as hydro alcohol extracts effective at low concentration against *S. aureus*, *S. mitis*, *S. Mutansi* and *C. albicans*. (Ramasubramania Raja R., 2010) Large scale isolation of secondary metabolites from *G. sylvestre* can be predicted to remain an essential component in the search for new secondary metabolites and its pharmacological activities. Its leaf extracts exhibit broad spectra of antimicrobial activity. However, further studies needed to isolation and identification of compounds responsible for antimicrobial activity.

The result of present investigation clearly indicates the antifungal activity of leaves and ascertains the value of this plant used in Ayurveda, which could be of considerable interest to the development of new drugs. The result of this study supports the use of plants as therapeutic agents for the treatment of several diseases caused by the pathogenic bacterial and fungal populations.

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