Available Online at http://www.resealert.com

International Journal of Current Advanced Research
Vol. 1, Issue, 1, pp.1 - 4, September, 2012

International Journal of Current Advanced Research

RESEARCH ARTICLE

Studies on Antibacterial Activity Of *Gymnema Sylvestre* Against Respiratory Infection Causing Bacteria

Jayamangala.swami[1] and Prabakaran[2]

[1]Research Development Centre, Bharathiyar University, Coimbatore, 641046, Tamilnadu
[2]PG& Research Department of Botany, Government Arts College, Dharmapuri-636705.Tamilnadu

*Corresponding author- G.Prabakaran, Email- gpbiotek@gmail.com

ARTICLE INFO

Article History:

Received 10th August, 2012
Received in revised form 25th, August, 2012
Accepted 15th September, 2012
Published online 27th September, 2012

Key words:

Gymnema sylvestre,Leaf extract,Antibacterial activity,Agar well diffusion method.

ABSTRACT

Increasing emergence of resistance to the currently available antibiotics has necessitated continued search for new antimicrobial compounds. The prime aim of the study was focused on the antibacterial potential of Gymnema *sylvestre* leaves against one Gram positive *(Staphylococcus aureus*)and three Gram negative, *(Escherichia Coli, Klebsiellapneumoniae and Pseudomonas aeroginosa*)bacteria by using different solvents namely Petroleum ether, chloroform, ethanol, methanol and acetone by Agar well diffusion method. The result showed that all the solvent extracts exhibited considerable activity against the tested bacteria. The antibacterial activity increased with the increasing concentration of the extract.

INTRODUCTION

Medicinal plants are the Touching Stone of Modern Science and India is a store house of it. During last few decades there has been an increase in the study of medicinal plants and their traditional use in different parts of the world (Lev, 2006). Prior to the development of modern medicine, the traditional systems of medicine that have evolved over the centuries within various communities, are still maintained as a great traditional knowledge base in herbal medicines (Mukherjee and Wahil, 2006).

Even today plants are almost exclusive source of drugs for the majority of world population. People in developing countries utilize traditional medicine for their primary health care needs (Palombo,E.A. and S.J.Semple,2001,Cowan, M.M., 1999). This is also true in Indiathat onlya small percentage of plants have been evaluated for antibacterial activity against human pathogen (Patwardhan, B., A.D.B. Vaidya and M.Chorghade, 2004).Thus considering the vast potentiality of plant as a source of new therapeutic agents hence detail investigations were conducted to test the efficacy of some plant extracts against human pathogenic bacteria.

According to World Health Organization (Santos *et al.*,1995)Medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties , safety and efficiency(Ellof*et al.*,1998) .Gudmaror*Gymnema sylvestre* (Asclepiadaceae)is a large , stout , much branched woody climber which is native to the tropical forests of southern and central Indiaand some parts of Africa(Anonymous 1997). It is used in the treatment of several diseases such as diabetes, cornealopacity, heart diseases, leucorrhoea, urinaryinfections, liver diseases, snakebite, stomach complaints and dental caries (Hiji Yasutake, U.S 1990). Its roots are used as astringent, emetic, expectorant, refrigerant, stomachic and tonic**(**Uniyal, M.R. (1993),Selvanayagam*et al.*,1998**)**In the present study, the selection of this plant for evaluation was based on its traditional usages. Although very few works have been done on Antimicrobial activity of this endangered medicinal plant(Satdive*et al.*,2003 andDevi, B. P*et al.*,2010) it needs further study for verification of its activityagainst*Respiratory infection* causing micro organisms. This paper describes the evaluation of the antibacterial potencyof *Gymnema sylvestre* against Respiratory infection causing bacteria.

MATERIALS AND METHODS

Collection of samples

The fresh plants of *Gymnema sylvestre* were collected and taxonomical identification of the plant was confirmed by Dept of Botany, University of Agriculture Science,G.K.V.K, Bangalore.

Preparation of plant extract

The leaves of *Gymnema sylvestre* were shade dried at room temperature, powdered by mixer and passed through sieves. Powdered leaves were extracted with various solvents such as Petroleum ether, chloroform, ethanol, methanol and acetone using soxhlet apparatus. All the extracts were concentrated

under reduced pressure by rotary vacuum evaporator. To evaluate the antibacterial properties through Agar well diffusion method, the different dried extracts were reconstituted with DMSO (Di Methyl Sulphoxide) to obtain stock solution of different concentrations (25mg/ml, 50 mg/ml, 100,mg/ml, 200 mg/ml).

Bacteria tested

The bacterial strains used to evaluate the antibacterial properties of different extracts of *Gymnema sylvestre*included *Staphylococcus aureus, Escherichia Coli, Klebsiella pneumoniae and Pseudomonas aeroginosa.*The bacterial samples were collected from patients referred to one hospital (Tamil nadu) with symptoms of respiratory tract infections. The primary identification for the Bacterial isolates was made based on the colony appearance and pigmentation. Gram's staining was carried out as Hockers modification (Rangasamy, 1975), Motility and Biochemical test were performed to identify the isolates.The cultures were maintained on Nutrient Agar (HiMedia) slant at 4 ºCand subcultured before use.
Some of the virulence factors in the isolates were examined by different types of tests such as: Cell Surface Hydrophobicity, Protease Enzyme Production Test, β Lactamase Test, and Slime (biofilm) activity test.

Antibacterial Activity

The antibacterial test was performed using the Agar well diffusion method (Nair *et al.*,, 2005) Overnight broth cultures of the respective bacterial strains were adjusted to turbidity equivalent to 0.5 Mc Farland standards. Muller Hinton Agar (Hi Media Lab pvt ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. Five wells (6mm in diameter) were made equidistance in each of the plates using a sterile cork borer. The cut agar was carefully removed by the use of sterile forceps. Up to 50µlof each concentration of the extract were respectively introduced into the wells using pipettes with the stock in one well. It was allowed to diffuse at room temperature for two hours and the plates were incubated at 37 ºCfor 24 hrs. The standard antibiotic discs were placed as control. The microbial growth was determined by measuring the diameter of the zone of inhibition in mm. The antibacterial activity was expressed as the mean zone of Inhibition (mm) produced by the plant extract. The experiment was repeated and the average values were recorded for antibacterial activity.

Antibacterial Stability Test

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial profiles of the isolates against the antibiotics like Tetracycline, Ciprofloxacin, Co-trimaxozole, Ampicillin Nitrofurantoin, Nalidixic acid, Norfloxacin , Penicillin, Tobramycin and Kanamycin. The nutrient broth was prepared and sterilized at 121°C at 15 min and inoculated with the isolates and incubated at 37°C for 24 hrs followed by swabbing on Mueller-Hinton agar plates and antibiotic discs were placed, then Plates were incubated at 37°C for 18 to 20 h. The zone of inhibition and resistance was measured, recorded and interpreted according to the recommendation of the disc manufacture's standard chart.

RESULTS AND DISCUSSION

Bacterial Profile for Respiratory Tract Infections in various samples.

In the samples investigated from 55 patients four bacterial species were isolated by using Selective culture medium and Standard Biochemical tests shown in table 1.

Virulence character of respiratory samples isolates Cell surface hydrophobicity

Allrespiratory Sample isolates were attempted to cell surface hydrophobicity with xylene.

Throat samples

In the Throat swab samples, among the 4 types of bacterial species *Pseudomonas aeruginosa* showed highest activity (93.72 ± 0.02) andlowest activity (68.04± 0.03) was shown by *Staphylococcus aureus.*

Nasal swab samples

In the nasal swab samples,among the 4 types of bacterial species,*Pseudomonas aeruginosa* showed the highest activity (93.72 ± 0.03) and *Staphylococcus aureus* showed the lowest activity (68.04 ± 0.04).

Sputum samples

In the Sputum samples, among the 4 types of bacterial species, *Pseudomonas aeruginosa* showed the highest activity (93.73 ± 0.02) and *E.coli* showed the lowest activity (69.57 ± 0.02).

Protease enzyme production

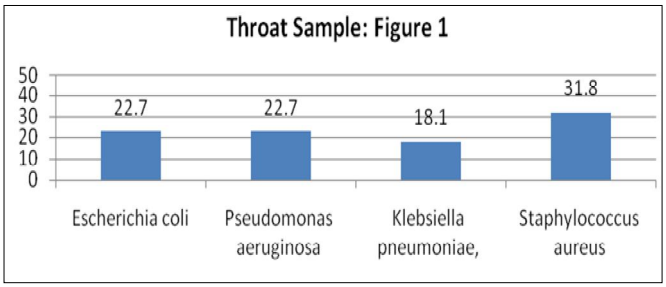
Totally 72.88% of protease producing isolates was observed. Sputum sampleisolates showed highest percentage ofprotease activity (78.94%) where as throat samples isolates showed lowest activity (66.66%).

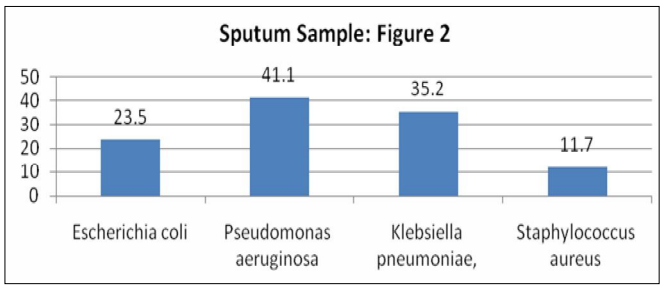
Organisms	Coagulase	Oxidase	Indole	MR	VP	Citrate	Urease	Catalase	TSI Slant/butt	G	S	L	M
Staphyloccus aureus	+ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	A/A	A^{+}	A^{+}	A^{+}	A^{+}
Escherichia coli	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	AG^{+}/AG^{+}	AG^{+}	AG^{+}	AG^{+}	AG^{+}
Klebsiella pneumoniae	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	AG^{++}/AG^{++}	AG^{++}	AG^{++}	AG^{++}	AG^{++}
Pseudomonas aeruginosa	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	AK/AK	-	-	-	-

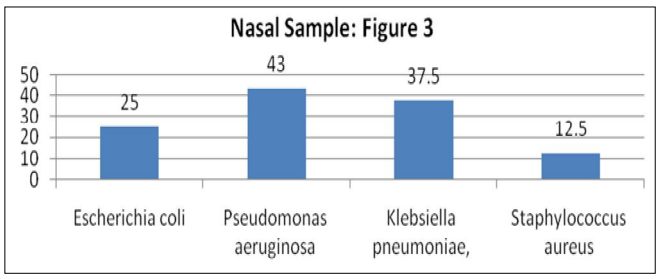
In species level,*Pseudomonas aeruginosa* and klebsiella *pneumoniae*showed highest enzymes activity (100%) and lowest activity was shown by *Staphylococcus aureus*(72.72%).

β lactamase production

Totally 88.13% of isolates showed β lactamase activity. The highestβ lactamaseactivity was observed in Sputum samplesisolates (94.73%) and lowest in Throat samples isolates (80.95%). In species level highest activity was observed in *Pseudomonasaeruginosa*, *E.coli* and *klebsiella pneumoniae* (100%) and lowest in *Staphylococcus aureus*(36.36%).







Prevalence of isolates from different types of Respiratory Tract infection was shown in **Fig 1-3.**

The microbiological analysis revealed that *pseudomonas aeruginosa* was the leading etiologic agent in both Sputum and Nasal Sample and *Staphylococcus aureus species* in Throat Sample**.**

Slime production (Biofilm)

In this study totally 42.37% of isolates produced slime (biofilm). Sputum samples had highest slime activity (47.36%) and lowest activity was observed in Throat samplesisolates (38.09%). In species level, *E.coli*had highest activity (100%) and lowest was observed in *klebsiella pneumoniae*(31.25%).

Antibacterial Activity

Antibacterial activity of Acetone, Chloroform, Ethanol, Methanol and Petroleum Ether extract were evaluated against various pathogens *(Staphylococcus aureus, Escherichia Coli, Klebsiellapneumonia,Pseudomonas aeroginosa*) by disc diffusion method. All the solvent extracts of*Gymnema sylvestre* leaf inhibited the growth of all the four bacterial species tested in a dose dependent manner. The dose dependent antimicrobial activity was also noted by other authors (Prescot *et al.*, 2002 and Vlietinck*et al.*, 1995).Among the five types ofsolvent extract Methanol and Ethanol extract showed the highest (100%) Antibacterial Activity followed by petroleum ether (88.13%), chloroform (76.27%) and acetone (52.54%).

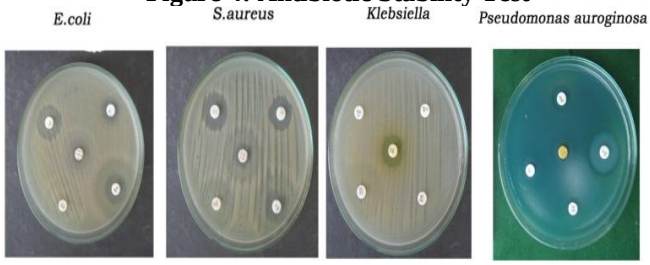
The susceptibility of microorganisms to plant extracts was compared with each other and with standard Antibiotics.
As a result, Methanol and Ethanol extract showed 100% activity against all isolates.Petroleum ether extract showed 100% activity against all isolates in nasal and sputum samples with 66.66% activity in Throat sample isolates.Chloroform extract showed 89.47% activity against Sputum sample isolates,followed by 84.21% activity against Nasal swab sample isolates and 57.14% activity against Throat swab sample isolates. Acetone extract showed 68% activity against Nasal and Sputum swab sample isolates followed by 23.80% activity against Throat swab sample isolates.

Antibiotic stability Test

The respiratory samples isolates were subsequently tested for antibacterial drug resistance based on Kirby-Bauer disk diffusion method. The drug resistance patterns of different types of organism were found to be highly variable. Almost all the isolates were resistant to one or more antibiotics.

In species level, *E.coli* showed the highest resistance against ciprofloxacin, co-trimaxozole and ampicillin (100%) and lowest antibiotic resistance against nitrofurantoin (30.76%), nalidixic acid.*klebsiella pneumoniae* showed the highest resistance against norfloxacin and lowest resistance against ciprofloxacin and tobramycin (31.25%).*Staphylococcus aureus* showed the highest resistance against penicillin (100%) andlowest antibiotic resistance by ampicillin (73.1%) and*Pseudomonasaeruginosa* showed100% resistance against kanamycin and tobramycin.

Figure 4: Antibiotic Stability Test



CONCLUSION

The activity of *Gymnema sylvestre* leaf extract against both Gram positive and Gram negativebacteria might indicate the presence of broad spectrum antimicrobial compounds. The present study supports the traditional use of the plant in the treatment of several diseases. Further studies are required to identify and characterize chemical compounds present in leaf

so that *Gymnema sylvestre*might be used as better alternative for synthetic antimicrobials.

References

1. Anonymous (1997).Medicinal plants categorized W/new IUCN Red list criteria under the Biodiversity Conservation Priorisation Project. In: CBSG, India news (pp.10).
2. Devi, B. P & Ramasubramaniaraja, R. (2010). Pharmacognostical and antimicrobial screening of Gymnema. Sylvestre R. Br and evaluation of Gurmar herbal toothpaste and powder composed of Gymnema. Sylvestre R. Br extracts in dental caries, Int. J.Pharma. Biosciences.
3. Ellof, J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J.Ethinopharmacol. 60,1-6,1998.
4. Hiji Yasutake, U.S (1990). Gymnemic acid for prevention of dental Caries. In: chemical abst.vol.113. No.46364b.
5. Lev, E. 2006. Ethnodiversity within current ethno-pharmacology as part of Israeli
6. Traditional medicine-A review. Journal of Ethnobiology and Ethnomedicine 2: 43.
7. Mukherjee, P.K. and Wahil, A. 2006. Integrated approaches towards drug development
8. from Ayurveda and other systems of medicine. Journal of Ethnopharmacology 103: 25-35.
9. Nair, R., Kalariya, T. and Chanda, S. (2005). Antibacterial activity of selected Indian Medicinal flora. Turk.J.Biol, 29:41-47.
10. Palombo,E.A. and S.J.Semple,2001.Antibacterial Activity of traditional medicinal plants. J.Ethnopharmacol, 77:151-157
11. Patwardhan,B., A.D.B.Vaidya and M.Chorghade, 2004.Ayurveda and natural products drug discovery. Current Sci., 86 (6):789-799.
12. Prescot, L.M., Harley, J. P. and Klein, D.A (2002). Microbiology, fifth edition, Mc Graw Hill Company, Inc. Newyork, pp 811.
13. Rangasamy, G.1975. Disease of crop plants in India. Prentice hall (p). Ltd., New Delhi P.520.
14. Santos. P.R.V; Oliveira, A.C.X; Tomassini, T.C.B. Control microbiogicode produtos. Fitoterapicos. Rev.Farm.Bioquim. 31,35-38,1995.
15. Satdive, R.K, Abhilash, P. & Fulzele, D.P. (2003). Antimicrobial activity of G.Sylvestre leaf extract. Fitoterapia, 74:699-701.
16. Selvanayagam, Z.E, Gnanavendhan, S.G., Chandrasekharan, P., Balakrishna, K. & Rao, R.B. (1995). Plant with antisnake venom activity – a review on pharmacological & clinical studies. Fitoterapia, 65:99-111.
17. Uniyal, M.R. (1993). Some popular on traditional ayurvedic herbs useful in family planning. Sachitra Ayurved, 45:665-668.
18. Vlietinck, A.J., Vanttoof, L, Totte, J., Lasure, A., Vanden Berghe, D., Rwangobo, P.C. and Mvukiyuniwami, J. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties, J. Ethnopharmacol, 46:31-47.

****