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Phytochemical investigation and antibacterial activity of *Gymnema sylvestre* and *Andrographis paniculata* from western ghats

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Abstract

In this study the antibacterial activity of *Gymnema sylvestre* (Retz.) and *Andrographis paniculata* collected from Western Ghats, Kanyakumari district, South India was made against common bacterial pathogens by filter paper disc-agar diffusion procedure. Both the plants showed considerable levels of antibacterial activity. Hexane extract of *Gymnema sylvestre* showed maximum inhibition against Serratia marcescens MTCC 86 and the *Andrographis paniculata* was effective against penicillin resistant Staphylococcus aureus MTCC 87. Phytochemical study revealed the presence of steroids/ terpenoids and coumarins in the extracts of both the plants. The bio-active compounds in the medicinal plants were extracted with Hexane and Chloroform (99 %) and further resolved by thin layer chromatography. Hexane extract of *Gymnema sylvestre* (Retz.) have 15 compounds and in its chloroform extract 25 compounds whereas 19 compounds in Chloroform extract and five compounds with Hexane extract was found in Andrographis paniculata. Further research is needed to characterize the compounds obtained and their mechanism of action on the bacterial pathogens.

Introduction

Historically, the medicinal value of plants was tested by trial and error. Modern approaches to determining the medicinal properties of plants involve collaborative efforts of ethno-botanists, anthropologists, pharmaceutical chemists, and physicians. Many modern medicines had their origin in medicinal plants. Examples include aspirin from willow bark (Salix spp.), digitalis from foxglove (Digitalis purpurea), and vinblastine from Madagascar periwinkle (Vinca rosea). More than 13,000 plants have been studied during the last 5 years period [Dahanukar et al., 2000]. The Indian Systems of medicine can be classified into traditional and classical systems. The traditional system is the local folk stream, which is prevalent in rural and tribal villages in India. The system like Ayurveda, Sidha, Unani, Yoga, and Naturopathy are expressions of classical systems. Thus the term Indian Systems of Medicine covers the system which originated in India or which originated outside but got adapted in the course of time [Sharma, 1995]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [Geissman, 1963]. Most are secondary metabolites, of which only 12,000 have been isolated, which are estimated to be less than 10% of the total [Schultes, 1978]. Of these, only small percentage has been investigated phyto-chemically and the fraction submitted to biological or Pharmacological screening is even lower. Since plants may contain hundreds or even thousands of metabolites, there is currently a great interest in the medicinal plant research as a possible source of new lead compounds for introduction into therapeutical screening

programmes. The new branch of Science, ethno-botany (or ethno-pharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous peoples about the plant and animal products they have used to maintain health [Borris, 1996]. The investigation was to analyze the bioactive potential and phytochemical constituents of two medicinal plants *Gymnema sylvestre* and *Andrographis paniculata* collected from the Western Ghats, part in Kanya kumari District, Tami Nadu, India. The motivation behind the study was detection of a phytoremedy for microbial infections as an alternative to chemotherapeutics and to have constructive exploitation of bioresources of a region thereby developing special protection over rare species.

Materials and Methods

Medicinal Plant Material: Leaves of *Gymnema sylvestre* and *Andrographis paniculata* were collected from the lower region of Western Ghats [Lat. 8^o 19‘N; Long. 77^o 10’ S] and authenticated taxonomy by standard procedures. *Gymnema sylvestre* [Retz.] R.Br. ex Roem. & Schult is medicinally important in antidiabetic activity. It has shown experimental or clinical anti-diabetic activity and it boosts the insulin level. Andrographis paniculata, a native medicinal plant commonly known as ‘Kalmegh’ (Hindi) ‘Kiriath’ (Malayalam); Nilavempui (Tamil).

Preparation of Medicinal Plant Extracts: The hexane and chloroform extracts of *Gymnema sylvestre* and *Andrographis paniculata* were used for the study. 500 gram leaves of both medicinal plant samples were shade dried and pulverized. From this 15 gram was mixed with 50 ml of hexane and was placed in a rotary shaker. The residual powder after hexane extraction was dried and was mixed with 50 ml of chloroform and placed in a rotary shaker followed by evaporation. The percentage yield of *Gymnema sylvestre* and *Andrographis paniculata* extracts were calculated.

Bacterial Pathogens: Eight different pathogenic bacteria viz., Staphylococcus aureus [PRSA] MTCC 87, Salmonella typhi MTCC 531, Pseudomonas aeruginosa MTCC 424, Clostridium perfringens MTCC 450, Serratia marcescens MTCC 86, Bacillus subtilis MTCC 1427, Enterobacter aerogenes MTCC 111 and Shigella flexneri MTCC 1457 obtained from Microbial Type Culture Collection (MTCC, Chandigarh, India] were used in the present study.

Antibacterial Activity Study: Filter paper disc-agar diffusion procedure (Kirby-Bauer method) was used to study the antimicrobial activity [Bauer et al., 1966; Dey and Harborne, 1991]. Filter paper discs (Whatman No.41) of uniform size (5 mm diameter), impregnated with specified concentrations of the different extracts (100µg/disc) and control were placed on the surface of the agar plate, that has been pre-seeded with the bacterial pathogens on Muller Hinton Agar (Himedia, India) plates with a pH of 7.4. The plates were incubated at 37°C for 17 hours. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in millimeter. Chloroform (95% v/v) and Hexane (95% v/v) were used as negative control and the antibiotic tetracycline was used as positive control for Salmonella sp and penicillin for Staphylococcus sp. All the experiments were done in triplicates. The average of triplicate values was calculated.

Phytochemical Investigation: The extracts were subjected to following phytochemical investigation such as Liebermann-Burchard test for steroids/ terpenoids, Shinoda's test for flavanoids, Molisch's test for carbohydrates, Wiefferering field test for iridoids, test for coumarins, Test for saponins, Dragendorff’s test for alkaloids [Sofowora, 1984 and Kepm, 1986].

Thin Layer Chromatography: The crude extracts were subjected to TLC with developing solvent systems using different ratios of Hexane, Chloroform and ethanol [Sadasivam and Manickam, 1996]. The TLC plates were exposed to iodine vapour in a glass chamber for locating

unsaturated compounds and Rf values were calculated.

Results

The extractive percentage was higher with hexane extract of *Gymnema sylvestre* 5 % whereas chloroform extracts of *Andrographis paniculata* showed 2% as its maximum (Fig 1).

The results of antibacterial activity of hexane and chloroform extracts of *Gymnema sylvestre* and *Andrographis paniculata* are shown in Table 1.

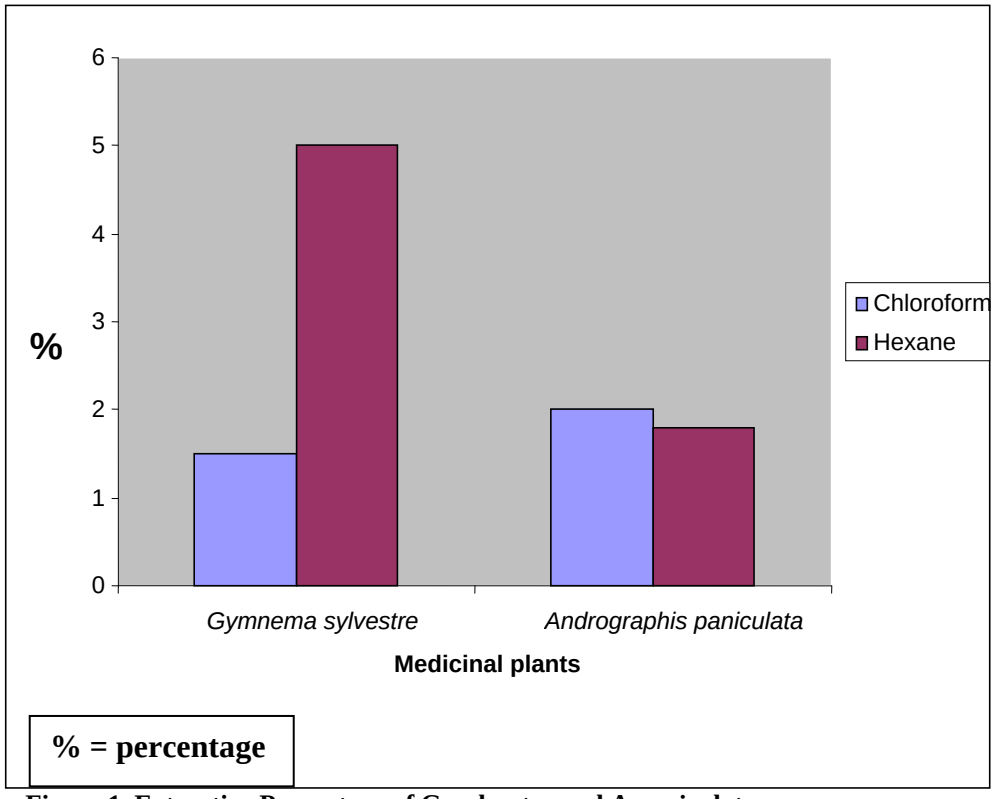

Figure 1. Extractive Percentage of G. sylvestre and A.paniculat.

Table 1. Antibacterial Effect of *Gymnema sylvestre* and Andrographis paniculata

Bacterial Pathogens	Zone of inhibition by *G. sylvestre* (mm)		Zone of inhibition by *A. paniculata* (mm)	
	Hexane extract	Chloroform extract	Hexane extract	Chloroform extract
***Staphylococcus aureus* [PRSA] MTCC 87**	12	7	17	19
***Salmonella typhi* MTCC 531**	8	T	9	11
***Pseudomonas aeruginosa* MTCC 424**	13	15	8	14
***Clostridium perfringens* MTCC 450**	10	10	T	8
***Serratia marcescens* MTCC 86**	18	T	17	14
***Bacillus subtilis* MTCC 1427**	-	T	12	9
***Enterobacter aerogenes* MTCC 111**	12	T	15	17
***Shigella flexneri* MTCC 1457**	12	T	8	11

'-'= No activity; T= Trace <7.0; MTCC= Microbial Type Culture Collection.
(Values are the average of Triplicates)

Zone of inhibition of hexane extract of *Gymnema sylvestre* ranges from 8.0 mm (*Pseudomonas aeruginosa* MTCC 424) to 18.0 mm for *Serratia marcescens* MTCC 86 whereas the activity by its

chloroform extract was maximum for *Pseudomonas aeruginosa* MTCC 424 (15.0 mm) followed by *Clostridium perfringens* MTCC 450 ((10.0 mm) and *Staphylococcus aureus* [PRSA] MTCC 87 (7.0 mm) but other pathogens showed trace response i.e., less than 7.0 mm.

Staphylococcus aureus [PRSA] MTCC 87 (19.0 mm), *Enterobacter aerogenes* MTCC 111 (17.0 mm), *Pseudomonas aeruginosa* MTCC 424 and *Serratia marcescens* MTCC 86 (14.0 mm each), *Salmonella typhi* MTCC 531 and *Shigella flexneri* MTCC 1457 (11.0 mm each) were the diameter of zone of inhibition by the chloroform extracts of *Andrographis paniculata*. Its hexane extract activity was maximum towards *Staphylococcus aureus* [PRSA] MTCC 87 and *Serratia marcescens* MTCC 86 (17.0 mm) followed by *Enterobacter aerogenes* MTCC 111 (15.0 mm), *Bacillus subtilis* MTCC 1427 (12.0 mm), *Salmonella typhi* MTCC 531 (9.0 mm) and *Pseudomonas aeruginosa* MTCC 424 and *Shigella flexneri* MTCC 1457 (8.0 mm each) but *Clostridium perfringens* MTCC 450 showed less sensitivity.

The phytochemical screening of *Gymnema sylvestre* showed the presence of steroids or terpenoids and coumarins in the chloroform and steroids or terpenoids alone was found in its hexane extract. Incase of *Andrographis paniculata* both the extracts showed the presence steroids or terpenoids and coumarins (Table 2).

Table 2. Phytochemical screening of G. sylvestre and A. paniculata.

Experiments	Phytochemical analysis of *G. sylvestre*		Phytochemical analysis of *A. paniculata*	
	Hexane extract	Chloroform extract	Hexane extract	Chloroform extract
Liebermann-Burchard test for steroids or terpenoids	+	+	+	+
Shinoda's test for flavanoids	-	-	_	_
Molisch's test for carbohydrates	-	-	_	_
Wiefferering field test for iridoids	-	-	_	_
Test for coumarins	-	+	+	+
Test for saponins	-	-	_	_

- = Absent; + = Present

Table 3. TLC of *Gymnema sylvestre* (Retz. R.Br.ex Roem. & Schult.

	Solvent system	No of spots obtained	Rf values
Chloroform Extract	Chloroform (100%)	5	0.137, ,0.168, 0.475, 0.893, 0.906
	Chloroform-Ethanol(9.5:0.5)	7	0.438, 0.451, 0.380, 0.651, 0.677, 0.845, 0.974
	Chloroform-Ethanol(9:1)	3	0.024, 0.325, 0.487
	Chloroform-Ethanol (7:3)	5	0.069, 0.333, 0.837, 0.852, 0.883
	Chloroform-Ethanol(8.5:1.5)	5	0.096, 0.103, 0.482, 0.848, 0.896
Hexane extract	Hexane-Chloroform (9.5:0.5)	6	0.192, 0.144,0.192, 0.269, 0.576, 0.932
	Hexane-Chloroform (7:3)	9	0.047, 0.085, 0.114, 0.171, 0.228, 0.571, 0.619, 0.809, 0.980

Table 4. TLC of *Andrographis paniculata* (Burm.f.) Wall. ex Nees.

	Solvent system	No of spots obtained	Rf value
Chloroform Extract	Chloroform (100%)	3	0.264, 0.052, 0.523
	Chloroform-ethanol (9:1)	5	0.160, 0.400, 0.120, 0.560, 0.600
	Chloroform-ethanol (9.5:0.5)	5	0.021, 0.425, 0.702, 0.709, 0.758
	Chloroform-Ethanol (9:1)	3	0.780, 0.950, 0.957
	Chloroform-Ethanol (7:3)	6	0.240, 0.263, 0.829 0.845, 0.914, 0.902
Hexane extract	Hexane-Chloroform (9.5:0.5)	3	0.144. 0.192,0.576
	Hexane-Chloroform (3:7)	2	0.056, 0.099

The result of thin layer chromatography of hexane and chloroform extract of *Gymnema sylvestre* Retz. R.Br.ex Roem. & Schult is shown in Table 3. The chloroform-ethanol (9:1) gave three fractions and the second fraction showed the highest Rf value (0.950). Totally Hexane extract of *Gymnema sylvestre* [Retz.] revealed to have 15 compounds and 25 compounds by its chloroform extract. The Hexane extract of *Andrographis paniculata* showed the presence of five compounds whereas 22 compounds were noticed in its Chloroform extracts (Table 4).

Discussion

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, flavanoids which have been found in vitro to have antimicrobial properties. Among these, only around ten percentages of all the plants had been investigated in detail for bioactive agents [Sanberg and Bruhn, 1979]. So it is imperative to characterize plants with bioactive properties to develop consumer friendly therapeutics with challenging effectiveness. The major advantage of this type studies may form a solution for emergence of multiple drug resistantence or even the complete cure or an alternative to diverse diseases. Various plant extracts are reported to be beneficial in the control of Hepatitis, and have antiviral, antibacterial, antimalarial and immune modulating factors and are with highly potent antibacterial and antimalarial ingredients [Mcrea and Towers, 1984]. The bioactive substances in plants though produced as secondary metabolites not only serves as developmental stage specific, but also organ and tissue specific [Daroker et al., 1988]. Selection of plant samples of this study considered all these remarkable properties of medicinal plants. The plant samples were collected from the Western Ghats is considered as one of the global biodiversity hot-spots. It comprises of over 5000 vascular plants, of which about 30% are endemic to the Ghats and this area has become a hotspot of discovery of new species in the last decade.

All the eight pathogenic bacteria viz., Staphylococcus aureus [PRSA] MTCC 87, Salmonella typhi MTCC 531, Pseudomonas aeruginosa MTCC 424, Clostridium perfringens MTCC 450, Serratia marcescens MTCC 86, Bacillus subtilis MTCC 1427, Enterobacter aerogenes MTCC 111 and Shigella flexneri MTCC 1457 obtained from Microbial Type Culture Collection (MTCC, Chandigarh, India] were sensitive to any of the extracts used (Table 1).

The Hexane extract of *Gymnema sylvestre* inhibited the growth of all the bacterial isolates analysed except Salmonella typhi MTCC 531 and Bacillus subtilis MTCC 1427. Its Chloroform extract was active against Pseudomonas aeruginosa MTCC 424, Clostridium perfringens MTCC 450, and Staphylococcus aureus MTCC 87 whereas Serratia marcescens MTCC 86, Bacillus subtilis MTCC 1427, Enterobacter

aerogenes MTCC 111 and Shigella flexneri MTCC 1457 and Salmonella typhi MTCC 531 showed less sensitivity. The chloroform and hexane extracts of *Andrographis paniculata* was inhibitory to all the bacterial pathogens. [Mishra et al., 2009] reported the antimicrobial activity of Cinnamonum zeylanicum extracts against human and plant pathogenic dermatiaceous moulds. The antibacterial effect of Azadirachta indica extracts against food borne pathogens and spoilage bacteria was reported by [Mahfuzul Hoque et al., 2007].

The highest inhibition towards Methicillin resistant S. aureus by both Chloroform and Hexane extracts of *Andrographis paniculata* is the remarkable finding of the study. The high antimicrobial properties of *Andrographis paniculata* (Burm.f.) are due to high phenolic composition [Tripathi et al., 2000; Patra et al., 2004]. Similar result was reported by [Dharmaratne et al., 1999] in which the sensitivity of MRSA strain with xanthones of Calophyllum sp. has been revealed. Further he suggested that the variations in activity among the organic solvents may be due to the polarity which determines the type of the reaction and solubility of the compounds. Antimicrobial activity of Syzygium aromaticum extracts against multiple drug resistant bacterial strains and fungus of clinical origin was reported by[Khan et al., 2009].

Presence of steroids/ terpenoids and coumarins (Table 2). The detection of 15 compounds in hexane and 25 compounds in chloroform extract of *Gymnema sylvestre* (Table 3) and five compounds of hexane and 22compounds in Chloroform extracts of *Andrographis paniculata* (Table 4) indicates that both plants studied are with higher numbers of compounds with potential antibacterial activity. Plants, rich in the secondary metabolites such as alkaloids, glycosides, steroids and relative active metabolites are used as drugs in pharmaceutical industry [Vlietinck et al., 1995]. Further research is needed to characterize the compounds obtained. More over, the presence of different active fractions in the TLC studies further reiterates the antibacterial properties of the selected plants. The antimicrobial activity of the extracts and their potency can be quantitatively assessed by determining the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The results suggest that the hexane and chloroform extracts of *Gymnema sylvestre* and *Andrographis paniculata* posses potent antibacterial activity. Further works are proceeding in Malankara catholic college laboratory to identify and characterize the compounds of these plants and also to check its antimycotic activity and response towards other than the bacteria studied.

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