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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF VARIOUS EXTRACTS OF *GUETTARDA SPECIOSA L.*

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ABSTRACT

The leaves of *Guettarda speciosa L.* is used to treat coughs, colds and sore throats. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in diarrhoea. The dried plant powder was extracted by Soxhelation and cold maceration process by using chloroform and ethanol. These extracts were subjected to various phytochemical analysis to identify alkaloids, flavonoids, triterpinoids, carbohydrates, tannins, phenols, gums and mucilage. Various crude extracts of *Guettarda speciosa L.* were investigated to screen the potential antibacterial activity against gram positive and gram negative bacteria like *Staphylococcus aureus, Bacillus subtilis, E.Coli,* and *Klebsiella pneumonia and* anti fungal activity was studied against *Aspergillus niger* and *Candida albicans* by using Muller-Hinton agar media by disc diffusion method .The result showed that both chloroform and ethanolic extract exhibit potent antifungal and anti bacterial activity.

Keywords: *Guettarda speciosa L.,* Antibacterial, Antifungal, Traditional Plant

INTRODUCTION:

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). So World Health Organisation is taking an official interest in this to develop the traditional system for health care. WHO especially focus on folk medicine as safety for microbial and non microbial diseases (WHO-tech report, 1978).

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Now a day the research is going on in herbal medicine against micro organisms. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem. The use of plant compounds for pharmaceutical purposes has gradually increased in India. So this present study to investigate the antimicrobial and antifungal activities of Guettarda speciosa.

Most of the herbal medicines are having a good immune response in the treatments. *Guettarda speciosa* Linn. (Family: Rubiaceae) is widely distributed from East Africa to India and throughout to Malaysia into the South Pacific. The decoction of the Guettarda speciosa leaves is used to treat coughs, colds and sore throats. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are

being traditionally used in epilepsy(Weiner, 1971; Weiner, 1984). Upon literature review it was found that the plant contains loganic acid and secologanin (Inouye *et al*., 1988; Cambie and Ash, 1994). Anti epileptic and antidiarrhoeal activity of Guettarda specoisa was reported (Saravanakumar *et al*., 2009; Gandhimathi *et al*., 2009). In the present study the preliminary phytochemical screening and microbiological activity against selected bacteria and fungi have done.

MATERIALS AND METHODS

Plant collection

The Plant material of *Guettarda Speciosa* used for investigation was collected from Tirunelveli District, in the Month of August 2007. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-GS-01) of the plant was deposited at the college for further reference.

Preparation of extracts

Inner bark of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (60gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanol and chloroform extract of *Guettarda speciosa* was found to be 17.5 % and 12%w/w respectively.

Phytochemical screening:

Dried extracts were investigated by various chemical tests (Harbore, 1973) and found alkaloids, flavonoids, triterpinoids, carbohydrates, tannins, phenols, gums and mucilage.

Antimicrobial Activity of *Guettarda speciosa L.*

The antibacterial and anti fungal activites of different extracts were studied by Disc Diffusion method against *Bacillus subtilis* (gram positive) ATCC 6633, *Staphylococcus aureus* (gram positive) ATCC 914, *Klebsiella pneumoniae* (gram negative) ATCC 29665, *E.Coli* (gram negative) ATCC 25922, and *Aspergillus niger* ATCC 9029, *Candida albicans* ATCC 2091.

Inoculum:

All the micro organisms were inoculated in SBCB media and incubated at 370°C for four hours. It produces a turbid solution and then it was diluted with same media and compared with the standard. This level is equivalent to 3.0 X 108CFU/ml. (John J Rojas *et al*., 2006)

Disc Diffusion Method:

Muller-Hinton agar media (Perz *et al*., 1990) was prepared and transferred to sterile Petri dishes and allowed to solidify. A suspension of inoculum was added to media and swabs the entire surface of the agar media. The inoculum was equally distributed in surface of the media by rotating the plate. Sterile discs 5mm in diameter dipped in solutions of the chloroform and ethanolic extracts of different concentration, standard and control were placed on the surface of agar plates. Leave the plates for 1 hour at room temperature as a period of pre incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Then the plates were incubated at 37°C for 18 hours and observed for antibacterial activity. The diameters of the zones of inhibition was observed and measured. The average area of zone of inhibition was calculated and compared with the standards.

RESULT AND DISCUSSION:

The phytochemical analysis of *Guettarda speciosa* extract showed the presence of alkaloids, flavonoids, triterpinoids, carbohydrates, tannins, phenols, gums and mucilage. Chloroform and ethanol extracts were subjected to antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, E.Coli, Klebsiella pneumonia, Aspergillus niger, and Candida albicans.* The results are showed in Table 1. In this study Chloroform extract exhibited significant activity against *E.coli* and moderate activity against *Klebsiella pneumoniae* at a concentration of 50µg/ml, 100µg/ml, and 200µg/ml when compared with the standard drug Ciprofloxin 5µg/ml. Ethanolic extract exhibited moderate activity against *E.coli* and significant activity against *Klebsiella pneumoniae* at a concentration of 50µg/ml, 100µg/ml, and 200µg/ml when compared with the same standard. Chloroform extract exhibited moderate activity against both *Aspergillus niger* and *Candida albicans* at a concentration of 50µg/ml, 100µg/ml and 200µg/ml when compared with the standard drug ketoconazole 5µg/ml. Ethanolic extract exhibited significant activity against both *Aspergillus niger* and *Candida albicans* at a concentration of 50µg/ml, 100µg/ml, 200µg/ml when compared with the same standard drug ketoconazole 5µg/ml. The result showed that both chloroform and ethanolic extract of *Guettarda speciosa* have potent antifungal and anti bacterial activity.

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Table 1: Antibacterial and antifungal activities of various extracts of *Guettarda speciosa L.*

Plant Name	Extract	Concentration	Zone of inhibition (in mm)					
			Staphylococcus aureaus ATCC9144	***Bacillus subtilis*** ATCC 6633	***E.coli*** ATCC 25922	***Aspersillus niger*** ATCC9029	***Candida albicans*** ATCC2091	***Klebsiella pneumonia*** ATCC 2966
Guettarda speciosa	Chloroform Extract	200µg/ml 100 µg/ml 50 µg/ml	22 19 17	21 19 17	22 19 17	23 22 17	29 22 19	23 17 15
	Ethanolic extract	200µg/ml 100 µg/ml 50 µg/ml	29 20 17	24 21 19	21 17 15	31 22 18	32 23 20	25 21 19
Control	Chloroform		0	0	0	0	0	0
	Ethanol		0	0	0	0	0	0
Standard Drug	Ciprofloxacin		34	27	25	_	_	28
	Ketaconazole		_	_	_	28	34	_

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