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Research Article

ANTIBACTERIAL POTENTIAL OF METHANOLIC EXTRACT OF *GYROCARPUS ASIATICUS* WILLD

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ABSTRACT

Objective: The present study was designed to evaluate the antibacterial activity of the methanolic extract of *Gyrocarpus asiaticus* Willd.
Methods: The *Gyrocarpus asiaticus* Willd extract under study has been screened for the antibacterial activity against with ***Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella paratyphi,*** *pseudomonas mirebelis, Pseudomonas auroginosa* and ***Klebsiella pneumoni*** human pathogenic bacteria by disc diffusion method.
Results: Methanolic extract showed satisfactory results against almost all the organisms, among them against *E.coli* it showed highest zone of inhibition 18.5mm (0.4g/ml) with the minimum inhibitatory concentration (MIC) value of 0.039 mg/ml and minimum bactericidal concentration (MBC) of 0.16mg/ml. Other Gram negative pathogens like *P. mirabelis, P. auroginosa* shows good zone of inhibition 13.9 mm and 17 mm (0.4g/ml) with less minimum inhibitatory concentration of 0.039 mg/ml and MBC values of 0.16 mg/ml for the above mention bacteria. But *klebsiella sp* was found to be resistant with no MIC, no MBC value and with a very less zone of inhibition of 7.5mm (0.4g/ml) when compared with the standards.
Conclusion: From the results, it is evident that *Gyrocarpus asiaticus* Willd is recommended as a plant of an antibacterial agent.

Keywords: *Gyrocarpus asiaticus* Willd, methanolic extract, antibacterial activity, MIC, MBC.

INTRODUCTION

Ayurveda and siddha medicines were alternative systems for medicine which were become popular in recent days [1]. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists [2].

Infectious diseases caused by bacteria, fungi, parasites and viruses are still a major threat to public health, despite the huge progress in human medicine. Their impact is particularly large in developing countries due to unavailability of medicines and the emergence of widespread drug resistance [3]. Natural products especially, those used in ethno medicine provide a major source of innovative therapeutic agents for various conditions including infectious diseases [4]. At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines [5-8].

Gyrocarpus asiaticus Willd commonly known as Taniki or Nalla poliki [9] is a tree belonging to the family Hernandiaceae. *Gyrocarpus asiaticus* is one of the species in the genus *Gyrocarpus* with the class Magnoliopside. The aim of this present study is to evaluate the anti-bacterial activity of the methanolic extract of *Gyrocarpus asiaticus* Willd.

MATERIALS AND METHODS

Collection of plant material

The *Gyrocarpus asiaticus* Willd plant (healthy and disease free plant samples) were collected from the nearby area of Tirunelveli District fields (Tamil Nadu) in December 2011, identified and authenticated by Dr. V. Chelladurai (Retired Research Officer-Botany, Central Council for Research in Ayurveda and Sidda, Govt. of India), Tiruneveli, Tamil Nadu, India. Herberium of the plant, *Gyrocarpus asiaticus* Willd, was prepared and preserved in the Department of Pharmacognosy, Koringa College of Pharmacy, Korangi, East Godavari District, Andhra Pradesh, India.

Preparation of extracts

A portion of dried aerial parts (100 g) of *Gyrocarpus asiaticus* Willd was placed in a soxhlet apparatus. Extraction was performed with 800 ml of methanol for 48 h at a temperature not exceeding the boiling point of the solvent. Extract was filtered through a 45 µm filter. The resulting solution was concentrated in vacuum to dryness to give methanolic extract (8.5 g). The extract was stored in a refrigerator for further use.

Anti-bacterial activity

Antibacterial sensitivity testing using disc diffusion method

Circular disc of 6 mm diameter were made from the whatman no 1 filter paper. Discs were impregnated with equal volume (50 µl) of plant extract at four different concentrations (0.05 g/ml, 0.1g/ml, 0.2g/ml & 0.4g/ml). The discs were aseptically placed over plates of Muller Hinton agar (MHA, Difco) seeded with each of test pathogens, and the inoculum was adjusted to 0.5 Mc Farland turbidimetry [10]. The extract under study was screened for the antibacterial activity with several human pathogenic bacteria such as ***Staphylococcus aureus,*** *Escherichia* ***coli, Salmonella typhi, Salmonella paratyphi***, *Pseudomonas mirebelis*, *pseudomonas auroginosa*, ***Klebsiella pneumoni.*** The plates were incubated in an upright position at 37^{0}C for 24 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 10 mm were considered as having low antibacterial activity. Diameters between 11 and 15 mm were considered moderately active, and these with >16mm were considered highly active. The clinical strains were also tested for their sensitivity against the standard antibiotics, ciprofloxacin (5 mcg) by the disk diffusion method.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) was determined by broth dilution method [11]. Twofold serial dilutions of the crude extract as well as the positive antibiotic control (Ciprofloxacin) were prepared in Mueller–Hinton broth [12]. A direct suspension of bacteria was prepared in 5 ml sterile distilled water from a 24-h-old suspension in Mueller–Hinton broth. The turbidity of the suspension

was adjusted to match a 0.5 McFarland standard [13] which corresponds to 1.5×10^8 cfu/ml. For broth dilution tests, 50 µl of standardized suspension of bacteria was added to each tube containing crude extract at a final concentration of 0.005–5.120 mg/ml and incubated at 37 °C. The lowest concentration that did not show any visible growth after macroscopic evaluation was considered as MIC. After the determination of MIC, the tubes which did not show any visible growth were diluted 100-fold with drug-free Mueller–Hinton broth and incubated at 37 °C for 48 h. The lowest concentration of the tube that did not show any visible growth was considered as the minimum bactericidal concentration (MBC). The assays were performed in triplicate.

RESULTS AND DISCUSSION

The methanolic extract of *Gyrocarpus asiaticus* tested for antibacterial activity on seven human pathogenic bacteria was presented on (Table 1 and Figure 1). The result showed that the antibacterial activity of the plant was increased with increasing the concentration of crude extracts. The extract showed prominent activity on almost all the pathogens specially on Gram negative *E.Coli*, *P. auroginosa*, *P. mirebelis, S. paratyphi* but only *klebsiella sp* appears to be resistance with less zone of inhibition. The methanolic extract of the plant showed highest activity against *E.coli* (18.5 mm for 0.4g/ml) and *P. auroginosa* (17 mm for 0.4g/ml) but on the other hand *klebsiella sp* which is considered as resistant showed only 7.5 mm inhibition zone at same concentration as above.

Table1: Antibacterial activity (Zone of inhibition) of methanolic extract of *Gyrocarpus asiaticus* Willd.

Extract	Conc.(mg/ml)	Disc diffusion method (inhibition zone, mm)						
		S.A.	E.C.	S.T.	S.P.	P.M.	P.A.	K.P.
Methanolic extract of *Gyrocarpus asiaticus*	50	8.4	10.1	7.2	9.3	10.3	10.0	--
	100	9.2	11.7	7.6	11.3	11.6	13.0	--
	200	10.2	15.3	9.3	14.8	13.2	15.5	6.2
	400	11.4	18.5	10.2	16.2	13.9	17.0	7.5
Ciprofloxacin	5 µg/ ml	24.0	24.2	22.8	27.5	25.5	28.3	14.6

-- indicates no zone of inhibition. **S.A.*: Staphylococcus aureus*, E.C.*: Escherichia coli*, S.T.*: Salmonella typhi*, S.P.*: Salmonella paratyphi*, P.M.*: pseudomonas mirebelis,* P.A.*: pseudomonas auroginosa,* **K.P.*: Klebsiella pneumoni.***

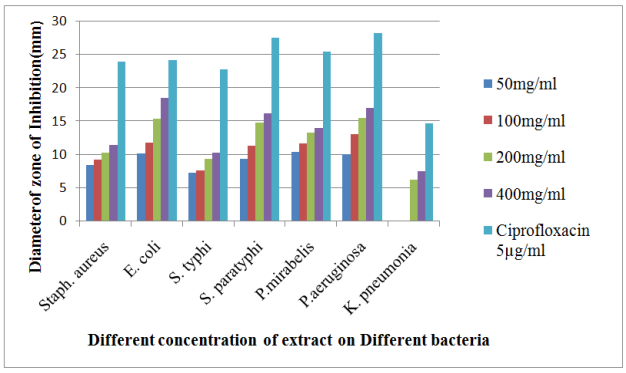

Fig. 1: Antibacterial activity of methanol extract of *Gyrocarpus asiaticus* Willd.

The MIC and MBC analysis of the methanolic extract showed satisfactory bacteriostatic and bcteriocidal concentration. The MIC of the plant extract was studied from the range of 0.039 to 0.625 to mg/ml. Plant extract for the bacterial strain *E.coli* appears to be sensitive with MIC value of 0.039 mg/ml with 18.5mm zone of inhibition and MBC value of 0.16 mg/ml. Gram positive *S. aureus* was moderately sensitive with MIC value of 0.32 mg/ml and MBC value of 0.625 mg/ml. Other Gram negative species like *P.mirebelis* and *P. auroginosa* were also found to be sensitive with MIC values of 0.039 mg/ml and MBC values of 0.16 mg/ml of the both when compared to standard ciprofloxacin 0.001 mg/ml for MIC and 0.04 mg/ml for MBC. The methanolic extract showed no MIC and no MBC against *klebsiella sp*. The results are cited in (Table 2). This shows satisfactory MIC, MBC as well as zone of inhibition against almost all pathogens except *klebsiella sp* which indicates its possible application against common human pathogenic bacterial infection.

Table 4: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of methanolic extract of *Gyrocarpus asiaticus* Willd.

Bacteria	*G. asiaticus* methanolic extract		+ve control Cf	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
S.A.	**0.320**	**0.625**	**0.002**	**0.16**
E.C.	0.039	0.16	0.002	0.04
S.T.	0.625	0.64	0.001	0.16
S.P.	0.156	0.32	0.002	0.04
P.M.	0.039	0.16	0.001	0.04
P.A.	0.039	0.16	0.001	0.04
K.P.	--	---	0.002	0.04

+ve control Cf = Ciprofloxacin. --: Not determined. **S.A.*: Staphylococcus aureus*, E.C.*: Escherichia coli*, S.T.*: Salmonella typhi*, S.P.*: Salmonella paratyphi*, P.M.*: pseudomonas mirebelis,* P.A.*: pseudomonas auroginosa,* **K.P.*: Klebsiella pneumoni.***

CONCLUSION

Gyrocarpus asiaticus represents a potent antimicrobial system for the development of natural drugs. Though in a crude form, the plant extracts have displayed broad spectrum activity towards the microorganisms. From these results, it could be concluded that "*Gyrocarpus asiaticus* Willd" contain antibacterial activity.

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