

EVALUATION OF IN-VITRO ANTIBACTERIAL ACTIVITY OF CASSIA SIAMEALEAVES

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ABSTRACT

Objective: Recently, natural plants have received much attention as sources of biological active substances. In the present study we investigated different extracts of *Cassia siamea* leaves for their *In-vitro* antibacterial activity.

Methods: *In-vitro* antibacterial activity was evaluated for ethanolic, ethyl acetate and hexane extracts against three Gram positive and three Gram negative bacteria by using cylinder plate assay.

Results: All the tested extracts of *Cassia siamea* showed significant zone of inhibition against tested bacterial strains in a dose dependent manner. All the extracts showed good zone of inhibition at a doses of 200 and 400µg/100µl. The highest zone of inhibition was shown by hexane extract against *Klebsiella pneumonia* and *Escherichia coli* at the dose of 400µg/100µl.

Conclusion: All extracts exhibited antibacterial activity against tested bacterial strains in a dose dependent manner but relatively low activity when compared to that of standard Rifampicin. The activity may be higher if larger dose levels were employed.

Keywords: *Cassia siamea*, Leaves, Microorganisms, *in vitro* antibacterial activity.

INTRODUCTION

Plants have the major advantage of being the most effective and cheaper alternative source of drugs [1]. Historically, pharmacological screening of compounds of natural origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics [2, 3]. As reported by World Health Organization (WHO), traditional medicinal plants are the best reservoirs to develop newer pharmaceuticals [4]. Researchers are increasingly turning their attention to natural products looking for leads to develop better drugs against many microbial infections [5-7]. The medicinal value of plant lies in some chemical substances present in them. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds [8]. The expanding bacterial resistance to antibiotics has become a growing concern worldwide [9]. Intensive care physicians consider antibiotic resistance bacterial a significant problem in the treatment of patients [10]. Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains [11, 12]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds [13]. Medicinal plant extracts offer considerable potential for the development of new agents effective against infections currently difficult to treat [14]. *Cassia siamea* is commonly known as the Kassod tree, belongs to family Fabaceae. The literature survey revealed that *C.siamea* contains different phytochemical compounds like lupeol, chrysophanol, cassiamin A, cassiamin, siameadin, lupeone, rhein, chrysophanol-antrone, barakol, cassia chromone (5-acetonyl-7-hydroxy-2-methylchromone), p-coumaric acid, apigenin-7-o-galactoside, β-sitosterol, cassia chromonone and cassiadinine [15-19]. *C.siamea* has been reported to possess medicinal effects [20-24]. In recent decades; antibiotic resistance of pathogens is an intensifying problem worldwide [25]. This has led to the search for new, safe and effective antimicrobial agents from natural products, so the present study was carried out to evaluate *in vitro* antibacterial activity of *C.siamea* leaves extracts.

MATERIALS AND METHODS

Preparation of extracts from leaves of *Cassia siamea*

The leaves of *Cassia siamea* were collected from Andhra University campus, Visakhapatnam, Andhra Pradesh, India during the month of December 2011 and authenticated by Dr. P. Prayaga Murthy, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Shade dried leaves of *Cassia siamea* was powdered and separately extracted in a Soxhlet

apparatus for 6 hrs successively with hexane, ethyl acetate, and ethanol were concentrated to dryness under vacuum at temperature of 45°C by using rotary evaporator (Buchi, Switzerland), dried completely and stored in desiccator.

Test organisms

The microorganisms used in the experiment were procured from MTCC, IMTECH-Chandigarh.

Gram-positive organisms

Staphylococcus aureus, *Bacillus subtilis*, *Bacillus pumilus*.

Gram-negative organisms

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*.

Evaluation of in-vitro antibacterial activity

The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds (extracts). A sterile borer was used to prepare the cups of 6 mm diameter in the agar medium spread with the microorganisms and 0.1 ml of inoculums. These cups were spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicates antibacterial activity [26].

RESULTS

Evaluation of antibacterial activity

Among all the tested *Cassia siamea* leaves extracts, hexane extract have shown significant antibacterial activity when compared to that of ethanol and ethyl acetate extracts. All the extracts of *Cassia siamea* leaves had produced a minimum zone of inhibition against some tested bacterial species at a dose of 50 and 100µg/100µl. Ethanol extract did not show any effect on *E.coli*, *B.subtilis*, *B.pumilis* and *P.aeruginosa* at lower concentrations. All extracts showed good zone of inhibition at a doses of 200 and 400 µg/100µl. The highest zone of inhibition was shown by hexane extract against *Klebsiella pneumonia* and *Escherichia coli* at the dose of 400µg/100µl. All the extracts of *Cassia siamea* leaves had produced a minimum zone of inhibition against *P.aeruginosa* species. The results were shown in the **Table 1**.

Table 1: Antibacterial activity of *Cassia siamea* leaves extracts

Plant material	Dose (µg/cup)	Zone of inhibition [#] (diameter in mm)					
		gram (+) ve			gram (-) ve		
		<i>B.s</i>	<i>S.a</i>	<i>B.p</i>	<i>E.c</i>	<i>P.a</i>	<i>K.p</i>
Ethanol extract	50	-	08	-	-	-	07
	100	08	09	07	07	-	09
	200	10	12	08	09	07	11
	400	12	13	10	11	08	13
Ethyl acetate extract	50	09	12	07	09	08	10
	100	09	13	10	10	08	12
	200	09	13	11	11	08	13
	400	11	14	12	12	09	14
Hexane extract	50	09	12	08	08	08	07
	100	10	13	08	12	08	08
	200	11	13	08	14	08	11
	400	14	14	09	15	08	15
Rifampicin	50	24	22	19	19	24	28
DMSO		-	-	-	-	-	-
Water		-	-	-	-	-	-

S.a=*Staphylococcus aureus*; *E.c*=*Escherichia coli*; *P.a*=*Pseudomonas aeruginosa*; *B.s* = *Bacillus subtilis*, *B.p*= *Bacillus pumilis*; *K.p* = *Klebsiella pneumoniae*.

--No activity

#Values are the average of triplicate; Includes the cup diameter (6mm).

DISCUSSION

Ethanol extract did not show any effect on *E.coli*, *B.subtilis*, *B.pumilis* and *P.aeruginosa* at lower concentrations – Table 1. Negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the extracts to show activity with the dose levels employed [27]. Lack of activity can thus only be proven by using large doses [28]. Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents [29]. With no antibacterial activity, extracts may be active against other bacterial species which were not tested [30].

CONCLUSION

All the extracts exhibited antibacterial activity against tested bacterial strains in a dose dependent manner but relatively low activity when compared to that of standard Rifampicin. The activity may be higher if larger dose levels were employed.

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