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Evaluation of *in vitro* antimicrobial activity of extracts from *Cassia obtusifolia* L. and *Senna sophera* (L.) Roxb against pathogenic organisms

Surekha R Deshpande[1], B. Shankar Naik[2*]

[1]Department of Zoology, Basaveshwar science college, Bagalkot, 587101, Karnataka, India. [2]Department of Biology, Govt science college, Chikmagalur, 577101, Karnataka, India.

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ABSTRACT

Cassia species have been of medicinal interest due to their good therapeutic value in folk medicine. In the present study petroleum ether, ethanol and chloroform extracts from leaf and stems of *Cassia obtusifolia* and *Senna sophera* were investigated for their antimicrobial activities against some pathogenic microbes in vitro. The *C. Obtusifolia* leaf extracts in pet ether and chloroform showed more sensitivity against *E. faecalis* (MIC 0.2725mg/ml and MIC 0.2647) and ethanol extracts against *A. fumigatus* (MIC 0.3116mg/ml). Similarly the stem extracts of *C. Obtusifolia* in pet ether showed more sensitivity against *E. faecalis* (MIC 0.407mg/ml), ethanol extracts against *E. faecalis* (MIC 0.3009mg/ml) and chloroform extracts against *E. faecalis* MIC 0.4946mg/ml). The leaf extracts of *S. sophera* in pet ether showed more sensitivity against *C. albicans* (MIC 0.3524mg/ml), ethanol extracts against *E. faecalis* (MIC 0.2738mg/ml) and chloroform extracts against *C. Albicans* (MIC 0.4239). *C. sophera* stem extracts in Pet ether showed more sensitivity against *E. faecalis* (MIC 0.254mg/ml), ethanol extracts against *E. faecalis* (MIC 0.2987mg/ml) and chloroform extracts against *E. faecalis* (MIC 0.5899mg/ml). This finding provides an insight into the usage of the leaves of *Cassia* species in traditional treatment of wounds or burns associated with bacterial and fungal infections. However, further work is needed in the form of phytochemical screening and pharmacological activity of some more extracts before one could conclude anything definite about the therapeutic potential of these extracts.

INTRODUCTION

Increased development of resistance to drugs by human pathogenic microbes forced the investigators to search new antimicrobials from various natural sources like medicinal plants (Tomoko *et al.*, 2000; Doshi *et al.*, 2011). Medicinal plants have been used to treat common infectious diseases for centuries and some of them are the source of traditional medicines (Raja, 2013). The use of plant extracts and photochemical both with known antimicrobial properties are of great significance. The plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. Which have been found to have vast antimicrobial properties *in vitro* (Cowan, 1999). In the past decades a number of investigations have been conducted worldwide. Among more than 250000 species of higher plants only 5-10% are chemically investigated (Nahrsted, 1996). World Health Organization (WHO) encourages countries to examine traditional medicine for providing safe and effective remedies for different diseases (Akinyemi *et al.*, 2002). *Cassia* species have been of medicinal interest due to their good therapeutic value in folk medicine. *Cassia obtusifolia* is an annual herb belongs to leguminosae native to tropical regions and grows throughout china US and elsewhere. The seeds of *C. Obtusifolia* have been used treat the eye problems, It lowers the cholesterol and blood pressure and prevents the formation of atherosclerotic plaque in the arterial wall and it also has the laxative and antibacterial effects. *Senna sophera* (L.) Roxb formerly called *Cassia sophera* is a shrub probably originated in India found in most tropical countries. It has been used in treating various respiratory disorders. In the present study leaves and stem extracts of *C. Obtusifolia* and *S. sophera* have been evaluated for their antimicrobial activity under laboratory conditions.

* *Corresponding Author*
Email: shankar_sbn@yahoo.co.in

MATERIALS AND METHODS

Plant material and extracts preparation

The leaves of *C. obtusifolia* and *S. sophera* were collected from in and around Bagalkot Dist located in North Karnataka region, in the month of September–October. A voucher specimen (BSC/Pharmacy/ 2015/1/12) was stored in the department for future reference. Leaves and stem were shade dried at room temperature. The shade dried and coarsely powdered plant material were successively extracted with petroleum ether (60-80^0 C), Chloroform and ethanol using Soxhlet apparatus. The extracts were dried under reduced pressure at temperature of 30^0 C to dryness to yield dried extract residue.

Antibacterial and antifungal activity

All the extracts were evaluated for antimicrobial activity against few clinical isolates, by serial dilution method in duplicate (Koneman, 1995). Antimicrobial activity tested against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC29212), *Klebsiella* sp. (ATCC-1705), *Escherichia coli* (ATCC 25922) and antifungal activity against *Aspergilus fumigatus* (ATCC102) and *Candia albicans* (ATCC10231).They are grown on blood agar media, sub cultured and isolated. On the other hand control strains of same organisms were also developed in suitable culture media. The inoculum of both control strains and clinical isolates were standardized by adjusting to McFarld scale (0.5) using Muller-Hinton (105CFU/ml). Ciprofloxacin and Fluconazole were used as reference standard. The plant extracts were initially dissolved in minimum quantity of DMSO and then were added to Muller-Hinton broth to reach final concentration of 1mg/ml, 300µl of these extracts were added to first and second tubes further dilutions were made from second tube to ninth tube using 2 fold dilution technique, so that the highest and lowest concentration of each extracts were 300 µl and 0.6 mg/ml respectively. To each of these tubes 100 µl of microbial culture (105CFU/ml) was added and incubated for 24 hrs at 37º C, and were examined from bottom using reflective viewer. The lowest growth was recorded as MIC for each organism.

RESULTS AND DISCUSSION

Leaf extracts of *C. obtusifolia* in pet ether showed more activity against *E. faecalis* (MIC 0.2725 mg/ml) and least sensitivity against *Klebsiella* sp. (MIC 1.0605 mg/ml). The ethanol extracts from *C. Obtusifolia* showed more sensitivity against *A. fumigatus* (MIC 0.3116mg/ml) and the chloroform extract was more sensitive against *E. faecalis* (MIC 0.2647mg/ml). Similarly the stem extracts of *C. Obtusifolia* in pet ether showed more sensitivity against *E. faecalis* (MIC 0.407mg/ml), ethanol extracts against *E. faecalis* (MIC 0.3009) and chloroform extracts against *E. faecalis* (MIC 0.4946mg/ml) Table 1.

The leaf extracts of *S. sophera* in pet ether showed more sensitivity against *C. Albicans* (MIC 0.3524mg/ml), ethanol extracts against *E. faecalis* (MIC 0.2738mg/ml) and chloroform extracts against *C. Albicans* (MIC 0.4239). *C. sophera* stem extracts in Pet ether showed more sensitivity against *E. faecalis* (MIC 0.254mg/ml), ethanol extracts against *E. faecalis* (MIC 0.2987mg/ml) and chloroform extracts against *E. faecalis* (MIC 0.5899mg/ml) Table 2. *Cassia* species containing anthraquinone, flavonoids and reducing sugar showed considerable antimicrobial activity against gram positive microorganisms (Abo *et al.*, 1998). The *in vitro* antimicrobial activities from extracts by Cassia species have been reported from various parts of the world (Anushia *et al.*, 2009). In our previous study leaf and stem extracts from *Cassia glauca* showed antimicrobial activity against bacterial and fungal pathogens in vitro(Kittur *et al.*, 2015) This finding provides an insight into the usage of the leaves of *Cassia* species in traditional treatment of wounds or burns associated with bacterial and fungal infections. However, further work is needed in the form of phytochemical screening and pharmacological activity of some more extracts before one could conclude anything definite about the therapeutic potential of these extracts.

Table 1: Antimicrobial activity of plant extracts by *C. obtusifolia* against pathogens.

Plant material	Extracts	MIC(mg/ml)					
		S. aureus	***E. faecalis***	***A. fumigatus***	***E. coli***	***Klebsiella sp.***	***C. albicans***
leaf extract	Pet ether	0.38425	0.3563	0.4581	1.395	0.5914	0.3524
	Ethanol	0.3193	0.2738	0.3802	1.4172	0.8453	0.3578
	chloroform	0.5523	0.426	0.5999	1.3391	1.0101	0.4239
stem extract	Pet ether	0.3342	0.254	0.4634	1.1314	0.8815	0.3526
	Ethanol	0.5069	0.29871	0.8858	1.1254	1.4421	0.4807
	chloroform	0.7472	0.5899	0.6066	1.4167	1.5215	0.6421

Table 2: Antimicrobial activity of plant extracts by *S. sophera* against pathogens.

Plant material	Extracts	MIC(mg/ml)					
		S. aureus	***E. faecalis***	***A. fumigatus***	***E. coli***	***Klebsiella sp.***	***C. albicans***
Leaf extract	Pet ether	0.3639	0.2725	0.6287	0.9574	1.0605	0.4834
	Ethanol	0.4317	0.3402	0.3116	1.1819	0.4211	0.394
	chloroform	0.2938	0.2647	0.4006	1.144	0.549	0.4834
Stem extract	Pet ether	0.4534	0.407	0.4946	1.397	0.6393	0.5062
	Ethanol	0.3791	0.3009	0.3168	1.3709	0.618	0.3058
	chloroform	0.4534	0.407	0.4946	1.397	0.6393	0.5062

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