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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *TRICHOSANTHES CUCUMERINA* L. AND *CASSIA DIDYMOBOTRYA* FRES. LEAVES

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ABSTRACT

The antibacterial activities of petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Trichosanthes cucumerina* and *Cassia didymobotrya* were screened against various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. The ethyl acetate, chloroform and methanol extracts of *Trichosanthes cucumerina* leaves as well as leaf ethyl acetate extract of *Cassia didymobotrya* exhibited pronounced activity on all organisms tested and their activity is quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions. The antimicrobial potency of these plant extracts is due to the presence of phenolic compounds, flavonoids and carotenoids. The study shows that the leaf ethyl acetate extract of *Cassia didymobotrya;* methanol, ethyl acetate and chloroform extracts of *Trichosanthes cucumerina* leaves can be used as a potential external antiseptic and can be incorporated into drug formulations.

Keywords: *Trichosanthes cucumerina*, *Cassia didymobotrya,* Antibacterial activity, Agar well diffusion method, Standard antibiotics, Drug formulation.

INTRODUCTION

The universal role of plants in the treatment of diseases is established by their employment in all important systems of medicine. There are many herbs on earth which lies unexplored in the field of medicine or Science. One such plant is *Trichosanthes cucumerina*, the fruit of which is mainly consumed as a vegetable. It is an annual climber belonging to the family Cucurbitaceae. It is commonly called as snake gourd, viper gourd, snake tomato or long tomato. The fruit is usually consumed as a vegetable due to its good nutritional value. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicine like Ayurveda and Siddha due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti inflammatory, larvicidal effects[1].

Trichosanthes cucumerina is used in the treatment of headache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrhoea, haematuria and skin allergy. *T. curcumineria* is used as an abortifacient, vermifuge, refrigerant, purgative, malaria, laxative, hem agglutinant, emetic, cathartic, bronchitis and anthelmintic[2]. A novel isoflavone glucoside, 5,6,6'-trimethoxy-3',4'-methylene-dioxyisoflavone7-O-beta-D-(2''-O-p-coumaroylglucopyranoside) has been characterized from the seeds of *Trichosanthes*[3]. The positive effects of the plant are due to the carotenoids, flavonoids, lycopene, phenolics and ß-carotene present in it[4]. The seed is said to be cooling. The dried seeds are used for its anthelmintic and anti-diarrhoeal properties. Seeds have anti-bacterial, anti-spasmodic and insecticidal properties. It is used as abortifacient, acrid, aphrodisiac, astringent, bitter, febrifuge, purgative, toxic, trichogenous[2, 5]. Hot aqueous extract of root tubers of *Trichosanthes cucumerina* exhibited significant anti-inflammatory activity[6]. The root extract of *Trichosanthes cucumerina* L. and the fruit juice tested cytotoxicity against four human breast cancer cell lines and lung cancer cell lines and one colon cancer cell line. The root extract inhibited more strongly than the fruit juice[7]. Crude ethanolic extract of *Trichosanthes cucumerina* showed significant blood glucose lowering activity in alloxan diabetic albino rats[8]. The acetone extract of leaves of *Trichosanthes cucumerina* showed moderate larvicidal effects[9]. Hot water extract of aerial parts of *Trichosanthes cucurmerina* has noted to improve glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats. Study showed the drug possess antidiabetic activity with volume improvement in oral glucose tolerance and glucose uptake in peripheral tissues [10].

Cassia didymobotrya a native to tropical Africa, prefers light to medium soils in a open sunny position. The flowers produce a scent similar to that of buttered popcorn, hence the common name 'Popcorn Cassia'. From suspension cultures of *Cassia didymobotrya* 7- acetyl chrysophanol, chrysophanol-physcion-10, 10'-bianthrone have been isolated along with several known metabolites[11]. Vitali et al[12] extracted, purified and characterized the peroxidase from plant cell cultures of *Cassia didymobotrya*. The catalytic activity on flavonodis and dibenzylbutanolides and the specificity of the peroxidase isolated from 29d-old cell cultures of *Cassia didymobotrya* are reported[13].

So far no data about the antibacterial activities of these plants were reported. In the present study antibacterial activities of the crude leaf extracts of *Trichosanthes cucumerina* and *Cassia didymobotrya* were investigated for the aim of discovering the medicinal potential of these plant extracts.

MATERIALS AND METHODS

Plant material

Leaves of *Trichosanthes cucumerina* and *Cassia didymobotrya* were collected from Kerala, South India and authenticated by Dr. A.K. Pradeep, Dept. of Botany, Calicut University. Voucher specimen is deposited in the specially maintained herbarium, Department of Chemistry, Vimala College, Thrissur, Kerala.

Preparation of plant extracts

Fifty grams of each of powered plant material were extracted successively with 150ml of petroleum ether, chloroform, ethyl acetate, methanol and water as solvents for 24 hours by Soxhlet equipment[14].

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96), *Enterobacter faecalis* (MTCC-5112) and *Streptococcus faecalis* (MTCC-439) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella*

pneumoniae (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50µl of the petroleum ether, chloroform, ethyl acetate and methanol extracts of the leaves of *Trichosanthes cucumerina* and *Cassia didymobotrya* were added in each well. Wells introduced with 50µl of pure petroleum ether, chloroform, ethyl acetate and methanol served as negative controls. The plates were incubated at 37^0C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm[15]. All the assays were performed in triplicate and expressed as average values.

The antibacterial spectra of the leaf extracts of *Trichosanthes cucumerina* and *Cassia didymobotrya*, showing the zone of inhibition in millimeters, against Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Enterobacter faecalis* and *Streptococcus faecalis* and Gram-negative bacteria such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi* and *Serratia marcescens* are summarized in table 1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in table 2.

Table1: Antibacterial activity of the leaf extracts of *Trichosanthes cucumerina* and *Cassia didymobotrya*

Microorganisms	Diameter of inhibition zones(mm/50µl)							
	T. cucumerina				*C. didymobotrya*			
	A	B	C	D	A	B	C	D
1. *Bacillus cereus*	15	12	30	9	16	14	10	--
2. *Enterobacter faecalis*	10	10	16	--	14	17	13	--
3. *Salmonella paratyphi*	25	11	16	--	16	13	11	10
4. *Staphylococcus aureus*	16	12	--	--	16	28	14	--
5. *Escherichia coli*	11	10	11	--	15	15	12	--
6. *Streptococcus faecalis*	16	28	12	--	16	21	11	--
7. *Proteus vulgaris*	14	16	11	--	14	15	10	--
8. *Klebsiella pneumoniae*	16	11	18	--	15	16	11	10
9. *Pseudomonas aeruginosa*	14	--	--	--	15	16	11	10
10. *Serratia marcescens*	18	26	18	--	11	24	16	12

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Table 2: Inhibition zones formed by the standard antibiotics and negative controls

Microorganisms	Diameter of inhibition zones (mm/50µl)				
	Tob 10µg	Gen 10µg	Oflo 10µg	Cip 10µg	Control A, B, C, D
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Streptococcus faecalis*	28	34	30	32	--
7. *Proteus vulgaris*	26	30	24	32	--
8. *Klebsiella pneumoniae*	26	32	32	36	--
9. *Pseudomonas aeruginosa*	26	24	32	28	--
10.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: petroleum ether

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

RESULTS AND DISCUSSION

As can be seen from table 1, the leaf ethyl acetate extract of *Cassia didymobotrya* showed pronounced antibacterial activity against all the microorganisms tested (13-28mm/50µl inhibition zone). Methanol (11-16mm/50µl inhibition zone) and chloroform (10-16mm/50µl inhibition zone) extracts of the leaves were found to be active against all the tested microorganisms such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens.*

Choloroform extract of *Trichosanthes cucumerina* leaves exhibited remarkable activity against *Bacillus cereus* (30mm/50µl inhibition zone) whereas it did not inhibit *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The leaf choloroform extract was also found to be effective on *Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae* and *Serratia marcescens* (11-18mm/50µl inhibition zone). *Trichosanthes cucumerina* leaf ethyl acetate extract showed appreciable activity on all test bacteria (10-28mm/50µl inhibition zone) except *Pseudomonas aeruginosa.* The leaf methanol extract inhibited the growth of all tested bacteria to a considerable extent (10-25mm/50µl inhibition zone).

The results obtained were compared with standard antibiotics and it was observed that *Cassia didymobotrya* leaf ethyl acetate extract at a concentration of 1mg/ml was more active against *Staphylococcus aureus* than tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin (10µg each). The activity of the leaf ethyl acetate

extract of *Cassia didymobotrya* against *Serratia marcescens* was comparable with that of standard antibiotic tobramycin (10µg).

The leaf petroleum ether extracts of both plants were found to be non effective or having little activity on all microorganisms tested. The Minimum Inhibitory Concentration (MIC) of methanol, ethyl acetate and chloroform extracts of *Trichosanthes cucumerina* leaves was found to be 0.5mg/ml and that of the ethyl acetate extract of *Cassia didymobotrya* was 1mg/ml.

Out of the eight herbal extracts examined for antibacterial activity, leaf chloroform extract of *Trichosanthes cucumerina* showed highest activity against *Bacillus cereus* and its activity is quite comparable with that of the standard antibiotics tobramycin and ciprofloxacin at a concentration of 10µg. Ethyl acetate extract of *Cassia didymobotrya* leaves exhibited remarkable activity against *Staphylococcus aureus* and *Serratia marcescens* whereas *Trichosanthes cucumerina* leaf ethyl acetate extract inhibited *Streptococcus faecalis* and *Serratia marcescens*. The methanol extract of *Trichosanthes cucumerina* showed appreciable inhibitory effect on *Salmonella paratyphi*. The antimicrobial potency of these plant extracts is due to the presence of phenolic compounds, flavonoids and carotenoids[4]. It is interesting to note that even crude extract of these plants showed prominent activity against various pathogenic bacteria where modern therapy has failed. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts.

CONCLUSION

Amongst the herbal extracts of *Trichosanthes cucumerina* and *Cassia didymobotrya* leaves examined for antibacterial activity, *Cassia didymobotrya* leaf ethyl acetate extract, *Trichosanthes cucumerina* leaf ethyl acetate, chloroform and methanol extracts showed significant activity against the different strains of bacteria. The activities of these extracts are found to be quiet comparable with the standard antibiotics screened under similar conditions. So these extracts can be used as an external antiseptic in prevention and treatment of bacterial infections. The incorporation of these extracts into the drug formulations is also recommended.

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