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ANTIMICROBIAL ACTIVITY OF *TINOSPORA CORDIFOLIA, TALINUM CUNEIFORMIS AND COCCINIA INDICA*

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ABSTRACT

The antimicrobial activity of alcoholic extracts of 3 medicinal plants from Andhra Pradesh were tested against three gram negative bacteria, four gram positive bacteria and three pathogenic fungi by the disc diffusion and tube dilution methods. Among the tested plants, only *Tinospora cordifolia* showed a significant control of the growth of all the tested microorganisms. *Talinum cuneiformis* was highly effective against pathogenic gram negative bacteria and moderately effective against gram positive bacteria. *Coccinia indica* showed significant activity only against pathogenic gram positive bacteria particularly against *Staphylococcus aureus*.

Key words: Antimicrobial activity, Alcoholic extracts, Human pathogens, MIC.

INTRODUCTION

Plants are known to contain innumerable biologically active compounds[1], which possess antibacterial properties[2,3]. Today, nearly 88% of the global population turn to plant derived medicines as their first line of defence for maintaining health and combating diseases. Currently, people of Asia are utilizing plants as part of their routine health management. New sources, especially plant sources, are also being investigated. It is estimated that there are 2,50,000 to 5,00,000 species of plants on Earth[4].

In the present study, an attempt has been made to enrich the knowledge of antimicrobial activity of 3 different plants traditionally used in Andhra Pradesh. *Tinospora cordifolia* Miers (Menispermaceae) is a deciduous woody climber, distributed throughout the plains of India. It is used in the treatment of jaundice, rheumatism, urinary diseases, intermittent fever, hyperacidity, eye and liver ailments[5]. The aqueous and alcoholic extract of the plant significantly reduced the blood glucose[6]; diuretic[7]; antiretroviral[8]; and antineoplastic[9]. *T.*

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cordifolia was reported to contain cardinane sesquiterpene glycoside, tinocordiside[10], daucane type sesquiterpene glycoside, tinocordifolioside[11], cordifolioside A, B[12], palmatosides C, F[13], arabinogalactan[14], isocolumbin, palmatine, tetrahydropalmatine and magnoflorine[15]. The ethnic tribal communities have been using the *Talinum cuneiformis* (Portulacaceae) from many generations and information regarding the efficacy remains primarily anecdotal. There is no previous record and research work available on the traditional medicinal values of *T. cuneiformis*. Most of the ancient knowledge systems continued to survive by oral communication from generation to generation in rural as well as in tribal communities. *Coccinia indica W.* & A (cucurbitaceae) is a perennial creeping herb with long tapering tuberous roots and grows abundantly in India. The plant occurs both in bitter and non–bitter forms. These fruits are reported to be useful in various ailments[16]. The plant is known to possess good hypoglycemic properties[17, 18] and antinociceptive and antiinflammatory activities[19]. *C. indica* was reported to contain flavanoid glycoside, ombuin 3–O–arabinofuranoside[20], triterpenoid saponin, Coccinioside–K[21] and 3–O–b–(a–L–arabinopyranosyl)–b–D–glucopyranosyl–b–hydroxylup–20(29)–en–28–oic acid[22].

As there is no reference in literature regarding the antimicrobial activity of these plants, it was, therefore, considered worthwhile to study the antimicrobial activity against various pathogenic gram positive bacteria, gram negative bacteria and pathogenic fungi

MATERIALS AND METHODS

Collection of plant samples : Fresh parts of *Tinospora cordifolia* (stems), *Talinum cuneiformis* (stems, roots, leaves) and *Coccinia indica* (fruits) were obtained from the medicinal plant garden of Padmabhushan Dr. B. V. Raju Foundation campus, Bhimavaram, Andhra Pradesh and they were dried under shade. The taxonomical identification of the plants was done at National Botanical Research Institute (NBRI), Lucknow.

Preparation of plant extracts of *T. cordifolia* and *T. cuneiformis*: Dried parts of the plants were pulverized by a mechanical grinder, then passed through 40–mesh sieve and stored in a closed vessel. Powdered material (500–1000 g) was extracted with alcohol using Soxhlet extraction apparatus for 8–10 h[23]. The alcoholic extract was completely evaporated at 40^0C under vacuum using a rotary evaporator. The weight of dry residues were recorded and stored in clean glass bottles for bioassay [yield: 18%, 18%, 9% and 12% w/w respectively for *T. cordifolia* (stems) and *T. cuneiformis* (stems, roots, leaves)]. They were dissolved in alcohol to get a concentration of 1mg mL^{-1}.

Preparation of fruit juice powder of *C. indica*: The fresh fruits of *C. indica* (1 Kg) were washed with distilled water to remove dirt and soil, size reduced (3–5 cm), crushed and soaked in alcohol (1L) for 24 h and extracted by a juicer and filtered through muslin cloth. One Kg of fruits yielded 850 mL of juice. The juice was passed through the SD–05 spray drier (Lab– lant Ltd. U.K.) and the solid content in terms of powder weight obtained was 12% w/w. The fruit

juice powder of *C. indica* was stored in a desiccator. It was dissolved in alcohol to get a concentration of 1mg mL^{-1}.

Microbial strains: All microorganisms, except *Escherichia coli*, were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India, whereas *E. coli* was obtained from Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

Gram negative bacteria: *E. coli* (enteropathogen), *Proteus vulgaris* (NCIB 8067), *Pseudomonas aeruginosa* (NCIM 8162); **Gram positive bacteria :** *Bacillus subtilis* (NCIM 2063), *B. pumilus* (NCIM 2327), *Staphylococcus aureus* (NCIM 2127), *Streptococcus faecalis* (NCIM 5025); **Yeast type fungi:** *Candida albicans* (NCIM 3471), **Mold type fungi:** *Aspergillus niger* (NCIM 616) and *Rhizopus oligosporus* (NCIM 1215).

Bacterial cultures were sub–cultured on nutrient agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock cultures. Fungal cultures were sub–cultured on Saboraud dextrose agar medium, incubated at 28°C for 120 h and stored at 4°C in the refrigerator to maintain stock cultures. Antimicrobial activity was assayed by the following standard methods –

Preparation of compounds for antimicrobial activity: The pure antibiotic powders or alcoholic extracts were stored at 4°C or held under desiccation. Antibiotics were weighed and dissolved in sterile distilled water or dimethyl sulfoxide (DMSO) to give appropriate concentrations of active substance. The standard antibiotics used were ampicillin (40 μg mL^{-1}) and nystatin (50 IU mL^{-1}).

Disc diffusion method: Antimicrobial activity of the alcoholic extracts was tested using the disc diffusion[24] and tube dilution methods[25]. Sterile nutrient agar media (pH 7.2) was inoculated and aseptically transferred into each sterile petri dish. Sterile filter paper discs of 6–mm diameter, impregnated with alcoholic extracts of plants, were introduced on the surface of the medium. Each disc absorbed 50 μg of extract. Petridishes were incubated at 37°C for 24 h to obtain inhibition zones. The tests were conducted in triplicate. Antifungal activities of plant extracts were tested using Saboraud dextrose agar, incubated at 28°C for 120–144 h.

The broth dilution method: The test was performed in sterile, plugged test tubes. Minimum Inhibitory Concentration (MIC) of all the alcoholic extracts was determined by tube dilution method. The varying concentrations of the extracts were mixed with nutrient or saboraud broth to give a concentration of 50–1000 μg mL^{-1}. All the tubes were inoculated with 0.1mL of each culture ($1x10^4$ cfu mL^{-1}) and incubated for 24 to 48 h. The positive control tube was not served with antimicrobial test compound.

The lowest concentration of the test compound that resulted in complete inhibition of visible growth represented the minimum inhibitory concentration (MIC). A very faint haziness or a small button of possible growth was generally disregarded, whereas a large button of growth was considered evidence that the drug had failed to inhibit growth at that concentration.

RESULTS AND DISCUSSION

The antimicrobial screening of alcoholic extracts of the different plants are reported in Table 1. These have been tested against 7 different bacterial strains and three fungal strains in agar diffusion assays. Those plants with an inhibition zone diameter 7 mm were tested in dilution assays. Direkbusarakom et al.[26] investigated the efficacy of *T. cordifolia* against fish and

Table 1. Antimicrobial activity of alcoholic extracts.

Family species	Org. →	*Ec*	*Pv*	Pa	Bs	*Bp*	*Sa*	*Sf*	*Ca*	*An*	*Ro*
MENISPERMACEAE *Tinospora cordifolia* (dried stems)	IZD	18	12	10	12	12	16	09	10	14	12
	MIC	200	225	250	175	150	100	225	250	125	150
PORTULACACEAE *Talinum cuneiformis* (stems)	IZD	23	20	18	22	17	25	22	0	0	0
	MIC	200	225	300	200	300	150	175	Nd	Nd	Nd
PORTULACACEAE *Talinum cuneiformis* (roots)	IZD	23	25	20	22	22	21	19	0	0	0
	MIC	150	125	200	150	175	225	250	Nd	Nd	Nd
PORTULACACEAE *Talinum cuneiformis* (leaves)	IZD	39	41	30	18	22	24	21	0	0	0
	MIC	50	50	75	200	175	150	200	Nd	Nd	Nd
CUCURBITACEAE *Coccinia indica* (fruits)	IZD	0	0	0	12	9	17	13	0	0	0
	MIC	Nd	Nd	Nd	300	400	150	300	Nd	Nd	Nd
Ampicillin (40 µg/mL)	IZD	17	22	16	21	22	23	24	Nd	Nd	Nd
Nystatin (50 IU/mL)	IZD	Nd	Nd	Nd	Nd	Nd	Nd	Nd	18	21	24
Control (60%v/v alcohol)	IZD	0	0	0	0	0	0	0	0	0	0
Control (DMSO)	IZD	0	0	0	0	0	0	0	0	0	0

IZD: Inhibition zone diameter (mm); MIC: Minimum inhibitory concentration (µg mL^{-1}); values are the mean of 3 replicates; Nd: not determined.
Ec: *Escherichia coli* (enteropathogen), *Pv*: *Proteus vulgaris; Pa*: *Pseudomonas aeruginosa*; *Bs*: *Bacillus subtilis; Bp*: *B. pumilus*; *Sa*: *Staphylococcus aureus; Sf*: *Streptococcus faecalis; Ca*: *Candida albicans; An*: *Aspergillus niger; Ro*: *Rhizopus oligosporus*.

shrimp pathogenic bacteria, *Aeromonas hydrophila*, a Streptococcus species and 10 strains of *Vibrio*. In the present study, *T. cordifolia* showed significant antimicrobial activity against all tested organisms, particularly against enteropathogen, *E. coli* (MIC = 200µg mL^{-1}) and *S.*

aureus (MIC = 100 μg mL^{-1}). There are no reports on the antimicrobial activity of *T. cuneiformis* and *C. indica*. In the present study, the agar diffusion tests of alcoholic extracts of root and stem extract of *T. cuneiformis* were highly inhibitory to gram negative bacteria and moderately effective against pathogenic gram positive bacteria particularly against *S. aureus*. The leaf extract formed zones of inhibition ranging from 18–41 mm against all selected organisms, suggests its broad spectrum of activity. *C. indica* showed moderate activity against gram positive bacteria, but a significant activity against *S. aureus* (MIC = 150 μg mL^{-1}).

The minimum inhibitory concentration of extracts of various plants are in the range of *T. cordifolia* ($100 \leq MIC \leq 250$ μg mL^{-1}); *T. cuneiformis* (stem: $150 \leq MIC \leq 300$ μg mL^{-1}; root: $125 \leq MIC \leq 250$ μg mL^{-1}; leaf: $50 \leq MIC \leq 200$ μg mL^{-1}) and *C. indica* ($150 \leq MIC \leq 400$ μg mL^{-1}).

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