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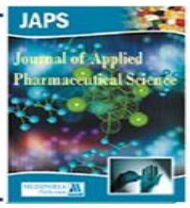

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Antibacterial activity of *Coccinia grandis* leaf extract on selective bacterial strains

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ABSTRACT

To assess the antibacterial activities of *Coccinia grandis* leaf extract on selective bacterial strains under *in-vitro* conditions. The antibacterial activity was tested against five bacterial strains by agar well diffusion method. The crude extract showed a broad spectrum of antibacterial activity by inhibiting both the gram positive and gram negative groups. The antibacterial activity of *C.grandis* leaf extract using solvents such as acetone, ethanol, methanol, aqueous and hexane was evaluated against five bacterial sp. Ethanol leaf extract of *C.grandis* showed high antibacterial activity against *S.aureus, B.cereus, E.coli, K.pneumoniae* and *S.pyogens.* Minimal inhibitory concentration of the leaf extract against each test organism was also studied by observing their growth on Mueller Hinton Agar containing the extract at various incremental levels, equivalent to 31.25µg/ml to 1000µg/ml of the extract. The highest activity was observed in ethanol extracts against *S.aureus, E.coli, and K.pneumoniae* with an inhibitory concentration below 31.5µg/ml. The significance of the study was conducted to investigate the *invitro* antibacterial activity of folklore medicinal plant and to evaluate scientific base of their applications.

Key words: Antibacterial, *Coccinia grandis*, Agar well diffusion, Minimal inhibitory concentration (MIC).

INTRODUCTION

The use of plants and plant products as medicines could be traced back from human civilization. The herbal wealth of India and the knowledge of their medicinal properties have a long tradition, as referred in Rig Veda and other ancient literature. The topography of India which is in the tropical belt with its varied climatic zones makes it a vast storehouse of medicinal plants. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use (Rojas *et al.*, 2003). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (mer ERT.RK1 *et al.*, 2006). Antimicrobials of plant origin have enormous therapeutic potential. The potential plant antimicrobials are combinations of the secondary metabolites such as alkaloids, steroids, resins, tanins, phenolics, flavonoids, steroids and fatty acids which have a potential effect on the physiological effects on the body.

Coccinia grandis L. (Family: *Cucurbitaceae*) is a climber herb cultivated throughout India. It is commonly known as Kovai in Tamil. In folklore medicine, the fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice and sore throats. It is also used as expectorant and astringent (Rastogi and Mehrota, 1993).

Phytochemical screening of *Coccinia grandis* reported the presence of saponins, cardenolides, flavonoids and polyphenols may be attributed to antibacterial activity. It provides the basis for further investigation on these plants to isolate active constituents and drug development. Phenolic compounds are generally noted for their antimicrobial activities (Evans, 1989). These phenolic constituents present in the exudates could then be responsible for its antibacterial effect. The leaves of the plant possess anti diabetic, anti-inflammatory, antipyretic, analgesic and antimicrobial properties (Asolkar *et al*., 1992; Nadkarni and Nadkarni, 1992). The leaves of the plant possess hepatoprotective effect (Vinoth kumar *et al.,* 2010).

MATERIALS AND METHODS

Plant material

The leaves of *Coccinia grandis* was collected during the month of January, (2011) from in and around Vellore District, Tamilnadu, India. The plant material was cleaned with distilled water and shade dried at room temperature. The plant material was authenticated and voucher specimens were kept at the Department of Zoology, C.Abdul Hakeem College, Melvisharam, Vellore Dt., Tamilnadu, India. The shade dried plant materials were powdered by using electric blender.

Preparation of extract

The herbal extract was prepared at the rate of 1g/5ml of solvent in a 250mL Erlenmeyer flasks with intermittent shaking. The flasks were closed with cotton plug and aluminum foil with intermittent shaking at 48 hours at room temperature, filtered through what man filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C).The extracts were preserved in airtight containers and kept at 4°C until further use.

Bacterial Strains

The following gram positive and gram negative bacterial species were used for *invitro* antibacterial studies; *E.coli* (MTCC 443), *Bacillus cereus* (MTCC 430), *Klebsiella Pneumoniae* (MTCC139), *Staphylococcus aureus* (MTCC 2940), *Streptococcus pyogenes* (MTCC 442). All the stock cultures were obtained from the Institute of Microbial Technology (IMTECH, Chandigarh, India).

Antibacterial assay

The antimicrobial activity of *C.grandis* extract was determined by agar well diffusion method against different bacteria as described by Okeke *et al*., 2001). In this method, pure isolate of each bacterium was sub cultured in nutrient broth at 37ºC for 24h. About 100Ml (10^6CFU/mL, standardized by 0.5 Mac-Farland) of each test bacterium was inoculated by pour plate method in sterile Muller-Hinton Agar plate (Hi Media, Mumbai, India) so as to achieve a confluent growth throughout the media. The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50µL volume of the extract was introduced in triplicate into wells of the inoculated Muller-Hinton Agar plates. Sterile DMSO served as negative control. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37ºC for 24h. The zone of inhibition was recorded to the nearest size in mm.

Determination of Minimum Inhibitory Concentration (MIC)

The method of Thongson *et al.,* 2004 was applied. The MIC for the crude extract was determined by agar-well diffusion method. In agar-well diffusion technique, a twofold serial dilution of the test extracts was prepared by first reconstituting it in dimethyl sulfoxide, then diluting it in sterile dimethyl sulfoxide only, to achieve a decreasing concentration range of 1000µg/mL to 31.25µg/mL. A 50µL volume of each dilution was added aseptically into Mueller Hinton agar plates that were already seeded with the standardized inoculums (10^6CFU/mL) of the test bacterial cells by spread plate method. Sterile dimethyl sulfoxide without herbal extract served as negative control. All the experiments were set in triplicate. The test plates were incubated at 37ºC for 24h. The lowest concentration of extract showing a clear zone of inhibition was considered as the MIC.

RESULTS AND DISCUSSION

In the present study the antibacterial activity of *Coccinia grandis* leaf extracts using solvents such as acetone, ethanol, methanol, aqueous and hexane was evaluated against five bacterial spp. (Table.1).

Table: 1 Antibacterial activity of *Coccinia grandis* by Agar well diffusion method.

Organisms	Zone of Inhibition (in mm)				
	Acetone	Ethanol	Methanol	Aqueous	Hexane
S. aureus	NA	13.2 ±0.30	13.8 ± 0.26	NA	NA
S. pyogenes	NA	9.90 ± 0.10	NA	6.23 ±0.25	12.2±0.26
B. cereus	6.96±0.15	11.13 ±0.32	4.86 ± 0.32	NA	12.86±0.32
E. coli	6 ± 0.10	14.83 ± 0.15	6.33 ± 0.41	NA	11.2 ± 0.43
K. pneumoniae	5.90± 0.36	13.13 ± 0.32	5.13 ± 0.32	5.33 ± 0.49	3.53±0.55

AE- Acetone, **ET** - Ethanol, **MT** –Methanol, **AQ**- Aqueous, **HE**- Hexane, **NA-**No activity.

Ethanol leaf extract of *Coccinia grandis* showed high antibacterial activity against *S.aureus, B.cereus, E.coli, K.pneumoniae* and *S.pyogens.*).The hexane leaf extracts showed high antibacterial activity against *B.cereus, E.coli, K.pneumoniae* and *S.pyogens.* Whereas, acetone, methanol and aqueous extracts showed intermediate activity against *S.aureus, B.cereus, E.coli, K.pneumoniae* and *S.pyogens*.

The minimal inhibitory concentration (Table.2) of ethanol extract was most potent against *S.aureus, E.coli, K.pneumoniae* at a concentration below 31.25µg/ml. The methanol and hexane extract was potent against *S.aureus, K.pneumoniae* at 31.25µg/ml. The least activity was recorded with the acetone, aqueous and hexane extracts against *s.aureus* and *B.cereus*. The most resistant organism was *S.pyogens* and *B.cereus* which had inhibitor concentration of more than 62.5µg/ml.

Table: 2 Minimum inhibitory concentration of *Coccinia grandis* leaf extract

Organisms	Acetone	Ethanol	Methanol	Aqueous	Hexane
S.aureus	>1000	31.25	31.25	>1000	>1000
S.pyogenes	>1000	125	>1000	500	62.5
B. cereus	500	62.5	250	>1000	62.5
E.coli	500	31.25	500	>1000	62.5
K.pneumoniae	500	31.25	500	500	31.25

Concentration expressed in µg/ml, Incubation temperature: 37ºC; Incubation period: 24h, Negative control- Dimethyl sulfoxide (DMSO).

The present study was conducted to investigate the *invitro* antibacterial activity of folklore medicinal plant used by people of India, to evaluate scientific base of their applications. The gram positive strains such as *S. aureus, B. cereus* and in gram negative strains such as *K.pneumoniae* causing serious infection in human and in other animals including superficial skin lesion, localized abscesses, and food poisoning were in first positions (Topley and Wilsons, 1998). In this study, the *C.grandis* exerted antibacterial activity against both Gram positive and Gram negative bacteria associated with different type of infections including pneumonia (*k. pneumoniae*), urinary tract infections (*S. aureus*) and wound infections. The demonstration of activity against both Gram positive and Gram negative bacteria is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections. Further investigations of its activity against a wider range of bacteria and fungi, identification and purification of its chemical constituents, and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

The *Coccinia grandis* plant was used for testing their antibacterial activity and showed high activity against those organisms such as *S.aureus, B.cereus,* and *K.pneumoniae.* These indicate that the herbal preparations could be used for preventing and treat the diseases caused by those selected organisms.

CONCLUSION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade (Patrick Ekong Ebong *et al.*, 2008). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Veeramuthu Duraipandiyan et *al*., 2006). According to World Health Report of Infectious diseases 2000; overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management. Plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address the therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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