PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Opuntia dillenii(Ker-Gawl)* AGAINST URINARY TRACT INFECTION CAUSING BACTERIA

*** Kavitha.V and Surya.S**

Post Graduate Department of Biochemistry, Marudhar Kesari Jain College for women, Vaniyambadi, vellore (Dt), Tamilnadu, India

Summary

Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The aim of the study was to investigate the bioactive chemical constituents and to evaluate the antimicrobial activity of the ethanolic extract of fruits skin of *Opuntia dillenii,* a traditionally used medicinal plant. The preliminary phytochemical screening of the fruits skin revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenol compounds, saponins and phytosteroids. Carbohydrate, protein and aminoacids, fixed oil and fats were absent. The antimicrobial studies of the extract were carried out on two clinical isolates *Staphylococcus aureus* and *Klebsiella Pneumoniae* using standard drug cefixime. 200 mg/ml in Dimethyl sulphoxide (DMSO) of the extract showed inhibitory activity with an inhibition zone of 10mm for *Staphylococcus aureus* and 10mm for *Klebsiella Pneumoniae*. The presence of above said phytochemicals might be responsible for these activities. The results suggest that the fruits skin contains bioactive constituents and its antimicrobial activity justifies its use in traditional medicine.

Keywords: *In vitro* Antimicrobial, *Opuntia dillenii*, Ethanolic Extract, Phytochemical Screening.

***Corresponding author:**
V.Kavitha. M.Sc, M.Phil.,
Lecturer
Post graduate Department of biochemistry
Marudhar Kesari Jain College for women,
Vaniyambadi, vellore (Dt), Tamilnadu, India

E-mail: **Kavitha827biocare@gmail.com**

Introduction

Plants have been used for the treatment of various diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs (1). Thus over 50% of these modern drugs are of natural products origin and as such these natural products play an important role in drug development in the pharmaceutical industry (2). Infectious diseases are the number one cause of death world-wide, and in tropical countries it accounts for approximately 50% of deaths. This may be due to poverty and increasing incidence of multiple drug resistance. Bacterial resistance to almost all antibacterial agents has been reported (3). This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used in treatment of infectious diseases. Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications, so there is urgent need to develop new antimicrobial agents that are very effective with minimal unwanted side effects, and higher plants represent a potential source of novel antibiotic prototypes (4).

Opuntia dillenii (Ker-Gawl) Haw is a cactus belonging to the family Opuntiae, which usually grows in semi-desert regions in the tropics and subtropics, including the Canary Islands. It is because of their edible fruit that they are known in vernacular as prickly pears. It has yellow flowers and red fruits. The red fruit contains high amounts of ascorbic acid. Canarian folk medicine has shown much evidence that the crude extract prepared from fruits of *O.dillenii* is useful in the treatment of gastrointestinal and liver disturbances (diabetes, hepatitis, intestinal spasm, etc.) (5,6). It is also used for treatment of cough, bronchial troubles and asthma (7). The fleshy leaf of the plant has been used externally against different types of inflamed wounds as a wound healer (5). However, the Antimicrobial activity of the fruits skin of *O. dillenii* has not been investigated. The objective of this research was to screen the phytochemical constituents and evaluate the potential of fruits skin extract onto cilinical isolates *S.aureus* and *K.pneumoniae,* which were isolated from patients with Urinary tract infection (UTI).

Material and methods

Plant material

The editable fruits of *O. dillenii* were collected in December 2008, at Thamalerimuthur village, Vellore(Dt),Tamil Nadu, India. They were brushed two minutes under distilled water with a nailbrush. The skin was removed from the fruit by peeling, cut up into small pieces. It was air-dried and made into powder using pestle and mortar.

Preparation of extract

Approximately 70 gm of air- dried powdered material was extracted with 80% ethanol using soxhlet apparatus; the solvent was removed in-vacuo to yeild a residue. The extract was referred as ethanolic extract (ODEE) and stored at 20°C in refrigerator until used.

Preliminary phytochemical screening

Phytochemical screening was carried out on the ethanolic extract for the qualitative determination of phytochemical constituents as described by Harborne, Trease and Evans, and Sofowora(8,9,10).

Preparation of sample

In the study of antimicrobial activity, the extract was dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in term of mg of extract per ml of solvent (mg/ml).

Isolation and identification of Bacteria

Fifteen urine samples from UTI patients were collected from different pathological laboratories in Vellore. For the isolation of UTI causing strains, loof full of urine sample was streaked on to nutrient agar plate and incubated at 37°C for 24 hours. Next day, the individual colonies were selected and identified on the basis of cultural, morphological and biochemical characteristics.

For the identification of gram negative Bacteria

To check morphological characteristics gram-staining, capsule staining and mortality test curve were performed. To check the growth pattern, different media including Mae conkey's agar No.3, Eosin methylene Blue agar (Bio Medical laboratories, USA) were used.

For the identification of gram positive Bacteria

Mac conkey's agar No.3, Nutrient agar, blood agar base supplemented with 5% sheep blood was used.

Maintenance of clinical Isolates

Stock culture was maintained in vials by growing the UTI isolates in 3ml nutrient broth and next day, culture was over layered with 3ml of 40% glycerol. Vials were treated at 70 °C.

Preliminary screening for antimicrobial activity – Paper Disc Method

The disc diffusion method was used to screen the antibacterial activity. Invitro antibacterial assay was screened by using Muller Hinton agar (MHA) obtained from Himedia, Mumbai, India. The MHA plates were prepared by pouring 15ml of molten media into sterile petridishes. The plates were allowed to solidify for 10 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculum was dried for 15 minutes. Sterile paper disc 6mm were soaked with ODEE juice at different concentrations (200 mg / ml, 100 mg/ml and 50 mg/ml). Each disc impregnated with different concentration of extract was dried at 37°C overnight. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. The antibiotic disc containing standard drug cefixime-5µg (Hi-Media-India) used as control. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the disc.

Results

Phytochemical screening

The results of the phytochemical screening of ODEE revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenol compounds, saponins and phytosteroids. Carbohydrate, protein and aminoacid, fixed oil and fats were absent. (Table1).

Antimicrobial Activity

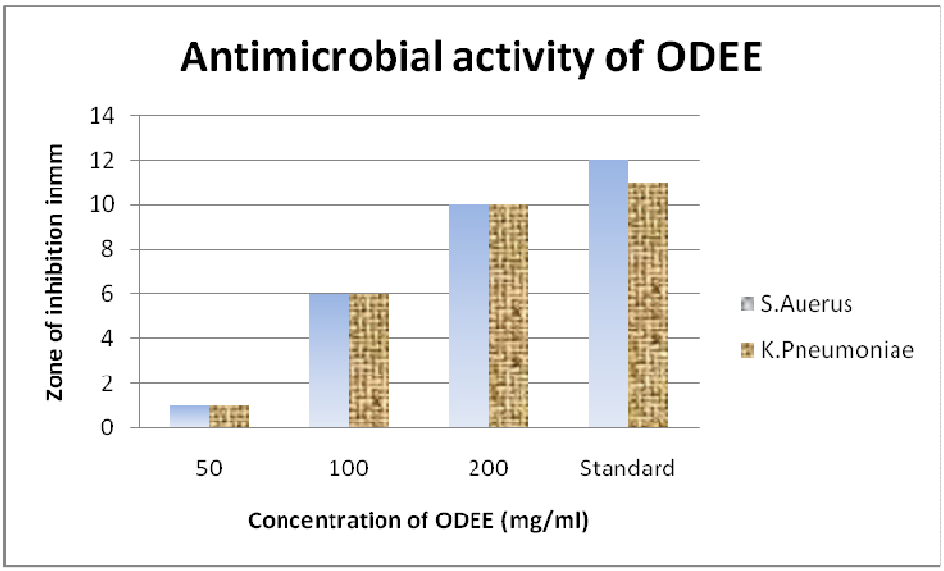
The antimicrobial activities of ODEE at different concentration against *S.aureus and K.pneumoniae* are shown in figure 1.The micro organisms screened were susceptible to 200 mg/ml ODEE with an inhibition zone of 10 mm for *S.aureus* and 10mm for *K.pneumoniae* respectively.

Table 1. Phytochemical constituents of the ODEE

S.No	Phytoconstituents	Ethanol Extract
1	Alkaloids	+
2	Carbohydrates	-
3	Glycosides	+
4	Phytosterols	+
5	Saponins	+
6	Fixed oils and fats	-
7	Tannin and phenolic compounds	+
8	Proteins and free amino acids	-
9	Gums and mucilage	-
10	Flavonoids	+

+ Indicates presence - Indicates absence

Figure 1. *In vitro* antimicrobial activity of ODEE against *Staphylococcus Aureus* and *Klebsiella Pneumoniae* at different concentration.



Discussion

All plant parts synthesize some chemicals in themselves which metabolize their physiological activities. These phytochemicals are used to cure the disease in herbal and homeopathic medicine. Nowadays, most of the people like to use the traditional methods to cure general diseases. The result of phytochemical screening of ODEE reveals the presence of secondary metabolites alkaloids, glycosides, flavonoids, tannins, phenol compounds, saponins and phytosteroids. These secondary metabolites have been reported to have antimicrobial activity (11).

Phytochemicals exert antimicrobial activity through different mechanisms; Tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes (12) in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (13), thus exhibiting antimicrobial activity. It has been reported that reviewed the biological activities of tannins and observed that tannins have remarkable activity in cancer prevention and anticancer, thus suggesting that medicinal plant has potentials as a source of important bioactive molecules for the treatment and prevention of cancer (14).In addition to its antimicrobial, anticancer activities, tannins have roles such as stable and potent antioxidants (15). The observations above support the use of *O.dillenii* in herbal cure remedies.

One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (16). In addition, alkaloids possess anti-inflammatory, anti-asthmatic and anti- anaphylactic properties with consequences of altered immunological status *in vivo* (17).

Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms (18). All these facts support the usefulness of fruits skin in folklore remedies and one of the reasons why this fruits is widely used for the treatment of many diseases.

The antimicrobial activity of ODEE was evaluated against two clinical isolates *K. pneumoniae,* a gram negative bacteria and *S.aureus,* a gram positive bacteria. Duguid et al(19) reported *S.aureus* as the causative agent of wide variety of disease of supporative infections such as boils and wound infections, superficial infection such as skin pustule, subcutaneous and sub-mucosa obscesses, osteomyelitis, bronchopneumonia and food poisoning, a common cause of vomiting and diarrhea . It is also the leading cause of UTI (20). *K.pnemoniae* is responsible for most urinary, gastrointestinal and respiratory tract infection and account for approximately 8% of all nosocomial infections. The klebsiella genus is notorious for causing many hospital acquired infections such as pneumonia, thrombophlebitis, urinary tract infection, upper respiratory tract infection and diarrhea.

In the present investigation, ODEE exhibited high antimicrobial activity at the concentration of 200 mg/ml with an inhibition zone of 10mm for *S. aureus* and 10mm for *K.pneumoniae*. The activity against both gram positive and gram negative bacteria afforded by ODEE may be attributed to the presence of secondary metabolites alkaloids, flavonoids and tannins and polyphenol present in the fruits skin.

Conclusion

From the result it can be concluded that fruit skin of *O.dillenii* has antimicrobial activity against *S.aureus and K.pneumoniae*, which are known to cause urinary and respiratory infections and other ailments. Therefore the results justify the use of the fruits skin in ethno medicine.

References

1. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., John Willey and Sons ltd., Ibadan, 1982: 8-14.
2. Jeyachandran R, Mahesh A. Antimicrobial evaluation of Kigelia Africana (Lam). Res. J. Microbiol 2007.2(8): 645-649.
3. Truiti M.C.T, Sarragiotto, M.H, Filho, B.A.A, Nakamura, C.V. and Filho, B.P.D. Invitro Antibacterial activity of a 7-O-β –Dglucopyronosyl- nutanocoumarin from Chaptalia nutans (Asteraceae) .Mem Inst Oswaldo Cruz, 2003: 98 (2):283-286
4. Maureer – Grimes B, Macbeth, D.L, Hallihan, B, and Delph S. Antimicrobial activity of medicinal plants of the scrophulariaceae and acanthaceae. Int. J. of pharmacognosy 1996: 34: 243 – 248

5. Bosh-Millares J, Siglo XVIII. La ciudad de Las Palmas en este siglo: otros remedios caseros en el siglo XVIII. In: Historia de la Medicina en Gran Canaria, vol. 1. Excmo. Cabildo Insular de Gran Canaria, Las Palmas 1967; 270– 271.

6. Loro, J.F. Estudios farmacolo´ gicos y toxicolo´ gicos de un extracto acuoso de frutos de *Opuntia dillenii* Haw. Masters Thesis, Faculty of Medicine, Universidad de Las Palmas, Las Palmas de Gran Canaria 1994.
7. Pe´rez de Paz, P.L., Medina, M, Cata´logo de Plantas Medicinales de la Flora Canaria (Aplicaciones Populares). Viceconsejerı´a de Cultura y Deportes del Gobierno de,1988.
8. Harborne JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London, 1973.
9. Trease GE, Evans WC. Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine. 1989. 13th Edition, Bailliere Tindall London.
10. Sofowora LA. Medicinal plants and traditional medicine in Africa.Spectrum Books Ltd, Ibaban 1993; 55-71.
11. Cowan M.M. Plant products as antimicrobial agents. Clinical Microbiology Review 1999; 12(4):564-582
12. Scalbert A. Antimicrobial properties of tannins. Phytochemistry 1991; 30:3875-3883.
13. Dharmananda S. Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of institute for Traditional Medicine,Portland, Oregon. 2003.
14. Li H, Wang Z, Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai.2003; 26(6): 444-448.
15. Trease GE, Evans WC. Textbook of Pharmacognosy, 12th edition (Balliere, Tindall, London), 1983: 343-383.
16. Nobori T, Miurak K, Wu DJ, et al. Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 1994; 368(6473): 753-756
17. Ganguly T, Sainis KB. Inhibition of cellular immune response by *Tylophora indica* In experimental models. Phytomed 2001 8(5): 348-355.
18. Hodek P, Trefil P, Stiborova M. Flavonoids-Potent and versatile biologically active compounds interacting with cytochrome P450. Chemico-Biol. Int 2002 ; 139(1): 1-21.
19. Duguid JP, Marmion BP, Swain RHA. Staphylococcus: in Mackie and McCartney Medical Microbiology 13th Ed. Longman group Ltd.U.K., 1978; 236-244.
20. Abdul F, Online AB. Bacterial isolate from the urine of women in Ilorin and their Antibiotic susceptibility patterns; J. Obst. Gynae 2001;18:16.