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Antibacterial Activity of *Cycas circinalis* Ovules - A Naked Seeded Gymnosperm

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ABSTRACT

Cycas circinalis L commonly known as Queen Sago a member of the cycad family, was investigated for antibacterial activity against important human pathogenic bacteria viz., *Bacillus cereus* (MTCC 1272), *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 7410) and important plant pathogenic bacteria *Xanthomonas axonapodis pv malvacearum* (Isolated from cotton plant). The ovule of *Cycas circinalis* L. has three layers. The outer most layer Sarcotesta, the middle layer Sclerotesta and the inner most layer endotesta. Endotesta is the edible part. All three different parts were separately extracted with water and successively with petroleum ether, chloroform and methanol. Each of these solvent extracts was subjected to antibacterial activity assay by agar cup diffusion method. Only methanol extract of Ovule-endotesta showed activity against all the test bacteria. The zone of inhibition against human pathogenic bacteria ranged between 14 mm to 18 mm. Comparison of the inhibitory activity of the extracts with the antibiotics Vancomycin and Penicillin against human pathogenic bacteria and Bacterimycin against plant pathogenic bacteria revealed that methanol extract of *Cycas circinalis* ovule was significantly higher than that of the antibiotics tested. The phytochemical analysis of the active extract revealed the presence of alkaloids, saponins and carbohydrates.

Keywords: *Cycas circinalis*, Ovule, Anti-bacterial Activity, Human and Plant Pathogenic Bacteria.

1. Introduction

The common view in the society and scientific community is that the natural agents are healthier, harmless and more reliable when compared to synthetic products. The World Health Organization (WHO) estimates that approximately 80% of the world populations presently use herbal medicine for some aspects of primary health care. Plant products play an important role in the health care systems of the remaining 20% of the population [1-2]. Herbal remedies are a part of Indian civilization. Nearly 70% of Indian population living in rural areas is dependent on plants for their primary health care (WHO, 2008). The search for newer sources of antibiotics is a global challenge which is preoccupying research institutions, pharmaceutical companies and academia. The development of resistance to most of the available synthetic drugs and the high costs of treatments has necessitated the search for alternative strategies, which will be useful in the development of new, safe, efficient, cost effective and eco-friendly ways of the management of pathogenic bacteria.

Phytochemicals of importance in human health care can be derived from different parts of the plant like bark, leaves, flowers, roots, fruits, seeds etc. Reports of many angiosperm plants possessing antimicrobial properties are available [3-5] however evaluation of gymnosperms for antibacterial property are very few, hence the present study. *Cycas circinalis*. L is a member of the cycad family. Cycad family plants are known to posses medicinal uses. One of five species of cycas folia has been reported for inhibition of cytochrome P-450 aromatase for use in the treatment of estrogen-dependent tumors [6]. Terminal shoots are considered as astringent and diuretic. Seeds are considered emmenagogue, expectorant. Cycad stems and seeds are known to be used for high blood pressure, headaches, congestion, rheumatism and bone pain.

2. Materials and methods

2.1 Collection of plant materials

Cycas circinalis L. ovules (Fig-1) were selected. The parts of the seeds i.e. sarcotesta, sclerotesta and female gametophyte (Endotesta) (Fig-2) were separated and washed thoroughly 2-3 times under running tap water and once with distilled water, air dried, homogenized to fine powder using waring blender and used for extraction.

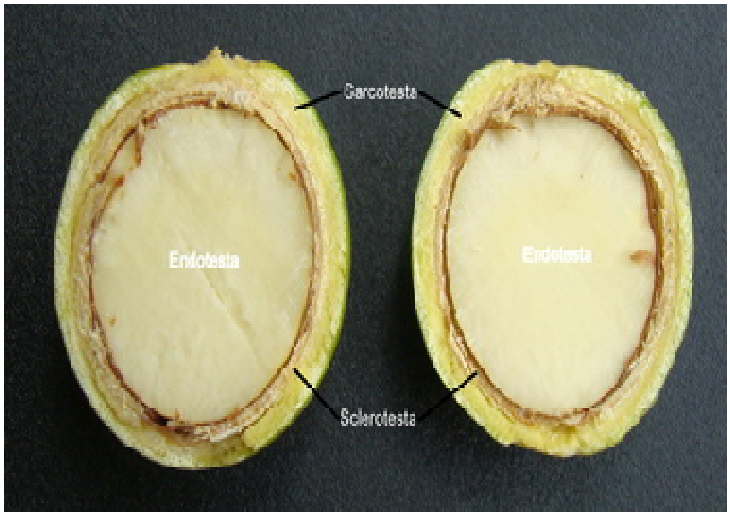

Fig 1: The cross section of *Cycas circinalis* ovule showing three regions.

Fig 2: Endotesta separated from ovule

2.2 Preparation of Extracts

2.2.1Aqueous extract

Ten grams of powdered sample were mixed with 50 ml of distilled water in conical flasks and kept on a water bath at boiling point for 15 min. They were first filtered through three layered muslin cloth, then centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120 ºC for 30 min. Extracts were preserved aseptically in brown bottles until use at 5 ºC.

2.2.2 Solvent Extract

Fifty grams of shade dried powder of each of the test plants were filled separately in the thimble and extracted successively with 200 ml each of petroleum ether, chloroform and methanol using Soxhlet extractor until clear colour is obtained. Each of the solvent extracts was concentrated separately under reduced pressure and the yield was calculated.[7] One gram of each of the solvent extract was first reconstituted in 9ml of their respective solvent and subjected to antimicrobial activity assay by agar well diffusion method.

2.3 Test Bacteria

Standard type cultures of human pathogenic bacteria viz., *Bacillus cereus* (MTCC 1272), *Staphylococcus aureus* (MTCC 7443), *Escherichia coli* (MTCC 7410) were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. *Xanthomonas axonopodis pv. malvacearum* a plant pathogenic bacteria was isolated from cotton plant served as test bacteria.

2.4 Antibacterial Activity Assay

Antibacterial activity was determined by agar well diffusion method [8]. Six mm diameter wells were made in sterile nutrient agar plate using sterile cork borer. Test bacterial inoculum containing 10^6 CFU/ml of test bacteria were spread uniformly on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl of aqueous and solvent extracts of four test plants were placed separately in the well. All the plates were incubated for 24 h. at 37 ºC and zone of inhibition if any around the wells was measured in mm. Four replicates were maintained for each treatment

2.5 Antibiotics

Ten mcg per disc of two positive controls i.e., Vancomycin and Penicillin were maintained against the human pathogenic bacteria and one 15 µl positive control of Bacterimycin for the plant pathogenic bacteria. Similarly, 50 µl of distilled water, petroleum ether, chloroform and methanol were maintained as a negative control.

3. Results & Discussion

3.1 Antibacterial Activity Assay

3.1.1 Aqueous Extract

Aqueous extract of all the test samples did not show any antibacterial activity against any of the test bacteria.

3.1.2 Solvent Extract

The yield of methanol extract of *Cycas circinalis* [Seed- Female gametophyte] and extract were 5.23 g. Data of the antibacterial activity of methanol extract of *Cycas circinalis* [Seed- Female gametophyte] against the test pathogenic bacteria are presented in Table- 1. Among the pathogenic bacteria *E. coli* was highly susceptible followed by *Staph. aureus*, *B. cereus* and *X. ax. pv. malvacerum.*

The Antibacterial activity of the antibiotics against the test bacteria is presented in Table-1. The inhibitory activity of methanol extract of *Cycas circinalis* [Seed- Female gametophyte] with that of synthetic antibiotics tested revealed that the methanol extract of *Cycas circinalis* [Seed- Female gametophyte] showed higher inhibitory activity than that of the test antibiotics.

Table 1: Antibacterial activity of solvent extract of *Cycas circinalis* L. and antibiotics against test bacteria.

Plant parts and antibiotics	Extract	*B. cereus*	*E. coli*	*Staph. aureus*	*X. ax.* pv. *Malvacerum*
		Zone of Inhibition in mm			
Sarcotesta	Petroleum ether	0.00	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00	0.00
Sclerotesta	Petroleum ether	0.00	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00	0.00
	Methanol	0.00	0.00	0.00	0.00
Endotesta	Petroleum ether	0.00	0.00	0.00	0.00
	Chloroform	0.00	0.00	0.00	0.00
	Methanol Extract	**14.67 ± 0.53**	**15.67 ± 1.53**	**18.33 ± 0.58**	**13.67 ± 0.58**
Antibiotics	Vancomycin	**10.34 ± 0.58**	**8.67 ± 0.53**	**13.67 ± 0.58**	-
	Penicillin	**15.67 ± 0.53**	**5.67 ± 0.58**	**11.34 ± 0.58**	-
	Bacterimycin	-	-	-	**21.67 ± 0.53**

*Values are the mean of three replicates ± standard deviation.

3.2 Phytochemical Analysis

The Phytochemical analysis [7] revealed the presence of alkaloids, saponins and carbohydrates in the endotesta of *Cycas circinalis*.

Parts of *Cycas circinalis* L seeds i.e. sarcotesta, sclerotesta and female gametophyte (Endotesta) were selected for in vitro antibacterial activity against three human pathogenic bacteria viz; *B. cereus*, *Staph. aureus*, *E. coli* and an important plant pathogenic bacteria X. ax. pv. malvacerum. Among the three parts screened, methanol extract of *C. circinali*s endotesta revealed significant antibacterial activity. The activity was observed against both plant pathogenic and human pathogenic test bacteria which included both gram positive and gram negative bacteria.

Saponins and Alkaloids are known to posses antibacterial potential property [9]. Phytochemical analysis reveals the presence of saponins and alkaloids which are reported to posses antibacterial activity suggesting that, the antibactirial property of endotesta of *C. circinalis* could be due to these compounds.

4. Conclusions

In the recent years, several reports are available on the antibacterial activity of plant extracts on human and plant pathogenic bacteria [10-13]. Reports on the evaluation of antimicrobial potential of gymnosperm plant *C. circinalis* are lacking. The present investigation has demonstrated the antibacterial potentiality of this naked seeded *Cycas circinalis* Ovule for the first time over human pathogenic bacteria belonging to both gram positive and gram negative bacteria and plant pathogenic gram negative bacteria Xanthomonas axonopodis pv. malvacearum. The results indicate that it is an important candidate plant for further work for isolation and characterization of the active principle.

5. Reference:

1. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD, Newman DJ, Sausville EA. International collaboration in drug discovery and development: the NCI experience. Pure Applied Chemistry, 1999; 71: 1619-1633.
2. Raskin L, Ribinicky DM, Komarnytsky S, Llin N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, Oneal JM, Cornwell T, Pastor L, Fridlender B. Plants and human health in the twenty-first century. Trends in Biotecnology, 2002; 20: 522-531.
3. Leven M, Vanen Berghe DA, Mertens F. Medicinal plants and its importance in antimicrobial activity. Planta Medica, 1979; 36: 311-321.
4. Erturk O, Demirbag Z. Scorzonare mollis Bieb (Compositae) bitkisinin antimikrobiyal aktivitesi. Ekoloji cevre Dergisi, 2003; 12: 27-31.
5. Mehlika Benli, Umit Bingol, Fatmagagul Geven, Kerim Giney, Nazife Yigit. An Investigation on the antimicrobial activity of some endemic plant species from Turkey. African Journal of Biotechnology, 2008; 7(1): 001-005.
6. Maria T, Kowalska, Yossef Itzhak, David Puett. Presence of aromatase inhibitors in cycads. Journal of Ethnopharmacology, 1995; 47(3):113–116.
7. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Champman and hall pub, London, UK, 1998, 3.
8. Perez C, Paul M, Bazerque P. An Antibiotic assay by the agar well diffusion method. Acta Biologica Medica, 1990; 15: 113-115.
9. Benziane Maatalah M, Kambuche Bouzidi N, Bellahouel S, Merah B, Fortas Z, Soulimani R, Saidi S, Derdour A. Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulate*. Journal of Biotechnology and Pharmaceutical Research, 2012; 3(3): 54-57.
10. Pietro RCLR, Kashima S, Sato DN, Januario AH, Franca SC. In vitro antimycobacterial activities of *Physalis angulata* L. Phytomedicine, 2000; 7: 335-338.
11. Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India. Journal of Ethnopharmacology, 2000; 69: 63-71.
12. Britto SJ, Senthikumar S. Antibacterial activity of *Solanum incanum* L. leaf extracts. Asian Journal of Microbiology Biotechnology and Environmental Science, 2001; 3: 65- 66.
13. Bylka W, Hajdrych MS, Matalawska I, Goslinka O. Antibacterial activity of Isocytisoside and extracts of *Aquilegia vulgaris* L. Letters in Applied Microbiology, 2004; 39: 93- 97.