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PREPARATION AND EXTRACTION OF ANTIBIOTIC FROM OVULE OF *CYCAS REVOLUTA*

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ABSTRACT

Cycas revoluta basically belongs from member of cycad family. Cycas carcinalis was identified having antibacterial property against human pathogenic bacteria like . E.coli, Pseudomonas, Staphlyococcus aureus, Bacillus cerius. According to some source of information every part of the gymnosperm plant have antibacterial properties. This is a native plant of Southern Japan including Ryukyu island. The Cycas revoluta has three layers the exterior layer is called Sarcotesta. The middle layer is called Sclerotesta and the internal layer is called Endotesta. The various parts of the the plant is extracted with distil water, petroleum ether, methanol, ethanol, chloroform. All the extraction of various layers of the plant shows the antibacterial property aginst various human pathogenic bacteria (E.coli, Pseudomonas, Staphlyococcus aureus, Bacillus cerius). The zone of inhibition obtained in the antibacterial assay ranges from 15 to 28. A comparative analysis of inhibition activity of the extracts with antibiotics like Vancomycin and Penicillin against human pathogenic bacteria was made for testing of the higher antibacterial property.

Keywords : *Cycas revoluta Ovule, Anti-bacterial Activity, Human Pathogenic Bacteria*

INTRODUCTION

The trending scenario in the scientific society is that natural compounds are more beneficial, healthier, non toxic and have more efficacy when compared to the artificial compounds(synthetic). According to the current estimation of World Health Organization about 80- 85 % of the world total population have dependency on the herbal medicine because of its healthy effects. Plant derived products or herbal products play a crucial role in the health care management of remaining 15-20% of the population [1],[2]. Cycads are naturally occurring variety of plant which have a mono evolutionary histological at both molecular as well as morphological level [3], [4].The scientists are still confused regarding the ancestral background of the cycads and we also don't their relationship with other families but it is quite well proved that they belong to ancient group of plants**.** Some changes in cycad taxonomy , member of the IUCN / SSC cycad main group have regularly published a world list of cycad provide on regular record of all accepting / recognize species . The first list was published in the Journal of the cycas society of South Africa [5].

Ayurvedic &herbal are component of Indian society and Indian civilization. Because just we talk about 1000 year ago our ancestral were very knowledgeable regarding the herbal and ayurvedic medicine. Around 72-75% of Indian population & society living in rural areas and they are totally dependent on plants directly & indirectly for there primary health care [6]. The alternative search

newer sources of antibiotic is a global challenge which is fulfilment by many research institution pharmaceutical companies & academia. The development of resistance to most of the available synthetic drugs and the high cost treatment have necessitated the search for alternative strategy, which is basically used for in the development of new, safe, efficient & cost fluctuating strategy which play important role in the development of new , unknown, safe, efficient, cost feasible & ecofriendly ways of the management of human & plant pathogenic bacteria.The importance of phyochemical in human healthcare can be derived from different part of plant body a like bark, stem, ovule ,seed, flower, fruits & every parts of plant. Many researchers find out that gymnosperm plant having antimicrobial properties against human pathogenic & plant pathogenic bacteria[7], [8], [9]. Basically evolution of gymnosperm for antibacterial property against human pathogenic bacteria are very few in numbers. Also cycad family plants are known to possess medicinal uses [10].

MATERIAL & METHODS

Collection of material.

The healthy plant (ovule) material was collected from Gautam buddha University Uttar Pradesh , India. The ovule of plant was thoroughly washed by potable water then it was kept in sunlight for 10-15 days for drying and after this process the dried material was crushed with help of mixer grinder and powdered form of ovule was obtained.

Fig . 1 Showing the cycas plant and its ovule and the powdered form

Source : Gautam Buddha University

Preparation of extracts.

Ten grams of powdered sample were mixed with 50 ml of distilled water, petroleum ether, methanol (80%, 90%, 100%), ethanol(80%, 90%, 100%) chloroform (80%, 90%, 100%) ,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.Solvent extract was satutared separately under lower pressure and then the yield was calculated[11]. 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 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. 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.

ANTIBACTERIAL ASSAY

Standard cultures of ***Escherichia coli*** (MTCC 1695), ***Staphylococcus aureus*** (MTCC *102) and ***Bacillus subtilis*** (MTCC 441) were collected from Microbial Type Culture Collection & Gene Bank (MTCC), Gautam Buddha university, Greater Noida (U.P).

Escherichia coli was revived in LB broth at 37^0C with a incubation period of 24 hrs, *Bacillus subtilis* were revived in nutrient broth at 30^0C with a incubation period of 24 hrs while *Staphylococcus* were revived in nutrient broth at 25^0C with a incubation period of 48 hrs. LB Agar and Nutrient Agar media were prepared as per manufacturer's instruction, autoclaved for 20 min at 121^0C and finally poured into sterilized petri plates. Paper discs of 5 mm diameter were prepared from whatman filter paper and autoclaved. 200µl of each bacterial strain were plated on their respective media plates. The dried extracts were further processed by dissolving 1g of the concentrates obtained from different solvents in 100 ml of autoclaved distilled water making a

final concentration of 10 mg mL^{-1}. The sterile paper discs were saturated with 10µl of the test compound, allowed to dry and finally introduced on the upper layer of the agar plates. The test plates were incubated according to their respective temperature for 24 - 48 hrs. The experiments were done in quadruplicates and the averages of the diameter of zone of inhibition (IZD) were measured at the end of incubation period. Similar tests were also carried with standard drug Chloramphenicol at 10 mg mL^{-1} concentration. Autoclaved distilled water was used as control.

RESULTS:

Plant parts	**Zone of inhibition**				
	Exract	E.coli	Pseudomonas.	Staph.aureus	B.cerius
Endotesta	P.ether	0.0	0.0	0.0	
Endotesta	Chloroform	0.0	0.0	0.0	
Endotesta	Methanol(100 %)	18.0	27.0	21.0	26.0
	Methanol(90 %)	17.0	18.0	20.0	20.0
	Methanol(80%)	19.0	19.0	18.0	20.0

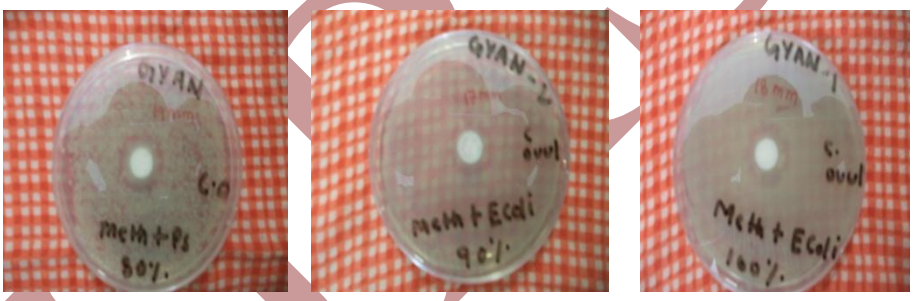

Fig 2 .Antibacterial activity of Ovules extracts(methanol)of cycas circinalis against *E.coli.*

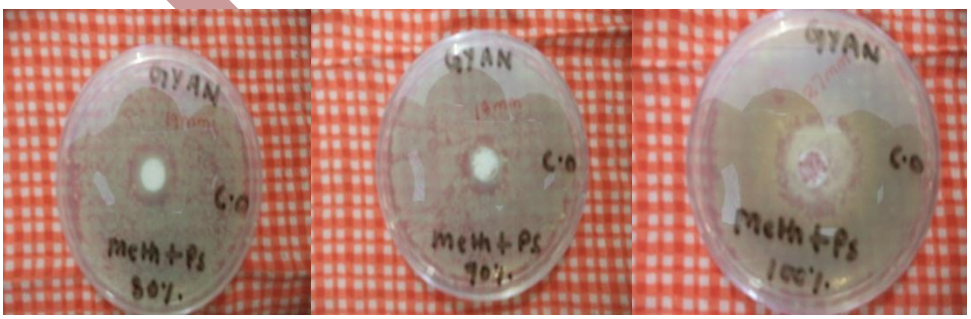

Fig 3 : Antibacterial activity of Ovules extracts(methanol)of cycas circinalis against *Pseudomonas*

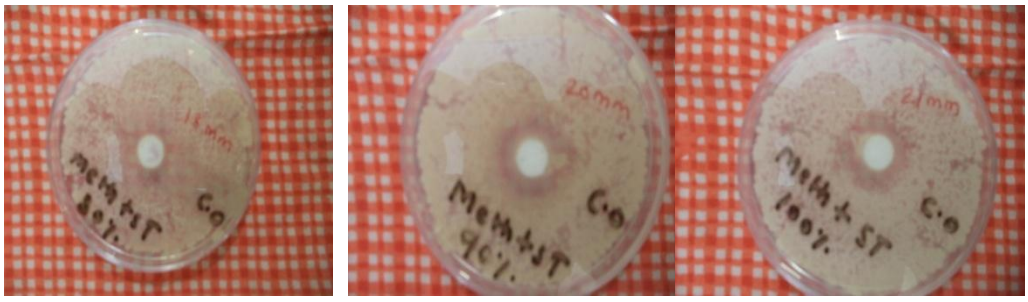

Fig 4 :Antibacterial activity of Ovules extracts(methanol)of cycas circinalis against *Staph. Aureus*

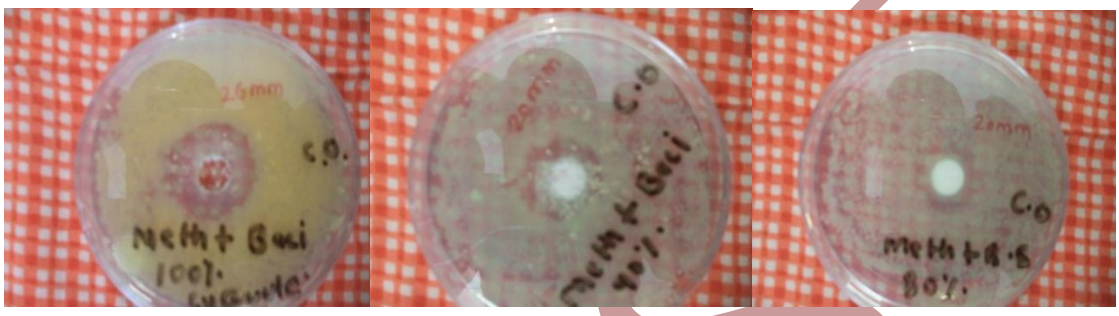

Fig 5: Antibacterial activity of Ovules extracts(methanol)of cycas circinalis against *B. cereus.*

CONCLUSION

From the results of antibacterial screening of four solvents (petroleum ether, chloroform, ethanol and water) used in this study the best antibacterial property was found in the case of 100% methanolic extract (100% methanol+Ovule) against various pathogenic bacteria. This potential extract (methanolic) property could be further utilize in the areas of pharmalogical research by implementation of different techniques and methods

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