



Antimicrobial And Qualitative Phytochemical Screening Of *Cycas Beddomei* Leaf Extracts

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Abstract - *Cycas beddomei* Dyer., (Cycadaceae) is a Gymnosperm of only living genus of Cycadaceae, enlisted as Critically Endangered (C.E) under IUCN Status of Red listed with confined (endemic) to Seshachalam Hill Ranges of Eastern Ghats. It is one of the medicinal plant with proven therapeutic studies against the specific selected bacteria is equal to that of the herbal uses of *C. beddomei* male and female cones as aphrodisiac, skin diseases, mosquito repellent, general debility, rheumatoid arthritis, wound healing, antidiabetic, antidiarrheal and tuberculosis. However, the leaves are not yet reported for the biological and phytochemical studies. Hence, the present study focused and on the determination of the *in vitro* antimicrobial properties of leaf extracts using organic solvents and aqueous extracts. The ethanol and ethyl acetate extract of leaf exhibited maximum inhibition, while petroleum ether expressed moderate activity. Water extract failed to inhibit the test organisms. The preliminary phytochemical studies also attempted revealed for the presence of important groups of secondary metabolites. The present study revealed the potential inhibition properties against the selected bacteria and one fungal strain.

Index Terms: *Cycas beddomei*; Endemic plant; Antimicrobial activity, Preliminary or qualitative phytochemical screening

Introduction

About Plant

Cycas beddomei Dyer (Cycadaceae) is an endemic medicinal plant, restricted to Seshachalam hill range of Eastern Ghats (1). It is an endemic, endangered and CITES (Appendix I) listed species from India's Southern Eastern Ghats. The species is known by multiple local names, "Peritha", "Kondaitha", "Erriitha" (for being similar in appearance to its associate "Phoenix"); "Srivari keeritamu" (about the male cones similarity to Lord Balaji crown) and *Madhana Kamakshi* (for its assumed aphrodisiac properties). It was described from the Cuddapah hills and later its presence was reported from the Tirumala hills. Rao & al. (2015) stated that the species had distributed outside the Seshachalam hills in the Velikonda hills of the

Nellore district. Its presence is now recorded in Chitivel hills of Cuddapah district and Penchalakona hill ranges of the Nellore district (Nethaji et al., 2022).

Common name

Beddome's Cycas (English); Peritha, Madanakamakshi and Kondaeetha (Telugu).

Common English names: Beddome's cycas, Cicas di Beddome.

Vernacular names: Tel.: Perita, Madhana - Kamakshi.

Trade name: Andhra Pradesh Cycas, Cycad.

Scientific classification

Kingdom: Plantae

Clade: Tracheophytes

Division: Cycadophyta

Class: Cycadopsida

Order: Cycadales

Family: Cycadaceae

Genus: Cycas

Species: *C. beddomei*

Binomial name: *Cycas beddomei* Dyer

Distribution, habitat and Ecology

This species is the global endemic of Seshachalam hills (formerly called as Tirupati-Kadapa Hills) and the most recently from Velikonda Hills covering Chittoor, Kadapa and Nellore districts of the Southern Eastern Ghats of Andhra Pradesh. This species is recorded only from the also known as the Seshachalam hills naturally. The occurrence of species belongs to dry, open hill slopes, in open grassy woodland or grass dominated forested hot sites in the scrublands at an altitude between 500–1165 m. Whereas, isolated trees are also recorded from the range of 500–600 m altitude. The entire area of distribution should probably be considered as a single locality due to the connection between the blocks as a single event such as a large fire, disease or pest outbreak could impact the entire population. These plants are fairly fire resistant, except as seeds and seedlings, which are very vulnerable to annual grass fires. It grows in an area with annual rainfall of 570–1,230 mm. Plants grow on skeletal soils, mainly in dry deciduous mixed type forest with patches of moist deciduous forest. The species is mostly restricted to the top slopes and predominantly in black soils. Common associate species are: *Phoenix humilis*, *Terminalia pallida*, *Syzygium alternifolium*, *Pimpinella tirupatiensis*, *Curculigo orchoides*, *Cymbopogon* spp. and *Dechaschistia* spp. Reproductive period ranges from the month of April–August.

Botanical description

Superficially, *Cycas beddomei* (Fig.1) has erect, solitary stems similar to *Cycas revoluta*. It has a crown like appearance decorated with 20-30 leaves of 90 cm long, stiff, lanceolate, pinnate, with 50-100 pairs of leaflets. Shrubs to 2 m high, dioecious; bark brown, exfoliating in rectangular scales 70–120 cm long

Stem: arborescent, appear like a small palm with a distinct trunk of up to 1.5m high. It is covered with the remnants of leaf bases.

Leaves: grey-green, dull, 90 cm long, flat (not keeled) in section (opposing leaflets inserted at 180° on rachis), tomentum shedding as leaf expands. Petiole 15 cm long, glabrous, spinescent. Basal leaflets not gradually reducing to spines. Leaves up to 1 m long;

Median leaflets: simple, strongly discolourous, 100-175 mm long, 3-4 mm wide; section slightly keeled; margins revolute; apex acute or aristate, spinescent; midrib raised above, flat below.

Cataphylls: narrowly triangular, soft, thinly sericeous or lacking tomentum, persistent.

Pollen cones: narrowly ovoid, orange, 30 cm long, 7.5 cm diam.; microsporophyll lamina firm, not dorsiventrally thickened, apical spine prominent, gradually raised.

Microsporophyll: oblong, deltoid, tapering, acuminate at apex, lower erect, upper strongly recurved. microsporophyll with an apical spine up to 3cm long.

Megasporophylls: 15-20 cm long, brown-tomentose; ovules 2, glabrous; lamina lanceolate, 75 mm long, 25 mm wide, regularly dentate, with 10-16 pungent lateral spines, apical spine distinct from lateral spines. Ovate-lanceolate, grow up to 4x2 cm and with pectinate margins; ovules usually 2-4, occasionally 6–8, inserted above the middle of the stalk, up to 4cm across.

Seeds: globose flattened-ovoid, 38 mm long, 34 mm wide; sarcotesta yellow; fibrous layer present; sclerotesta smooth. Spongy endocarp absent with fibrous sarcotesta, up to 5×5 cm, green, turning yellow on maturity.

Male cone: oblong-ovoid, up to 35 x 16 cm, orange, with a short peduncle; Petioles up to 15-20cm long, with minute spines on upper portion, base clothed with tufted tomentum.

Leaflets: narrow, linear, 10–18 x 0.2–0.4 cm, margins revolute, apex pointed.

Rachis: quadrangular

Kundansing R. Jadhao (2020)

Edible Uses

Cycads have often been used traditionally as a source of food starch, obtained either from the seeds or from the stems, and it is probable that the seed kernels and stem pith of all cycads can be used as food after treatment to remove any toxic principle that may be present. The starch would appear to be of particular importance as a source of sustenance during hard times or in areas where the food supply is naturally limited. The starch-rich seeds can be used for food. The starch-rich pith from the stem is eaten. The small size of the stems discourages the use of this species for food.

(<https://tropical.theferns.info/viewtropical.php?id=Cycas+beddomei>)

Medicinal Properties

The young male cones are used in ayurvedic medicine. The male cones are known as 'per ita', and are supposed to have a cooling effect on the body when prepared in a dried and sugared form. They are also used to treat rheumatism and muscular pains. The pith from the stems is used as a treatment in the case of stomach pains and debility.

(<https://tropical.ferns.info/viewtropical.php?id=Cycas+beddomei>)

C. beddomei Bark paste is used to cure boils and skin diseases by the local herbalists. Male cones used to cure arthritis. Male and Female cones used to increase sexual potency (aphrodisiac), and also used as an ideal food supplement and rejuvenator. It is also helped to cure diabetes. Pith is used in case of debility. Pith flour along with rice flour used to prepare traditional food for young couple. *C. beddomei* plant parts used against various ailments like rheumatism, skin diseases, muscle pains, ulcers, acidity, aphrodisiac, rejuvenator, worm infestations and constipation by the local herbalists. Fresh mature male cone grinded with ghee and gingelly oil the paste is banded on the swelling parts of arthritis and inflammation in experimental male arthritis rats (Alekhya and Yasodamma 2016). Alekhya et al., (2013) demonstrated antimicrobial activity of male cones along with its groups of phytochemicals.

Plant used in ethno-medicine for different ailments like cough, stomach ache, diarrhea, gastric troubles and abortifacient (2).

Chemical Constituents:

Secondary metabolites from the cones of *Cycas beddomei* has reported by Das et al., (2005), namely 2,3-dihydro-4"-O-methyl amentoflavone, along with 2,3,2",3"-tetrahydro hinokiflavone, 2,3,2",3"-tetrahydro amentoflavone, 2,3-dihydro amentoflavone (biflavonoids). 7,7"-di-O-methyltetrahydrohinokiflavone together with tetrahydrohinokiflavone were isolated from the stems (Jayaprakasam et al., 2000).

Previously isolated class of constituents Flavonoids (3, 4, 5).

Material and Methods

The plant material (leaf) was collected from Tirumala hills and identified specimen was deposited at S.K. University herbarium (SKU), Anantapur.



Fig.: *Cycas beddomei* India: Andhra Pradesh; Attribution: JS Khuraijam; License: [CC BY-NC-SA 4.0](#);

Outing or collection (Brahms) Code: 1549; Date: 27 November 2014

Image code: [3495](#)

Tested material

The leaves were cut into small pieces and shade dried. The shade dried material was powdered (100g) and successively extracted with petroleum ether, ethyl acetate, ethanol and water using a Soxhlet apparatus. The extracts were distilled under reduced pressure to give crude samples. Crude extracts were subjected to preliminary phytochemical studies (6, 7).

Antimicrobial studies

Studied activity

Antimicrobial activity by disc diffusion method (8, 9), and determination of minimum inhibitory concentration (MIC) (10, 11).

Used microorganisms

The microbial strains viz., *Bacillus cereus* MTCC 1429, *Staphylococcus aureus* MTCC 737, *Escherichia coli* MTTC 1687, *Pseudomonas aeruginosa* MTTC1688, *Klebsiella pneumoniae* MTCC 109, *Micrococcus luteus* MTCC 2522 and yeast, *Candida albicans* MTTC 183, were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India, were used in the study.

Results

Cycas beddomei leaf petroleum ether, ethyl acetate and ethanol extracts were subjected to qualitative phytochemical screening and antimicrobial studies using disc diffusion method and MIC using broth dilution methods. The preliminary phytochemical analysis of extracts revealed the presence of alkaloids, flavonoids and steroids in three extracts (Table 1). Results on antimicrobial studies showed that petroleum ether extract showed very feeble activity against the tested pathogens. Ethyl acetate extract at higher concentration showed moderate activity. Ethanol extract exhibited concentration dependent activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* *Candida albicans* (Table 2). Minimum concentration of the extracts required to inhibit the pathogens are tabulated (Table 3). It revealed that ethanol extract strongly inhibited the tested pathogens followed by ethyl acetate and petroleum ether extracts.

Table1. Preliminary phytochemical screening of *Cycas beddomei* leaves

Components	Extracts		
	PE	EA	ET
Alkaloids	+	+	+
Anthracene glycosides	-	+	+
Anthrax quinines	-	-	+
Emodins	-	-	+
Flavonoids	+	+	++
Tannins	-	-	-
Steroids	+	+	+

Extracts: PE: Petroleum ether; EA: Ethyl acetate; ET: Ethyl acetate

Phytochemical tests: ++: quantitative; +: positive; -: negative

Table 2. Antimicrobial activity of *Cycas beddomei* leaf extracts

Extracts	Concentration µg/disc	Micro organisms						
		<i>B.c.</i>	<i>S.a.</i>	<i>M.l.</i>	<i>E.c.</i>	<i>P.s.</i>	<i>K.p.</i>	<i>C.a</i>
Petroleum ether	A	7	-	-	7	-	-	-
	B	7	8	-	8	7	9	8
Ethyl Acetate	A	8	7	-	8	8	-	7
	B	10	11	8	10	10	-	9
Ethanol	A	8	7	-	9	10	-	10
	B	10	14	10	12	12	12	13
*Standards	30	22 ^A	23 ^K	36 ^T	22 ^T	28 ^T	23 ^T	25 ^V

Organisms: *B.c*: *Bacillus cereus*; *S.a*: *Staphylococcus aureus*; *M.l*: *Micrococcus luteus*; *E.c*: *Escherichia coli*; *P.s*: *Pseudomonas aeruginosa*; *K.p*: *Klebsiella pneumoniae*; *C.a*: *Candida albicans*. Concentration: A: 250 µg/disc; B: 500 µg/disc; -: Not active

*Standards: A: ampicillin; K: kanamycin; T: tetracycline; V: vancomycin

Table 3. Minimum inhibitory concentrations (µg/ml) of *Cycas beddomei* leaf extracts

Extracts	Micro organisms						
	<i>B.c.</i>	<i>S.a.</i>	<i>M.l.</i>	<i>E.c.</i>	<i>P.s.</i>	<i>K.p.</i>	<i>C.a</i>
Petroleum ether	625	625	1250	625	625	625	1250
Ethyl Acetate	312	312	625	625	625	1250	1250
Ethanol	312	156	312	312	312	312	625

Micro organisms: *B.c*: *Bacillus cereus*; *S.a*: *Staphylococcus aureus*; *M.l*: *Micrococcus luteus*; *E.c*: *Escherichia coli*; *P.s*: *Pseudomonas aeruginosa*; *K.p*: *Klebsiella pneumoniae*; *C.a*: *Candida albicans*.

Discussion

The genus *Cycas* belongs to the family Cycadaceae, contains about 117 species, native to tropical and sub-tropical regions of the world. It grows in woodlands, slopes of drier mountains (Afifi et al., 2021) (12). *C. beddomei* is the global endemic of Seshachalam hills (formerly called as Tirupati-Kadapa Hills) and the most recently from Velikonda Hills covering Chittoor, Kadapa and Nellore districts of the Southern Eastern Ghats of Andhra Pradesh. Scientifically *C. beddomei* has been reported for antimicrobial (Alekhya et al., 2012) (13), anthelmintic (Alekhya et al., 2013a) (14), antibacterial (Alekhya et al., 2013b) (15) anti-inflammatory (Alekhya et al., 2014) (16), antioxidant (Mahendra Nath et al., 2014 and David and Selvaraajan, 2015) (17,18), analgesic and anti-arthritic activity (Alekhya et al., 2016) (19) and anticancer (Hari et al., 2022) (20). Very few and sporadic reports were noticed on antimicrobial activity of *C. beddomei*. The present work is initiated to evaluate the antimicrobial activity of petroleum ether, ethyl acetate and ethanol extracts of *C. beddomei* leaves.

Qualitative phytochemical analysis of petroleum ether, ethyl acetate and ethanol extracts of *C. beddomei* leaves showed the presence of seven types of components i.e. alkaloids, anthracene glycosides, anthrax quinones, emodins, flavonoids, tannins and steroids. Among the tested extracts ethanol extract showed positive results for six components. This may be due to the more components extractable capacity of ethanol. *In vitro* antimicrobial study results revealed that ethyl acetate and ethanol extract showed moderate to strong antimicrobial activity against the tested pathogens. Whereas petroleum ether extract exhibited very feeble activity. *Staphylococcus aureus* is a skin infection causing organism, moderately inhibited (11 mm) by ethyl acetate extract and strongly inhibited (14mm) by ethanol extract followed by *Candida albicans* (13 mm) *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (12mm each). Extracts and bioflavonoids of *Cycas* species have been reported as antimicrobial agents (Hosam et al.,

2023; Moawad et al., 2010) (21, 22). Biflavoloids isolated from *C. beddomei* leaves may be responsible for antibacterial activity of petroleum ether, ethyl acetate and ethanol extracts.

Conclusions

Among the tested extracts ethyl acetate and ethanol extracts exhibited broad spectrum of antimicrobial activity against tested pathogens. The phytochemical reports suggested that the antimicrobial activity was mainly due to the presence of flavonoids and other natural phenolic compounds (12). Phenolic components were reported for antioxidative, antidiabetic, anticarcinogenic, antimicrobial, anti allergic, anti mutagenic and anti-inflammatory activities (13, 14). Based on results obtained, the traditional uses of *Cycas beddomei* for the treatment of infectious diseases is promising, mainly against bacteria and yeast.

Acknowledgements

The authors are thankful to the University Grants Commission, New Delhi for financial assistance.

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