molecular studies/DNA barcoding to resolve taxonomic issues which go beyond morphological appraisal in Indian cycads.

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Citrus macroptera Montrouz var. *annamensis* Tanaka: a potential nutraceutical for ethno-fishery

In the northern part of Tripura, various aspects of ethno-fishery have been documented. Fruit peels of *Citrus macroptera* Montrouz. var. *annamensis* Tanaka are often used as nutraceuticals for indigenous fishery along with various botanicals, viz. leafy vegetables, fronds of ferns, fruits and seeds (Figure 1). During documentation of ethno-fishery practices, some garland-like structures were found floating on the aquatic bodies (Figure 2). This was a practice against rot diseases of fishes. The observation and documentation persisted for 0–60 days after the botanicals were applied as garland-like structures. Two different seasons were chosen when maximum disease outbreak was reported.

After a time-interval of 60 days, the garland-like structures had almost vanished, indicating that they were totally consumed by the fishes. This practice is useful and beneficial for major and minor Indian carps; it is also cost-effective. The plants used were *Alternanthera philoxeroides* Griseb., *Monochoria hastata* (L.) Solms, *Bryophyllum pinnatum* (Lam.) Oken, along with the major part of the garland-like structure containing fruit peels of *C. macroptera* Montrouz. var. *annamensis* Tanaka. Ethnic people sliced them and picked up with fine bamboo needle to sew them together into a garland-like structure. The sewing was done perfectly using fine jute rope

Figure 1. Fresh fruits of *Citrus macroptera* Montrouz. var. *annamensis* Tanaka.

Figure 2. Garland of *Alternanthera philoxeroides*, *Monochoria hastata, Bryophyllum pinnatum* and *C. macroptera* Montrouz. var. *annamensis* Tanaka floating on local aquatic body (scale bar = 25 cm).

Table 1. Nutraceuticals of *Citrus macroptera* Montrouz. var. *annamensis* Tanaka

Nutraceutical	Amount	Reference for analytical procedures
Total dietary fibre (TDF)*	0.5 g	14
Insoluble dietary fibre (IDF)	0.4 g	14
Soluble dietary fibre (SDF)	0.1 g	14
Moisture	40.0 g	15
Total protein	1.5 g	16
Free fatty acids	1.0 g	17
Minerals	0.7 g	18
Calcium	77 mg	18
Phosphorus	21 mg	18
Iron	0.7 mg	18
Magnesium	17 mg	18
Potassium	280 mg	18
Copper	0.07 mg	18
Manganese	0.08 mg	18
Zinc	0.06 mg	18
Cromium	0.007 mg	18
Nitrogen	0.24 g	19
Total free amino acids	0.8 g	20
Methionine	50 mg/g N	17
Tryptophan	100 mg/g N	17
Lysine	600 mg/g N	17
Oxalic acid	4 mg	21
Ash	7.71 g	18
Carotenoids	12 mg	22
Volatile oils	4%	22
Alkaloids	0.35 mg	22
Tannins	0.04 mg	22
Saponins	0.34 mg	23, 24
Total phenolic content	0.10 mg	22
Flavonoids	0.30 mg	22
Anthocyanins	10 mg	22
Total carotene	14 µg	17
Thiamine	0.03 mg	17
Riboflavin	0.05 mg	17
Niacin	0.07 mg	17
Ascorbic acid	50 mg	17
Crude fibre	1.4 g	17
Carbohydrates	40.5 g	25

*All values are per 100 g of edible fruit part (n = 3).

covered by *A. philoxeroides* hollow twigs. They were often thrown in the middle of the pond and number of garland-like structures varied depending on the size of the pond and population of the fishes present.

For experimental evidence of the nutraceutical property of aqueous extract, fresh fruits were collected, washed, shed-dried and ground to powder using a mechanical grinder. One hundred grams of powder was taken in equal volume of distilled water. Micro syringe filter was used for filtering the fruit extract to obtain bacteria free extracts and preserved at 4°C for analysis of different nutraceuticals[1–3]. The fruits were subjected to hydrodistillation for isolation of volatile oils and dried with anhydrous Na_2SO_4. Fruit peels of *C. macroptera* Montrouz. var. *annamensis* Tanaka are a rich source of nutraceuticals (Table 1), antioxidants[1] and secondary metabolites[4–7].

For antimicrobial property, fungal-infected samples of *Cyprinus carpio* var. *communis* were collected from the local aquatic bodies. Infected tissues were separated aseptically and sterilized double-distilled water was used for homogenization in an aseptic mortar[1,2]. Isolation of a piece of fungal mycelium was done in aseptic condition, followed by rinsing with sterilized double-distilled water. Then the fungal culture plates were aseptically transferred to Sabouroud's dextrose agar (SDA) medium[1–3]. This process was repeated to obtain pure fungal culture. The fungi were identified as *Saprolegnia parasitica* and *Branchiomyces sanguinis*[1,3,8,9] causing gill rot and ulcer. Disc diffusion method was used for determination of fungal inhibition zones[5,10,11]. The diameter of the inhibition zones was measured using a disc made of Whatman No. 1 filter paper for the antimicrobial activity[5,10–12]. Clotrimazole (10 µg/disk) was used as reference standard[5,10–12]. Broth micro-dilution method was employed to determine minimal inhibitory concentration (MIC) values[5,10,11,13]. Experiments were performed in three sets each and the results were considered as mean values of standard error[5,10–13].

Aqueous extracts of *C. macroptera* var. *annamensis* showed high antimicrobial activity against fungal pathogens *S. parasitica* and *B. sanguinis* (Table 2). This is a new report of fish antimicrobials and nutraceuticals from fruit peels of *C. macroptera* Montrouz. var. *annamensis*

Table 2. Antifungal activity of *C. macroptera* Montrouz. var. *annamensis* Tanaka against fungal pathogen of *Cyprinus carpio* var. *communis*

Fish pathogen	DD[a] (mm)		MIC[c] (µg/ml)
	Aqueous extracts of *C. macroptera*	RSC[b]	
Saprolegnia parasitica	10 ± 0.40	09 ± 0.30	90
Branchiomyces sanguinis	12 ± 0.55	10 ± 0.25	75

[a]DD: Diameter of inhibition zone (mm) ± standard error of means around the disks impregnated with 20 µl of aqueous extracts of *C. macroptera*.
[b]RSC: Reference standard compound as clotrimazole against fish pathogens.
[c]MIC: Minimal inhibitory concentration (µg/ml).

Tanaka, with leaves of *B. pinnatum* and twigs of *A. philoxeroides*. Identification of antimicrobial compounds from aqueous extracts of *C. macroptera* is in progress.

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