

Proximate composition, fatty acid profile, elemental, zoo-chemical and FT-IR based functional group analysis of *Meretrix casta* **and** *Donax cuneatus* **from Puducherry coastal waters, southeast coast of India**

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ABSTRACT

The proximate composition, fatty acid profile, elemental, zoochemical and FT-IR- based functional groups of *Meretrix casta* and *Donax cuneatus* of Puducherry coastal waters were analyzed. The moisture was found to be the major content and remains 74.9±0.03 in *M.casta* and 77.0±0.05 in *D.cuneatus.*The total protein content of *M.casta* was found to be high (9.75 ±0.05 %) when compared to *D.cuneatus* (7.79±0.04%). The lipid, carbohydrate and energetic values were high in *M.casta* than *D.cuneatus.* The energetic value of the *M.casta* and *D.cuneatus* was calculated as 4.96 kJ/g-1 and 4.08 kJ/g-1 respectively. 13 different fatty acids were reported in *M.casta* and 16 different fatty acids were detected in *D.cuneatus.*The percentage of PUFA was high in *M.casta* than *D.cuneatus.* The FT-IR established the presence of proteins, lipids, carbohydrates and their functional groups in both *M.casta* and *D.cuneatus* and the zoochemical analysis revealed the presence of alkaloids, polyphenols, saponins, terpenoids, anthroquinone glycosides. Elements like Al, Mn and Zn in *M.casta* and *D.cuneatus* were found to be exceptionally high. Therefore, the present study proved that the clams *M.casta* and *D.cuneatus* are not only the right choice of nutrient-rich food but also a potential source of nutraceuticals for good health and vigour.

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1. Introduction

Nutrient-rich food not only plays a pivotal role as the source of energy and metabolic activities but also provides healthpromoting effects. Since human beings are heterotrophs they rely upon other organisms for their nutritional requirements. Mollusca, the second largest animal phylum with comprehensive biodiversity identified as one of the inevitable natural resources for human consumption. The rich and significant nutrient contents of molluscs were reported to play prominent roles in the enhancement of the immune system of humans (Khan B.M *et al.,*2019). Bivalvia one of the classes of the phylum mollusca representing oysters, mussels, clams and scallops enjoyed significant attention not from the food industry and also from the research community in pursuit of nutraceuticals. Bivalves such as *Meretrix casta, Crassostrea rhizophorae, Perna viridis, Cerastoderma edule, Ostra edulis, Protothaca theca,* and *Mytilus galloprovincialis* are the major worldwide nutritionally important molluscs (Periyasamy *et al.,*2011).

The bivalves, *M.casta* (Backwater or yellow clam) and *D.cuneatus* (wedge clam) are edible molluscs belonging to the family Veneridae and Donacidae respectively found in the east coast of India, come under the category of suspension filter feeders inhabiting estuary and seawaters (Karthikeyan M,2009 and Varadharajan D, 2010). The *Meretrix casta* and *Donax cuneatus* are a good source of alternate food commonly consumed by the native people of the Puducherry coastal region with the belief of curing ailments based on traditional knowledge. But, the available scientific literature relating to the chemical composition and functional groups of these bivalves in meagre. Therefore, the present study was carried out to investigate the proximate composition, energetic value, fatty acid profile,

elemental composition, zoochemical and functional groups of *Meretrix casta* and *Donax cuneatus* from puducherry coastal waters.

2. Materials and Methods

The present study was carried out from January 2020 – December 2021 at the Department of Zoology, Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry, India.

2.1. Sample Collection, Dissection and extraction

Medium- sized fresh samples of intertidal bivalves *M.casta, D.cuneatus* were collected from the estuary and intertidal zone along the east coast of Nallavadu, Puducherry (11.847497 ̊ N-79.809012 ̊ E and 11.857901 ̊ N – 79.81267 ̊ E). The collected samples were kept in an insulate ice box and brought to the laboratory for species identification. Identification up to species level was carried out by a thorough examination of the morphological and shell characteristics based on the Standard keys (Woods,1961, Linnaeus 1758, Prashad 1932, Senthil Kumar, 2009). After identification, clams were washed with running water and de-shelled carefully using a scalpel. The entire body mass excluding digestive glands was excised and dried at 55 ̊C (Constant temperature) for one hour in the hot air oven (Srilatha *et al.,* 2023). The dried body tissue was powdered using a mortar and pestle and stored at 0 ̊C for further studies.

2.2. Proximate composition

The total protein (Lowry *et al.,* 1951), lipid (Folch *et al.,* 1957), Carbohydrate (Hedge *et al.,* 1962), moisture, ash and fatty acids (AOAC, 2015) of *Meretrix casta* and *Donax cuneatus* were analysed.

2.2.1 Energetic value

The energetic value of *Meretrix casta* and *Donax cuneatus*

were ascertained using Rubner's coefficients, especially for aquatic organisms: 9.5kcal $g⁻¹$ for lipids, 5.65 kcal $g⁻¹$, 4.1 kcal g^{-1} for proteins and carbohydrates respectively (Winberg,1971). The energy value was expressed in KJ g^{-1} wet mass by multiplying the 4.184 conversion factor as described by Eder and Lewis (2005).

2.3. Elemental analysis

The elements of *Meretrix casta* and *Donax cuneatus* were quantified after microwave-assisted acid digestion by inductively coupled plasma mass spectrometry (ICPMS Model Agilent 7700 series) and expressed in mg/kg (AOAC.2015).

2.4. Zoo-chemical analysis

The qualitative analysis of the zoochemicals viz. alkaloids, coumarin, flavonoids, polyphenolics, saponins, steroids, anthraquinone glycosides, triterpenoids, cardiac glycosides, tannin, quinines, pholobatannins, oil and fats present in the methanolic extract of *Meretrix casta* and *Donax cuneatus* was done based on the standard protocol described by Walag AMP *et al., (*2019) and Joenilo E *et al.,*(2019).

2.5. FT-IR analysis

10 mg of the sample of *Meretrix casta* and *Donax cuneatus* were mixed with 100 mg of KBr and made into a pellet and placed in a Golden cell cavity of an FT-IR Spectrophotometer. The FT-IR (Nicolet- iS5,Thermo Scientific, US) spectra were recorded from 500-4000 cm⁻¹ for both samples.

3. Results and Discussion

The proximate composition of *M.casta* and *D.cuneatus* are listed in Figure 1 and Table 1. Results are presented as percentage mean \pm SD. The moisture content was found to be the major component and remains 74.9 ±0.03 in *M.casta* and 77.0 ± 0.05 in *D.cuneatus.* Approximately molluscan shellfish contain 75% of water (USA 2018 a). This moisture content may vary between species and individuals within one species based on the differences in the thickness of the shell (Gafari, 2011), the physiology of the animal and the texture of the meat etc. (Vineetha *et al.,*2020). The protein content of *M.casta* (9.75 \pm 0.05) was found high when compared to *D.cuneatus* (7.79 \pm 0.04). The food and feeding behaviour of clams caused differences in the total protein content and reported that the protein content of clams has been found between 9.0% -13.0 % (Venugopal and Gopakumar (2017). The lipid content in *M.casta* and *D.cuneatus* were 5.33 ± 1 0.2 and 4.83 ± 0.2 respectively. Emiati *et al.*, (2023) reported the lipid content of *Meretrix* sp. and *D.cuneatus* were 3.54 ± 0.75 and 4.54 ± 0.74 respectively from the North Aceh district, Aceh province. In the absence of carbohydrates, the lipid is an important alternate source of energy for an organism and is usually stored in gonads in bivalves (Gabbott,1976). Therefore, depending upon the animal age, maturity and size of the gonads, the lipid content may vary between species and within the species. The level of carbohydrate content remains at 3.15 ± 0.05 in *M.casta* and 1.86 ±0.01 in *D.cuneatus.* Molluscan shellfish such as scallops, oysters, and mussels are generally considered low in carbohydrate content and exhibited large fluctuations between 3% and 5% carbohydrate (USDA 2015). The ash

content of *M.casta* and *D.cuneatus* were 6.7 ± 0.2 and 6.9 ± 0.2 respectively. Swapnaja *et al.,*(2013) have mentioned the ash content of *M,meretrix* which varied from 6.77% to 22.25% at Ratnagiri coast. A similar range of ash content was reported in *Gafrarium divaricatum* at Mumbai coast by Eswar *et al.,*(2016). The energetic value of the *M.casta* and *D.cuneatus* was calculated as 4.96 kJ/g⁻¹ and 4.08 kJ/g⁻¹ respectively.

In the present study,13 different fatty acids belonging to saturated (5), monounsaturated (3) and polyunsaturated (5) fatty acids were reported in *M.casta* (Table 2). The percentage availability of SFA, MUFA and PUFA were 41.89%,14.41% and 43.73% respectively. Among these, docosahexaenoic acid (DHA) C22:6 (25:16%), palmitic acid C16:0 (20:53%), eicosapentaenoic acid (EPA) C20:5 $(11:79%)$ and stearic acid C18:0 $(11:68%)$ represented as the dominant fatty acids. In *D.cuneatus*, 16 different fatty acids were detected, among these, seven of them belong to saturated fatty acids (SFA), four belong to monounsaturated fatty acids (MUFA) and five polyunsaturated fatty acids (PUFA). The SFAs C16:0 was the major fatty acid. In MUFA, C18:1 and in PUFA C22:5 and C22:6 were the major fatty acids. The percentage availability of SFA content was 49.37 followed by PUFA (35.7%) and MUFA (14.9%) in *D.cuneatus.* The percentage of PUFA was high in *M.casta* when compared to *D.cuneatus* (Table 2).

In the present investigation, saturated fatty acids such as myristic acid, palmitic acid, heptadecanoic acid, stearic acid, and tricosanoic acid were found in both *M.casta* and *D.cuneatus*. But, pentadecanoic acid, arachidic acid and margaroleic acid were found exclusively in *D.cuneatus* Among the SFA, C16:0 palmitic acid occurred in large quantities in *M.casta* and *D.cuneatus.*Mono-unsaturated fatty acids such as palmitoleic acid, cis-9 oleic acid and eicosenic acid were found in lesser quantities in *M.casta* and *D.cuneatus.* Further, the polysaturated fatty acids such as linoleic acid, gamma-linolenic acid, eicosadienoic acid, eicosapentaenoic acid and docosahexaenoic acids were found in appreciable quantity in *M.casta* and *D.cuneatus.* Polyunsaturated fatty acids play an important role in the prevention and management of cardiovascular diseases, hypertension, inflammation, diabetes (Finley and Shahidi, 2001), improvement of learning ability (Suzuki *et al.,*1998),modulators of gene expression and precursors for ecosanoids (self-healing agent) (Pollero RJ *et al.,*1979). Periyasamy *et al.,*(2014) have reported 28.13% of C16:0 Palmitic acid and 18.74% of Linolenic acid found in *D.incarnatus* from Cuddalore southeast coast of India.

Table 1. Proximate composition of *M.casta* and *D.cuneatus*

		Content in $\%$ Mean \pm SD	
	S.No Parameter	M.casta	D.cuneatus
	Moisture	74.9 ± 0.03	77.0 ± 0.05
2.	Protein	9.75 ± 0.05	7.79 ± 0.04
3.	Lipid	5.33 ± 0.2	4.83 ± 0.2
4.	Carbohydrate	3.15 ± 0.05	1.86 ± 0.01
5.	Ash	6.70 ± 0.2	6.99 ± 0.2
6.	Energetic Value (kJ/g^{-1})	4.96	4.08

Each value represents the mean \pm SD of three determinations on dry meat weight (DW) basis

Fig. 1. Proximate composition of *M.casta* and *D.cuneatus*

The result of the present investigation concerning MUFA coincides with the study made by Shanmugam *et al.,*(2007) in *D.cuneatus.*

The fatty acid profile of all the edible bivalve molluscs demonstrated that the palmitic and stearic acids were the predominant fatty acids found in the body mass. Palmitic acid played a role in cell membrane physical properties, biosynthesis of palmitoylethanolamide and surfactant activity in the alveoli of the lungs and stearic acid is probably increasing the low-density lipoprotein levels (Finley JW, Shahidi F(2001). The omega-9 oleic acid has beneficial effects on insulin sensitivity in type II diabetes, is an agent to treat Alzheimer's disease (Carrillo *etal.,*2012), anti-inflammatory, wound healing, and antioxidant properties (Tzu-Kai Lin,2018). Therefore, the *M.casta* and *D.cuneatus* can be treated as valuable natural sources for n-3 PUFAs and the best alternative to the finfishes.

The result of the elemental analysis revealed the presence of nineteen elements in the *M.casta* and *D.cuneatus* and expressed in mg/kg body weight (Table :3). All these elements belong to the category of essential minerals and play an important role in the metabolic functions but they

do not have any energy value. Generally, the elemental profile of an organism is based on its habitat and its food and feeding behavior (Lall,2002; Chakraborty and Joseph, 2015). In the present study, the Al content in *M.casta* (327.29 ± 28.72) and *D.cuneatus* (327.38 ± 35.38) was found to be exceptionally high and the same in both the clams. Therefore, *M.casta* and *D.cuneatus* were considered good sources of this mineral and utilized to treat antiperspirants and hyperhidrosis. The quantity of manganese was 91.07 ± 5.26 in *M*.*casta* when compared to *D.cuneatus* (14.09 ± 0.76) but Zn was high in *D.cuneatus*(104.36 \pm 0.79) when compared to *M.casta* (46.22 \pm 0.92). Manganese has considerable biological significance due to their ability to prevent heart attack, stroke and cardiac arrest. Mn supports the formation of bone is involved in enzyme function and is even a component of several enzymes, such as arginase, concanavalin A, glutamine- synthase, and many others (Homing *et al.,*2015). There appear to be several roles that manganese plays in osteoporosis, diabetes, and seizure disorders. Zinc is playing a major role in wound healing, growth-promoting and immunity, regulation of the ageing process, atherosclerosis (Samman, 2007), other roles in

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$S.\overline{NO}$	Fatty acids	% of Composition			
	Saturated fatty acids (SFA)	M.casta	D.cuneatus		
1.	Myristic acid C14:0	2.31	3.69		
2.	Pentadecanoic acid C15:0		0.83		
3.	Palmitic acid C16:0	20.53	22.89		
4.	Heptadecanoic acid C17:0	1.71	1.64		
5.	Stearic acid C18:0	11.68	12.93		
6.	Arachidic acid C20:0		1.61		
7.	Tricosanoic acid C23:0	5.66	5.78		
		Σ SFA 41.89	49.37		
	Mono-unsaturated fatty acids (MUFA)				
8.	Palmitoleic acid C16:1	5.97	5.46		
9.	Margaroleic acid C17:1		0.47		
10.	$Cis-9$ Oleic acid $C18:1$	4.23	6.33		
11.	Cis-11 Eicosenic acid C20:1	4.21	2.64		
	Σ MUFA 14.41		14.9		
	Poly-unsaturated fatty acids (PUFA)				
12.	Linoleic acid C18:2	1.86	1.95		
13.	Gamma-linolenic acid C18:3	2.59	2.56		
14.	Eicosadienoic acid C20:2	2.33	2.26		
15.	Eicosapentaenoic acid (EPA) C20:5	11.79	11.37		
16.	Docosahexaenoic acid (DHA) C22:6	25.16	17.56		
	Σ PUFA 43.73		35.7		

Table 2. Fatty acid composition of *M.casta* and *D.cuneatus*

				raph 9. Elemental analysis 01 <i>m</i> , casta and <i>D</i> , cameanas
Element	Isotopes	Concentration in mg/kg $Mean \pm SEM$		Reference with Max Permissible limit (ppm)
		M.casta	D.cuneatus	
B	11	4.78 ± 0.07	9.32 ± 0.05	1-13 (WHO, 1996)
Al	27	327.29 ± 28.72	327.38 ± 35.38	60 (WHO, 1989)
$_{\rm Cr}$	52	3.83 ± 0.48	5.35 ± 1.68	0.1 (WHO.1989)
Mn	55	91.07 ± 5.26	14.09 ± 0.76	0.01 (WHO 1985)
Co	59	1.65 ± 0.03	0.78 ± 0.007	0.30-1.77 (Underwood, 1977)
Ni	60	7.07 ± 0.26	2.86 ± 0.09	70-80(USFDA 1993 b)
Cu	63	17.17 ± 2.27	15.68 ± 0.73	30(WHO/FAO 1983)
Zn	66	46.22 ± 0.92	104.36 ± 0.79	40(WHO/FAO 1989). 100(WHO 1989)
As	75	2.97 ± 0.009	10.17 ± 0.13	2.0(WHO, 1985)
Se	78	0.24 ± 0.13	1.33 ± 0.19	1.0(MHSAC,2005)
Sr	88	42.49 ± 0.94	27.81 ± 0.75	130 (IAEA, 2003)
Mo	95	0.25 ± 0.01	2.37 ± 0.01	---
Ag	107	0.06 ± 0.05	0.26 ± 0.09	---
C _d	111	0.43 ± 0.008	0.10 ± 0.002	$0.05-0.3$ (EU,2006)
Sn	118	0.39 ± 0.18	0.31 ± 0.13	
Sb	121	0.009 ± 0.00	0.03 ± 0.006	---
Ba	137	8.02 ± 0.64	4.25 ± 0.58	
Hg	201	< 0.03	< 0.03	0.5 (FSSR, 2011).
Pb	208	0.99 ± 0.18	1.00 ± 0.02	1.0 (Heavy metals regulations 2003)

Table 3. Elemental analysis of *M.casta* and *D.cuneatus*

Blank cells indicate that no citable information is available.

Each value represents the mean \pm SD of three determinations on dry weight (DW) basis

cancer, diabetes and Alzheimer's disease (Sandstead, 2012, Guan *et al.,* 2015).

The strontium (Sr) content in *M.casta* (42.49 ± 0.94) and *D.cuneatus* (27.81 ± 0.75) remain high and indicates these edible clams are good sources of strontium. Strontium is usually more in filter-feeding marine organisms since strontium is found in marine waters. Strontium can increase bone mineral density, improves bone micro-architecture, and decreases the risk of fracture in a postmenopausal women with osteoporosis (Ikeda *et al.,*2002). The quantity of Ni and Cu concentration in both the edible clams was low when compared to the maximum permissible limit. Copper is an essential metal necessary for haemoglobin synthesis and a major component of many enzymes that are necessary for energy production, and the formation of red blood cells, bone and connective tissues. Apart from these minerals, the *M.casta* and *D.cuneatus* also comprised either a low or insignificant level of B, Cr, Co, As, Mo, Se, Ag, Cd, Sn, Sb, Ba, Hg and Pb concerning maximum permissible limit. The variation in the composition of minerals in the edible clams might be based on the feeding behavior of clams and the rate of mineral deposition in the water body through river influx (Schaule and Patterson, 1981). Therefore, the elemental analysis of the present study revealed that *M.casta* and *D.cuneatus* are valuable sources of minerals.

The results of the zoochemicals revealed that alkaloids, polyphenols, saponins, terpenoids, anthroquinone glycosides, protein, oil and fats were detected in the whole mussel tissue of *M.casta* and *D.cuneatus* (Table.4). The results of this study coincides with the study of Sreejamole and Radhakrishnan (2011) that alkaloids, saponins, sterols, and polyphenols were present at detectable amounts in the crude methanolic extract of *P.viridis* whole mussel

tissue. These saponins exhibited anti-microbial, antiinflammatory, anti-feedent and hemolytic activities at higher concentrations (Eswar *et al.,*2016). Polyphenols with its phenol rings can act as electron scavenger to reduce free radical activity. Anthraquinone glycosides has anti-microbial, anti-diuretic, laxative properties (Mireille Fouillaud, 2016). Secondary metabolites are a type of biomolecules usually referred as zoochemicals found in animals. Bivalves are filter and detritus feeders, they feed algae and plankton, unable to synthesis secondary metabolites (bioactive substances) *de novo,* they depend on a dietary intake. Therefore, it is plausible that zoochemicals detected from *M.casta* and *D.cuneatus* may be the biotransformed products arising from further metabolism of dietary compounds (Joy, 2017: Sadjadi, 2018).

The FT-IR spectra of *M.casta* and *D.cuneatus* flesh sample were recorded from 4000 to 500 cm⁻¹ to assess the specific functional groups and structural components found in organic compounds (Fig. 2, 3 and Table 5). The O-H stretching at 3799.18, 3757.11, 3748.67, 3742.56, 3731.11, 3673.51,3654.44,3418.44 cm-1 in *M.casta* and 3853.07, 3411.46 cm-1 in *D.cuneatus* were characteristic peaks for the presence of alcohol, phenol, carbohydrate/aromatic rings. The N-H strong stretching vibration of amine salt and C-H small stretching vibration of the methylene group are evidenced by the signals at 2925.59 and 2847.67 cm-1 respectively in *M.casta.* The medium bands observed at 2926.78 and, 2851.76 cm⁻¹ are attributed to the methylene asymmetric stretching vibrations of protein in *D.cuneatus.* The characteristic peak at 1651.80 cm⁻¹ indicates a C=C stretching vibration of the olefinic of alkenes of alkenes which denotes higher content of unsaturated fatty acid and the peaks at 1557.33, 1404.47,1327.58 cm⁻¹ are

betokened the presence of N-O vibration of aliphatic nitro

Note: "+" indicates positive reaction; "-"indicates negative or undetectable

Fig. 2. FT-IR spectrum of *M. casta*

Fig. 3. FT-IR spectrum of *D. cuneatus*

Table 5. The observed wave number, intensity and the probable vibrational assignments of the various compounds present in *M.casta* and *D.cuneatus*

compounds in *M.casta* . In *D.cuneatus* the specific peaks at 1634.78,1544.84 and 1403.72 cm-1 showed the presence of C=C vibration of alkane, small N-O stretch of aliphatic nitro compounds and O-H bending vibration of phenol or tertiary alcohol. The signals at 1335.21, 1220.68, 1084.57, 1043.38, 933.78 and 898.39 cm-1 are evidenced the presence of C=N stretching vibrations of aromatic primary amine, broad C-O stretching of alkyl aryl ether, C-O of primary alcohol or S=O bond of sulfate ion, S=O of sulfoxide, C-H out of plane bending of alkene of a vinyl compound and the signal at 566.83 to 505.82 cm⁻¹ overlaps the presence of C-I stretch of aliphatic iodo compounds.

The peaks at 1258.12 to 508.70 cm⁻¹ are shown the presence of medium C-O stretching vibration of aromatic ester, strong C-O stretching of aliphatic ether, C-O stretch of primary alcohol or cyclic ethers, C-N stretch of amine, P-O-C stretch of aromatic phosphates, C-H 1,4-disubstitution (Para) vibration of the aromatic ring (aryl), C-H monosubstitution of the aromatic ring (phenyl), small stretching vibration showed the presence of benzene derivative and strong C-I bond of halo compound (Nandiyanto, A.B.D.*et al.,* 2019). Therefore, the FT-IR spectral information obtained in this study established the presence of proteins, lipids, carbohydrates and their functional groups in both *M.casta* and *D.cuneatus* and this information may be valuable for quality assurance and selection for human consumption.

4. Conclusion

Reliable scientific information on the nutritional profile of a particular food ensures the consumer's choice of healthy

food. Molluscan shellfishes like clams, mussels and oysters are an exceptional source of nutrients with enormous health-related benefits. The present study revealed that the commonly available edible clams *M.casta* and *D.cuneatus* contain appreciable amounts of protein, polyunsaturated fatty acids and minerals. The presence of different functional groups and zoochemical like alkaloids, polyphenols, saponins, terpenoids, anthroquinone glycosides proved that these clams are not only the right choice of nutrient-rich food but also a potential source of nutraceuticals for good health and vigour.

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