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Eco-biological interaction of Polyphenols among *Acanthus ilicifolius*, detritus, and *Uca lactea annulipes* in estuarine-ecosystem

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ABSTRACT

Bio-cycling of polyphenols in estuarine ecosystem have not been documented adequately among the flora and fauna thereof. Total phenolic contents, antioxidative potential and free-radical scavenging activity have been analysed from three types of structural components (leaves of *Acanthus illicifolius*, and detritus loaded estuarine soil and a benthic fauna, *Uca lactea annulipes*), of the estuarine mangrove ecosystem of the North Eastern coastal belt of India. HPLC and MALDI-TOF Mass spectrometry analysis were performed to identify and quantify the phenolic compounds. Results reveal that all the three samples contain polyphenols that can be related through the process and pathways of eco-biological transformation. Though quantitatively less, the presence of deprotonated polyphenols enriches the detritus laden soil. Interestingly, very few parent ions of phenolic acids from leaf sample show up in the crab hemolymph. It contained new metabolites consisting of fragmented ions. This establishes the role of the fiddler crabs as agents participating in the bio-cycling of polyphenols and also depicts the interdependence of plants, animals, and soil for the exchange of essential life supporting ingredients as an outcome of coevolutionary process.

Key words : *Phenolic compounds, Eco-biological transformation, Estuarine mangrove ecosystem, Benthic fauna*

Introduction

Diversified forms of flora, fauna, and microbes inhabiting coastal mangrove-estuarine ecosystem have been found to display varied patterns of ecological succession, distribution, and trophic interactions in order to maintain the eco-dynamics of the said ecosystem in tune with the changing ecological gradients (Chakraborty, 2011, 2017). The cyclical movement of nutrients after being facilitated by the food chain-food web dynamics from organic to inorganic form from one trophic level to another is ensured by the microbes (Costanza *et al.,* 1997; Chapin *et al.,* 2000; Chakraborty, 2017) . Intertidal benthic fauna has been found to render valuable contribution in the physiological transformation of nutrients from one form to another through their feeding, digestion, assimilation and even by releasing nutrient enriched faecal matters which promote the growth of several microbes for undertaking litter decomposition process of mangrove litter (Chakraborty, 2011, 2017).

Polyphenols are secondary metabolites of plants and are abundant in nature. The versatility in their functions contributes to their ubiquity. The enormous roles played by polyphenols can be expressed under two broad categories, viz., polyphenols play roles as antibacterial (Simon *et al*., 2020; Erica and

Marchese, 2014), and antifungal (Giovanna *et al*., 2020) agents and also in nutrient cycling (Hättenschwiler and Vitousek, 2020). Phenolic substances from leaf litter and humus also play effective role in allelopathy (John, 2012) tending to influence species composition and dynamics of forest vegetation (Kuiters, 1990). They function as antioxidants that scavenge endogenous free radicals (Pandey and Rizvi 2009). Debilitating diseases namely cancer, diabetes, atherosclerosis, neurodegenerative disorders, aging etc., have been directly related to free radicals and oxidative stress (Pham-Huy *et al*., 2008). They have broad spectrum anti-inflammatory properties, including boosting of the immune system (Tangney and Rasmussen, 2013; Khurana *et al.,* 2013). They also contribute to form body structures (Wigglesworth, 1988) and coloration (Krishnan, 1951) in plant and animal bodies. Phenolic acids are considered as important precursors of humic substances in soils (Ziolkowska *et al.,* 2020). They play multiple roles in soil formation and pedogenesis, that can directly influence the recycling of minerals as well as organic matter dynamics (Seneviratne and Jayasinghearachchi, 2003), by producing organic metal complexes, and also serve to increase the availability of micro and macro nutrients (Micales, 1997).

All these properties of polyphenols have mostly been studied in stable biomes, where they are present in the humus laden soil and from where they get distributed and redistributed to various trophic levels. These roles of polyphenols have been less studied in an unstable estuarine ecosystem where the top soil is regularly removed by the ebb and flow of the ocean tides. Also, only fragmentary studies have so far been made on the transmission of plant biochemicals to detritivorous animals and their bioconversion thereafter.

Previous studies reported regarding bio-cycling of nutrients in mangrove ecosystems involved microbial transformation of nutrients to simpler, elementary forms (Singh *et al*., 2005). Conceptual models have also been built to elucidate the detritus pathway in the estuaries (Lopez and Levinton 1987; Mann, 1988; Holguin *et al*., 2001). Study related to crab bioturbatory activities reveals that it enhances labile organic C in the sediment, efficiently remineralizing detritus to CO_2 and dissolved organic C (Fanjul *et al.,* 2015). Role of fiddler crabs in the nutrient cycling in mangrove ecosystems has recently been investigated. It has been found that the carapace of the fiddler crabs, associated with microbial biofilms is hotspots of microbial N transformation (Zilius *et al*., 2020).

The present study aims at investigating the functional roles of a bioenergetically significant intertidal benthic fauna, fiddler crab (genus *Uca*) in a tropical estuarian ecosystem at the confluence of Subarnarekha River and Bay of Bengal in ecobiological cycling of polyphenols and build a conceptual model of the pathway thus investigated (Fig. 7). Similar studies have been conducted by our laboratory colleagues in the coastal Midnapore (East) district to understand the mode of bioconversion and biotransformation of lipids (Das *et al.,* 2017).

Materials and Methods

Sample collection

The leaves of *Acanthus ilicifolius*, an estuarine plant and the detritus derived from decomposition of its leaves, fiddler crab, *Uca lactea annulipes* and the soil from its habitat consisting of concentric semi-circular mounds formed by the bioturbatory activities with the deposition of rejected feeding pellets, were collected from Talsari, the exposed intertidal belt during low tide from the mangrove estuarine region, at the confluence of Subarnarekha River with Bay of Bengal, at junction of two eastern states West Bengal and Odisha, India. Geographically Talsari is situated between 21°35′48" Northern Latitude and 87°27′17" Eastern Longitude. The map depicting study site has been presented in Fig. 1.

Extraction of polyphenols

The body fluid of the crab was collected from the body cavity (hemocoel) of the crab with the help of an injection syringe and stored under refrigeration. Desired amount of fresh acanthus leaves was washed with distilled water and dried. Then the dried leaf sample was crushed in mortar and pestle with liquid nitrogen and thus powdered. These powdered samples were stored in air tight plastic containers. Four grams of each stored samples were weighed and mixed with 10ml of 80% methanol in a ratio 1:5 (w/v- weight/ volume) and mixed well. Extracted samples were allowed to centrifuge at 5000 rpm for 20 minutes and the supernatants were taken. This supernatant was again centrifuged at 4700 rpm for 10 minutes and concentrated in

Fig. 1. The map depicting location of the study site (image downloaded from Google Maps indicates the study region at Talesari, estuary of Subarnarekha River.

vacuum drier. The concentrated samples were stored at 4 °C until further use.

Separation of extracted polyphenols by High Performance Liquid Chromatography

Separations were performed through high performance liquid chromatography (HPLC), following a procedure standardized by standard method by Dawei Wen *et al.* (2005) (Wen *et al.*, 2005). HPLC analysis was performed in an AGILENT 1100 series model system, equipped with a binary pump, diode array detector (DAD), online vacuum degasser and CHEMSTATION software (Agilent Technologies, Santa Clara, CA, USA). The extracted samples were filtered through 0.45ìm membrane filter for HPLC analysis. 20µl of each filtered extract samples were injected into the HPLC system and separation was performed in reverse-phase Zorbax Eclipse XDB-C18 column (4.6×150 mm i.e., 5 µm particle size; Agilent Technologies), with varying proportion of solvent A (Methanol) to solvent B [1% TFA in water (v/v)]. The total run time was 30 minutes and the spectra were recorded at 200 nm to 600 nm. The separated and purified compounds were collected in tubes from HPLC analysis that corresponds to the respective peaks.

Identification of the separated polyphenols

Identification of the separated and purified compounds was performed through Matrix Assisted Laser Desorption/Ionization Time Of Flight (MALDI TOF) mass spectrometry and Fourier Transform Infra-Red (FTIR) spectroscopic analysis.

MALDI TOF mass spectrometry analysis

For MALDI-TOF mass analysis, 2µl of each sample was spotted onto a MALDI stainless steel plate. The purified samples were mixed with a suitable matrix material, 2, 5-dihydroxy benzoic acid and applied to the metal plate. Matrix-assisted laser desorption/ionization (MALDI) analysis were performed on an Applied Bio-system Voyager-DE PRO MALDI TOF mass spectrometer with a nitrogen laser (337nm) operated in an accelerating voltage (20kv). Each spectrum was collected in the positive ion reflector mode as average of 100 laser shots. The data has been externally calibrated using angiotensin and ACTH (Applied Biosystems, USA).

FTIR spectroscopic analysis

Two microliters of extracted samples were taken on a KBr pellet and scanned in a Fourier Transform Infrared Spectrometer (instrument model: NEXUS-870) in ATR (Attenuated Total Reflectance) mode in the region of 4000-600 cm^{-1} and 32 scans were collected with a spectral resolution of 0.5 cm^{-1}.

Total polyphenol content

Folin–Ciocalteu reagent assay was carried out based on the standardized methods used by Prior *et al* (Prior *et al.*, 2005). 100 µl of samples for test and 100µl of 80% methanol for blank were dissolved in 500µl (1/10 dilution) of the Folin–Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using UV–Vis spectrophotometer. The results were expressed in mg of gallic acid per g (GEA) of dry weight of plant. For each sample, three replicate assays were performed. The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation: T=C×V/M, where T is the total phenolic content in mg/g of the extracts as GAE, C is the concentration of gallic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract.

DPPH free radicals scavenging activity assay

Determination of the free radical scavenging activity

of the different extracts was carried out using a modified quantitative DPPH (1, 1-diphenyl-2-picrylhydrazyl; Sigma Aldrich, St. Louis, MO, USA) assay. Various concentrations of sample extracts in methanol were prepared (1000, 500, 250, and 100 μgml^{-1}). Gallic acid was used as a positive control at concentrations of 100, 50, 25, and 10 μgml^{-1}. Blank samples were run using 1 ml methanol in place of the test extract. One ml of 0.2 mM DPPH in methanol was added to 1 ml of the test solution, or standard, plus 1 ml of methanol for dilution and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 517 nm using UV–Vis spectrophotometer, Thermoscientific, model- Evolution 201. Inhibition of free radical in percent (I %) was calculated according to the equation:

$I\ \% = [(A_0 - A_1)/A_0] \times 100$

A_0 being the absorbance of the control reaction (containing all reagents except for the extract) and A_1 the absorbance of the extract. Measurements were carried out in triplicates.

ABTS free radicals scavenging activity assay

The free radical scavenging activity was determined by ABTS radical cation decolorization assay described by Re *et al.* (Pellegrini *et al.*, 1999). ABTS was dissolved in water to a 7 µM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 µM potassium per sulfate (final concentration) and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion, the samples containing the $ABTS^{+}$ solution were diluted with redistilled water to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30 °C. A reagent blank reading was taken (A_0). After addition of 3.0 ml of diluted $ABTS^{\cdot+}$ solution (A734nm = 0.700 ± 0.02) to 30 µl of polyphenolic extracts, the absorbance reading was exactly 6 min after initial mixing (A_t) using UV–Vis spectrophotometer, Thermoscientific, model- Evolution 201. The absorbance of the reaction samples was compared to that of Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. The results were expressed in µM Trolox per 100g dry weight (dw). All determinations were performed in triplicates.

FRAP reducing power assay

The antioxidant activity of polyphenolic extracts was determined using a modified method of ferric reducing/antioxidant power (FRAP) assay, used by Benzie and Strain (Benzie and Strain, 1996). The FRAP reagent contained 2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$, $6H_2O$ and 25 ml of 0.3 M acetate buffer at pH3.6. Freshly prepared FRAP reagent (3.0 ml) were warmed at 37 °C and mixed with 40 µl of extracted samples and the reaction mixtures were later incubated at 37 °C. Absorbance at 593 nm using UV–Vis spectrophotometer (Thermoscientific, model- Evolution 201) was read with reference to a reagent blank containing distilled water which was also incubated at 37 °C for up to 1h instead of 4 min, which was the original time applied in FRAP assay. Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. The results were expressed in µM Trolox per 100 g dry weight (DW). All determinations were performed in triplicates.

Results

HPLC analysis

All the samples contained phenolic acids that revealed different pattern of elution as shown in Fig.2. The leaf sample peaks eluted at ≈ 18 to 28 minutes and different fractions are obtained. Last peak is eluted at 27 min, where maximum absorption occurs at 17 and 27 minutes. Detritus sample peaks eluted at ≈ 17-27 minutes. These peaks are of lower height than leaf sample. In the crab sample we find peak in the first five minutes. Comparative peaks are listed in Fig. 1 which revealed that the samples contain same type of polyphenols as compared with their elution times.

The *Acanthus* leaf sample contains maximum number of polyphenols than *Acanthus* detritus sample while the fiddler crab sample contains polyphenols in smaller quantities. The reason that detritus samples contain lower quantity of polyphenols than leaf samples can be attributed to the fact that bacteria and the benthic fauna consume the polyphenols of the leaves and thus convert them to detritus. The various microorganisms and detritivores of benthic fauna including millipedes, burying beetles, fiddler crabs etc., break down the

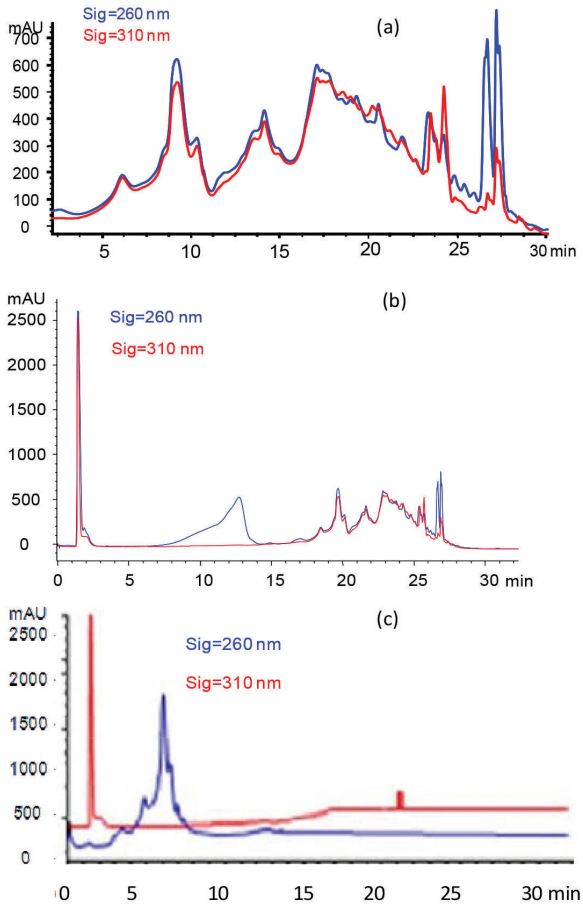


Fig. 2. HPLC chromatogram of phenolic acid mixtures from different samples monitored at 280 nm (shown in blue line) and 310 nm (shown in red line). The obtained spectra represent as *Acanthus* leaf (a), detritus (b), and crab sample (c).

organic materials to gain the resources required for their own survival and proliferation. Also, a greater ecosystem service is imparted in the recycling of the nutrients and energy.

MALDI TOF mass spectrometry analysis

Mass of the polyphenols has been determined by the method as described earlier (Mandal and Dey, 2008). The positive ion linear mode MALDI-TOF spectrum of all the samples represents a series of ions (Fig. 3). The observed ions are listed in the Table below. The identity of the observed ions was confirmed through Phenol Explorer Software. The identified phenolics are gallic acid (*m/z* 170), catechol (*m/z* 110), bergapten (*m/z* 216), avenanthramide K (*m/z* 315), p- coumaroyl quinic acid (*m/z* 338), peonidin 3-o arabinoside (*m/z* 449), petunidin 3-o glucoside (*m/z* 479), rosmarinic acid (*m/z* 360), dihydromyricetin 3-o rhamnoside (*m/z* 466), quercetin 3-o glucoside (*m/z* 478), and (+) catechin 3-o gallate (*m/z* 442).

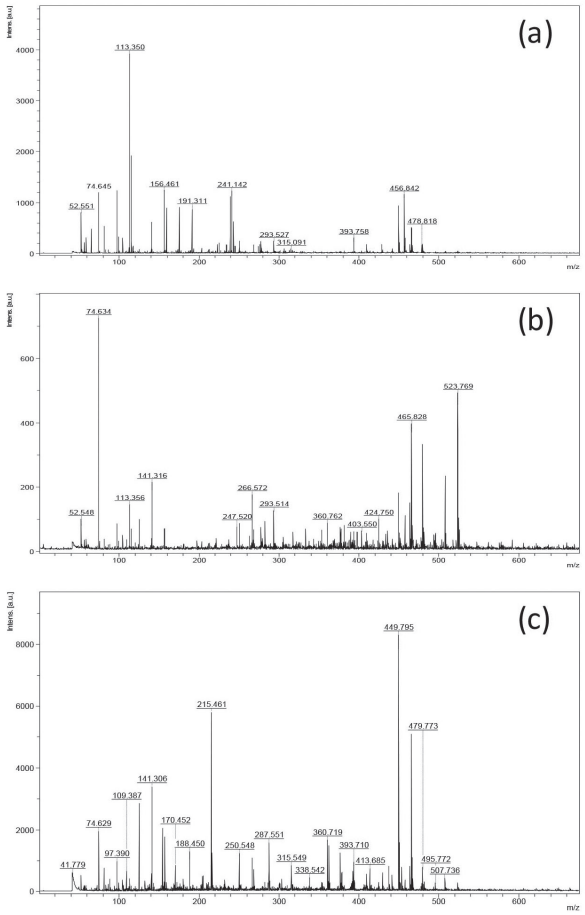


Fig. 3. MALDI-TOF mass spectrum obtained from different samples in positive ion linear mode, showing a difference between two intense fragment ions. The obtained spectra represent as *Acanthus* leaf (a), detritus (b), and crab sample (c).

FTIR spectroscopic analysis

The acanthus leaf, detritus and crab's hemolymph samples contain, [as shown in Figure 4] a complex mixture of many different acids containing carboxyl (wavenumber 1500-2000 cm^{1}) and phenolate groups so that the mixture behaves functionally as a dibasic or, occasionally, as a tribasic acid. A high intensity peak is observed in all the samples at 3200-3400 cm^{-1} belongs to stretching vibration of phenolic hydroxyl group (-OH) which represents hydrogen bonding. Appearance of broad band at

wavenumber 1250 $cm^{-1}$and 1200 cm^{-1} indicates presence of vibration stretching of carboxylic (-COOH) group and phenolic hydroxyl group (-OH) respectively, whereas the appearance of two medium and weak bands at 1615cm^{-1} and 1500 cm^{-1}represents stretching vibration of aromatic (C=C) group,

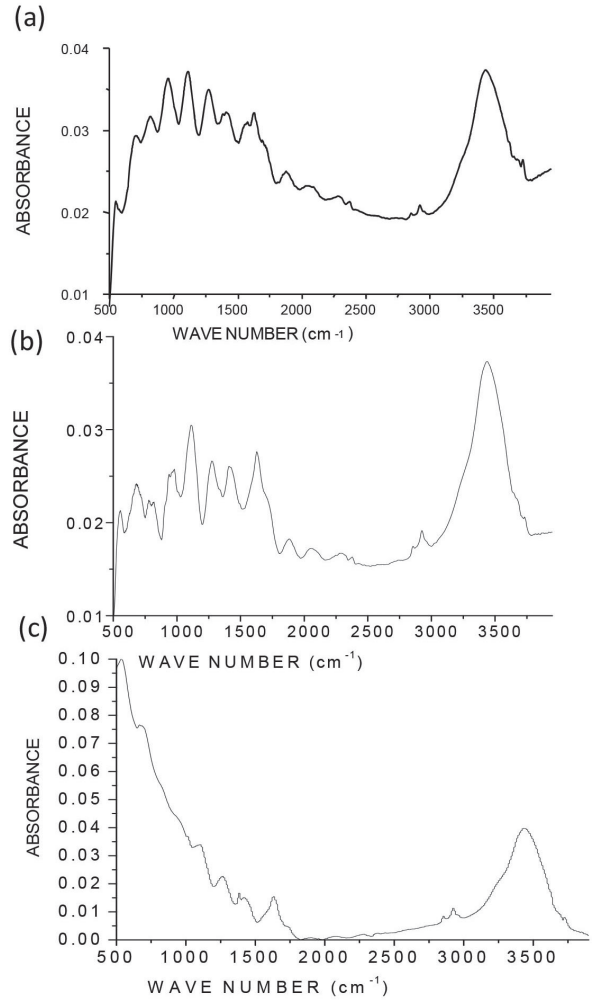


Fig. 4. The infrared absorption spectra of polyphenols from different samples. The obtained spectra represent as *Acanthus* leaf (a), detritus (b), and crab sample (c).

Antioxidant Activity

Total phenolic content (F-C reagent assay)

Methanolic (80%) extracts from the leaves and detritus of the *Acanthus sp.* and fiddler crab were standardized for their contents of phenolic compounds (Fig. 5). Leaves of *Acanthus* (104 ± 2. 0mg.g^{-1}) contained the high content of phenolics than the *Acanthus* detritus which contained lesser amount of phenolics (14.9 ± 0. 6mg.g^{-1}). On the other hand, hemolymph of crab (9.12±0. 16mg.g^{-1}) contained lowest amount of phenolics.

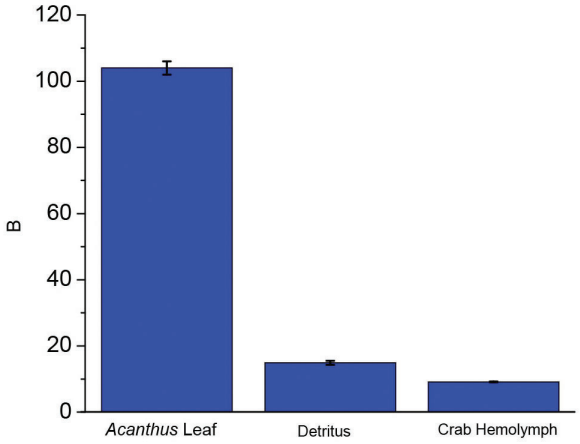


Fig. 5. Total phenolic content in *Acanthus* leaf (1), detritus (2), and fiddler crab hemolymph (3) determined by the Folin-Ciocalteu assay. Calculated as GAE in mg·g^{-1}extract based on dry weight. Results are the average of triplicates ± SD.

DPPH assay

The standardized extracts were assessed for their capacity to scavenge DDPH free radical along with gallic acid as a positive control. The antioxidant activity data are presented as percent of free radical inhibition in Table 1. It was previously reported that non-phenolic antioxidants might also contribute to the antioxidant activity of plant extracts. Thus, com-

Table 1. Antioxidant Activity of Sample Extracts Assayed by the DPPH Assay

Conc. of standard (µg/ml)	Acanthus Leaf	Acanthus Detritus	Crab Hemolymph	Conc. of standard (µg/ml)	Gallic acid
z1000	73.5± 3.2	50.7±0.2	71.1± 0.4	100	90.8± 1.5
500	67.3± 0.2	41.7±1.4	68.4± 0.2	50	83.7± 0.6
250	60.3± 2.0	38.3± 0.4	53.5± 0.2	25	76.3± 0.2
100	48.9± 3.7	35.9± 0.8	46.7±0.2	10	65.4± 0.1

pounds other than phenolics might be responsible for the pronounced antioxidant activity observed with crab extracts, which requires further investigation.

ABTS Assay and FRAP assay

Table 2 shows great differences in total antioxidant capacity measured by the ABTS and FRAP method between the species. There are many methods that differ in terms of their assay principles and experimental conditions and particular antioxidants have varying contributions to total antioxidant potential (Cao and Prior, 1998).

Discussion

Coastal environment being the interface between the land and sea, represents a potential eco zone along with several other geo-morphological components like estuaries, mangroves, dunes, deltas, lagoons, intertidal zones, etc. The breakdown of mangrove leaves is brought about by the activities of both macro and microfauna. Brachyuran crabs acting as a macro-decomposer accelerate the decomposition influencing the ecosystem functioning to a large extent. Interactions among different structural components of the ecosystem ensure ecological integrity and proliferation of biodiversity including the fruitful survivability of mangrove flora and fauna which in turn serve so many ecosystem goods and services (Chakraborty, 2011, 2017). The intertidal belt of an estuarine-mangrove ecosystem harbour so many diversified form of flora, fauna, and microbes in the form of halophytic mangroves, benthos, nekton and plankton and out of so many of these biodiversity components, brachyuran crabs (fiddler crabs) acting as a macro-decomposer accelerate the decomposition of litter and thereby which influence the ecosystem functioning to a large extent after being morphologically, physiologically and behaviourally well adapted to their respective habitats (Kristensen, 2008).

Energy and materials of primary production can be transferred to benthic habitat through its essential fauna and microbes. This transfer process that helps nutrient enrichment of soil is called decay of detritus or aging of detritus (Mann, 1988). The microorganisms metabolize the plant nutrients within the detritus for their own metabolism, converting a major fraction to CO_2 and thereby reducing the efficiency of the transfer. Whereas, the nutrient rich vegetable organic matter when directly ingested by the fiddler crabs have a relatively high efficiency of energy transfer. Also, the utilization of detritus by microor-

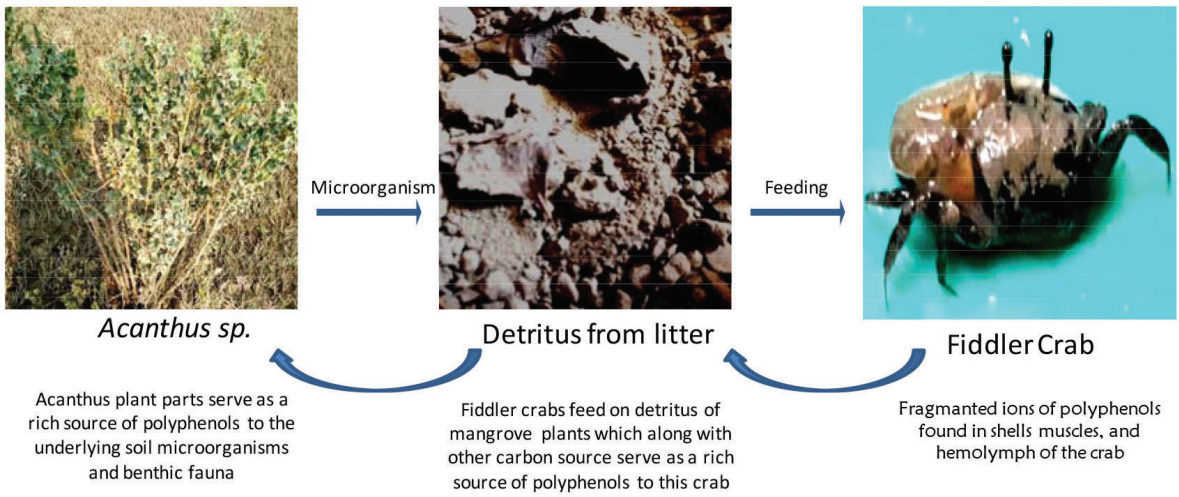
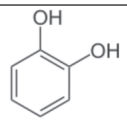
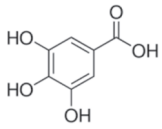
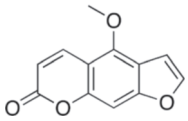
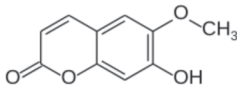
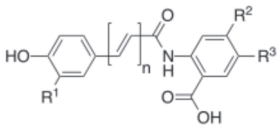
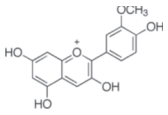
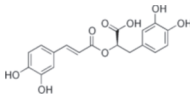
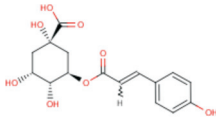
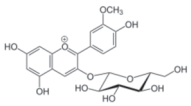
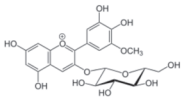
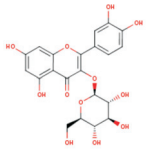
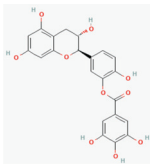


Fig. 6. Eco-biological cycling of polyphenols through trophic interaction among *Acanthus* sp., Detritus, Fiddler Crab.

Table 2. Antioxidant Capacity of Extracted Samples Assayed by ABTS and FRAP Method.

Samples	ABTS Assay (l Mtrolox/100g dw)	FRAP Assay (l Mtrolox/100 g dw)
Acanthus Leaf	8.25 ± 0.09	61.8 ± 0.91
Acanthus Detritus	6.74 ± 1.01	15.9 ± 3.10
Fidler-crabhemolymph	12.3 ± 0.01	51.7 ± 2.01

Table 3. Identified Polyphenols in *Acanthus* leaves, Detritus and Crab Samples

Sl. No.	Name of Polyphenols	Mass of Polyphenols (*M/Z*)	Structure
1	Catechol (Crab)	110	
2	Gallic Acid (Crab)	170	
3	Bergapten (Crab)	216	
4	Scopoletin (Acanthus)	192	
5	Aventhramide K (Crab)	315	
6	Peonidin (Acanthus)	301.4	
7	Rosmarinic Acid (Acanthus)	360.7	
8	5p-coumaroyl Quinic Acid (Crab)	338	
9	Peonidin 3-o Glucoside (Crab)	449.7	
10	Petunidin 3-o Glucoside (Crab)	479.4	
11	Quercetin 3-o Glucoside (Detritus)	478	
12	-catechin 3o Gallate (Detritus)	442	

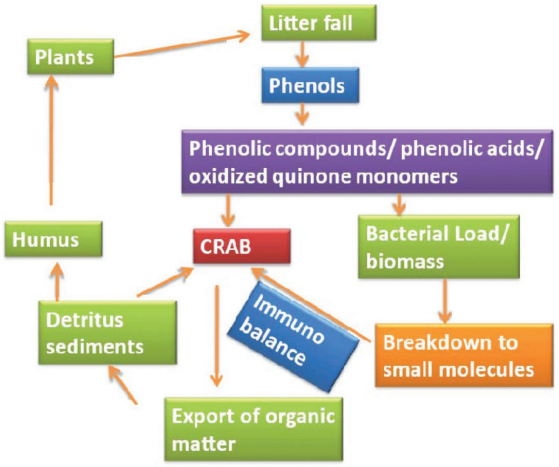


Fig. 7. Conceptual Model of Bio-cycling of polyphenols among mangrove plants and fiddler crabs and sediment in estuarine environment.

ganisms increases the available soil nutrients that promote fiddler crab productivity (Fig. 7).

The anti-oxidant activities of the three major ecological components such as *Acanthus* leaves, detritus and fiddler crab's hemolymph assessed through Folin-Ciocalteu, DPPH, ABTS and FRAP assay shows that maximum activity is found in the leaves followed by detritus and crab hemolymph. While the Infra-red spectroscopic analysis data indicate the presence of carboxylic –COOH, and phenolic –OH groups as well as aromatic –C=C- groups. Polyphenols found in the leaves are rosmarinic acid, and dihydromyricetin 3-o rhamnoside while that found in the detritus are Catechin 3-o gallate and Quercetin 3-o glucoside. A wide variety of polyphenols found in the crab's body includes catechol, gallic acid, bergapten, avenanthramide, p-coumaroyl quinic acid, peonidin 3-o arabinoside, and petunidin 3-0 glucoside. Interestingly, in the crab's hemolymph, spectroscopic data shows very few parent ions of the phenolic acids that were present in the leaves. The data as shown in Table 3, contains new metabolites consisting of fragmented ions that have been incorporated in the body fluids of the crab. This transformation is a critical part of recycling, enabling ongoing movement and dispersion of nutrients across the ecosystem. This further establishes the role of the fiddler crabs as agents participating in the bio-cycling of polyphenols. Fiddler crabs, an important faunal component of the mangrove ecosystem, consume detritus of mangrove vegetation which serve to increase its biomass and also lend immune boosting support thereby ensuring their fruitful survivability (Mak and Saunders, 2006). The crabs while transforming these polyphenols into the antioxidant potential of their shells, muscles and body fluids (Soundarapandian, *et al*, 2014) participate in the eco-biological cycling of these polyphenols through trophic interactions as depicted in **Fig. 6 & 7.** By acting as an agent in eco-biological cycling of polyphenols from mangroves through detritus, fiddler crabs shift the dominant pathway of nutrient cycling from mineral to organic forms, participating in minimizing potential nutrient losses from the ecosystem and maximizing litter-nutrient recovery by the symbiotic microflora.

Conclusion

The presence of polyphenols in the detritus laden estuarine soil is very significant. In spite of their presence being challenged by the ocean tides, the soil serves as an important link between the producers and consumers, the benthic fauna, ensuring ecobiological cycling of polyphenols. Most of the phenolic compounds undergoing decomposition get transformed into different forms. This nutrient loaded bottom soil is not only the primary source of nutrition for the benthic fauna on estuaries but also support primary producer communities such as mangrove plants, macro and microalgae, etc. Therefore, a vivid pattern of estuarine trophic interactions involving active functional roles of fiddler crabs not only ensure the biotransformation of a plant biochemical, polyphenols, but also can be cited an important example of co-evolution with the complementary dependence of so many actors (plant, animal and detritus) in the laboratory of nature.

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