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Qualitative and quantitative analysis of phytochemicals of artificial mangroves from Vellar estuary, Tamil Nadu, India

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Abstract
The preliminary screening of phytochemical constituents of artificial mangroves in vellar estuary has been studied. Different plant parts such as leaves, flowers and bark were extracted and the maximum extract was found in the leaf extracts of *R. mucronata* (35.86 %). All phytochemicals tested found to be present in all the species except phlobatannins and anthroquinones. However, anthroquinones found to be present in the leaf of *A. officinalis* and *A. marina*. The leaf part showed maximum proximate composition in all mangrove species followed by the flower and bark. It was also found that, the gross energy and caloric values was found to be higher in the leaves of all mangrove species and the bark exhibited lower energy content

Keywords: mangroves; man-made; phytoconstituents; proximates; gross energy

1. Introduction
Mangroves are the woody plants that grow in the mud flats at the interface between land and sea of tropical and sub-tropical regions, where the water is generally brackish. Mangrove forest is among the most productive ecosystem occurring in 112 countries and territories, where the global coverage of mangroves was estimated at 10 million hectares. Since mangroves are circumtropical in distribution, it can able to adapt under harsh conditions in terms of anatomy, physiology and morphology. They enrich coastal waters, yield commercial forest products, protect coastline and support coastal fisheries. It also protects the coastal areas from erosion, storm surge especially during hurricanes and tsunami (Mazda *et al*., 2005). Mangroves contain many bioactive compounds of ecological, toxicological and pharmaceutical importance. Extracts from mangrove plants and associates has been used for medicinal purpose worldwide and have been recorded around 349 metabolites with rich source of steroids, diterpenes, triterpenes, saponins, flavonoid, alkaloids and tannins (Wu *et al*., 2008) [20]. Artificial mangroves are the man-made mangroves developed in order to protect the coastal areas from natural disasters. The knowledge of the chemical constituents of artificial mangroves is desirable to understand the variations among the wild mangroves. Numerous studies have referred the usefulness of mangrove plants in traditional medicine (Kokpsl *et al*., 1990; Premanathan *et al*., 1996) [13]. Mangrove plant extracts have been used as folk medicine by the local people as it cures many health disorders.

Many studies have been reported that the mangrove plant derived extracts may be considered as a rich source of novel compounds with potential biological activity. Biochemical composition or the phytoconstituents of artificial mangroves have not been studied yet and hence the present study delineated to qualitate and quantitates the potential phytochemicals of artificial mangroves of vellar estuary.

2. Materials and Methods
2.1 Collection of Plant materials
Different plant (leaf, flower and bark) parts of man-made mangroves were collected from Vellar estuary and were authentified by Dr. Kathiresan (CAS), Annamalai University. The collected samples were washed thrice with distilled water to remove salts and sand particles.

2.2 Phytochemical analysis
About 500 g of each sample was homogenized to a coarse powder and was defatted with petroleum ether (50-60℃). It was then extracted with 1 L of 70% of ethanol: water mixture by percolation method. The extract was filtered using Whatmann No.1 filter paper and the filtrated were concentrated using rotary evaporator and further lyophilised to remove the excess organic residues. The percentage of the extract was calculated by the following formula.

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material}} \text{ x } 100$$

Table 1: The following phytochemical test was performed for the different plant parts of mangrove extracts.

References	Test (s)	Observation	Inference
Siddiqui and Ali, 1997	0.5 g of each mangrove extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1 ml of the filterate.	Formation of turbidity or precipitation	Presence of alkaloids
Iyengar, 1995	0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added	Decolourization of bromine water	Presence of tannins

Siddiqui and Ali, 1997	0.5 g of extract was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added	Formation of red colour	Presence of flavonoids
Brinda *et al*., 1981	0.5 g of mangrove extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added.	Formation of pink or red coloration in ammoniacal phase	Presence of anthroquinone
Siddiqui and Ali, 1997	0.5 g of mangrove extract was mixed with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly	Formation of red violet colour	Presence of terpenoids
Siddiqui and Ali, 1997	0.5 g of mangrove extract was mixed with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly	Formation of green bluish colour	Presence of steroids
Brinda *et al*., 1981	0.5 g of ethanolic extract was mixed with distilled water and adds few drops of ferric chloride.	Formation of violet colour	Presence of phenolic group
Brinda *et al*., 1981	0.5 ml of alcoholic extract was mixed with concentrated HCl.	Formation of pink colour	Presence of catachin
Brinda *et al*., 1981	0.5 ml of ethanolic extract was mixed with Fehlings I and II solutions and boiling for half an hour in water bath.	Formation of red precipitation	Presence of reducing sugars
Jigna and Sumitra, 2007	A small portion of the extract was mixed with 2 mL of glacial acetic acid containing 1-2 drops of ferric chloride solution. The mixture was then poured into another test tube containing 2 mL of concentrated sulphuric acid	Appearance of brown ring	Presence of glycosides

The other phytochemicals were tested for their presence in the plant saponins (Evans, 2002) [19], phlobatannins (Trease & Evans, 1989) [19], ketoses (Seliwanoff's test) (Edeoga *et al*., 2005) [6], starch (Iodine tests), arginine (Sakaguchi's Test), cysteine (Lead sulfide test), aromatic amino acids (Xanthoproteic test), phenolic amino acids (Million's test).

2.3 Determination of proximate composition
The proximate composition of various parts of mangroves were determined using the recommended methods of the Association of Official Analytical chemists (AOAC, 2005), gross energy values (GEV) were calculated using the methods of Livesey (1990) and caloric values (CV) were estimated using the methods of Ooi *et al* (2012) [11].

2.4 Estimation of Carbohydrates
Sample (500 mg) was subjected to hydrolysis with 5 mL of 2.5 N HCl by keeping the tubes in a boiling water bath for 3 hours. It was then cooled to room temperature and neutralized with sodium carbonate until the effervescence ceases. The volume of the sample was then made upto 100 mL and was centrifuged. The supernatant was collected for phenol- suphuric acid method. 0.1 ml of sample was pipetted out and the volume was made upto 1 ml with dilstilled water. Phenol (1 mL) and sulphuric acid (5 mL) was added to each tube and mixed well. After 10 min, the sample was placed in a water bath at 20 – 30˚C for 20 min and the absorbance was read at 490 nm. Glucose was used as a standard (Krishnaveni *et al*. 1984) [21].

2.5 Estimation of Protein
Samples (1gm) were extracted with diethyl ether and water (1:4) for 3 h in a shaker. It was the centrifuged and the supernatant were discarded. To the pellet, 1N NaOH was added to the pellet and kept in a shaker for 3 hours. The reaction mixture was centrifuged at 7000 rpm for 10 min and the supernatant was precipitated with 10% TCA at pH 4.0. The precipitated protein was washed and dried. The pellet was dissolved in 0.1 N NaOH and the concentration of protein was determined according to Lowry *et al*. (1951) using bovine serum albumin as standard. (Krishnaveni *et al*. 1984) [21].

2.6 Estimation of Lipids
The total lipid content was determined by the modified method of AACC (2000). Samples (3 gm) were defatted with petroleum ether and extracted with chloroform: methanol (2:1) in a soxhlet apparatus for 6 hours. The extracts obtained were evaporated in an oven at 80˚C for overnight and the lipid content was determined gravimetrically. (Krishnaveni *et al*. 1984) [21].

2.7 Estimation of amino acid
Sample (1gm) was extracted with phosphate buffer (pH 7.0). Total amino acid contents were determined after hydrolysis of sample wit 6N HCl at 100˚C in a vaccum hydrolysis tubes for 24 hours. It was then centrifuged at 3500 rpm for 15 minand the supernatant was filtered. The filtrate was neutralized with 1N NaoH and diluted to 1:100 of the volume with distilled water. The analysis was performed by reverse phase HPLC, HP-1101 Agilent Technologies with UV and fluorescent detector. (Krishnaveni *et al*. 1984) [21].

3. Results
The percentage of extraction from different parts of mangrove species is summarized in Table.1. Of the four species collected from the vellar estuary, different plant parts such as leaves, flowers and bark were extracted and the maximum extract was found in the leaf extracts of *R. mucronata* (35.86%), followed by the leaf extracts of *A. marina* (32.16%), A. officinalis (28.32%), *R. apiculata* (28.31%), flower extracts of *A. marina* (21.37%), *A. officinalis* (19.05%), *R. mucronata* (12.81%), *R. apiculata* (10.13%), bark extracts (9.34%) of *R. apiculata* (8.34%), *R. mucronata* (6.32%), *A. officinalis* (5.16%) and *A. marina* (5.07%). Among the plant parts of mangrove species, the maximum percentage of extraction was found in leaf (30.69%), flower (23.61%), whereas the bark (17.36%) showed minimum extraction (Figure.1). It was found that *R. mucronata* (32.5%) showed the maximum yield of extraction, followed by *A. marina* (30.59%) and the lowest yield of extraction was found in *A. officinalis* (22.68%), *R. apiculata* (19.05%) respectively (Figure.2).

The phytochemical analysis of different plant parts of mangroves were analysed and listed in Table.1. All phytochemical tested found to be present in all the species except phlobatannins and anthroquinones. However, anthroquinones found to be present in the leaf of *A. officinalis* and *A. marina*. The proximate composition of various parts of different man-made mangroves in the vellar estuary was represented in Figure. 3 (A-D) and Table 1. The leaf part showed maximum proximate composition in all mangrove species followed by the flower and bark. In *R.*

mucronata, the carbohydrate (59.18±2.1 g/100 g) and Lipid (4.39±0.92 g/100g) content was found to be higher in the flower, whereas the moisture (25.8±1.05 g/100g), crude fibre (21.3±1.31 g/100g), ash (9.73±0.87 g/100g) and protein (7.23±0.72 g/100 g) were found to be higher in the leaves. In case of *R. apiculata*, moisture (30.5±1.84 g/100 g), ash (11.25±1.1 g/100 g), fibre (23.5±1.29 g/100 g), lipid (4.37±0.29 g/100 g) and protein (5.13±0.97 g/100 g) found to be higher in the leaf, whereas the carbohydrate (50.28±2.19 g/100 g) in the bark.

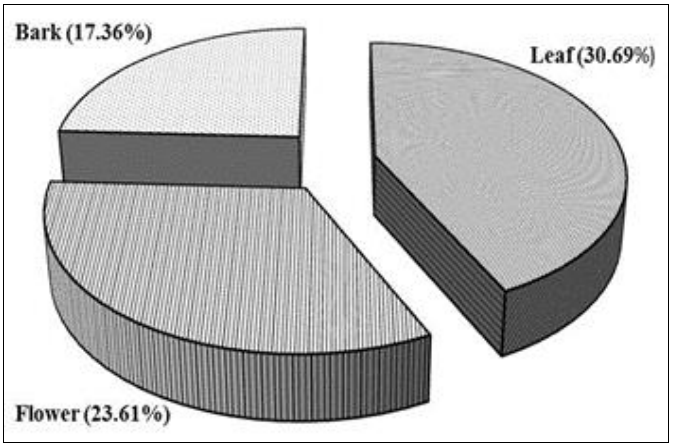

Fig 1: Percentage of extraction between mangrove plant parts

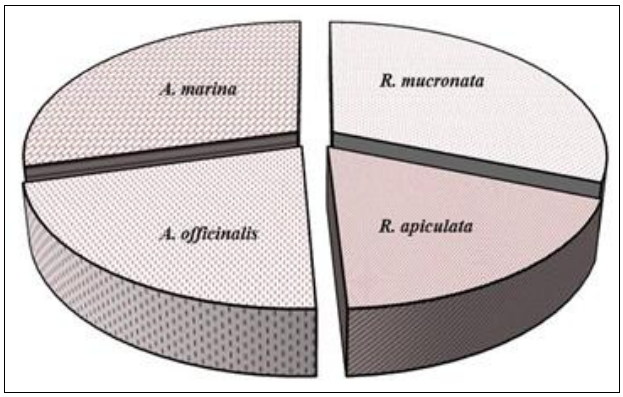

Fig 2: Precentage of extraction between the mangrove species

The leaf of *A. officinalis* showed higher moisture (37.9±1.67 g/100 g), ash (13.1±1.19 g/100 g), fibre (15.26±1.31 g/100g) and carbohydrate (60.25±3.14 g/100 g) content and the maximum accumulation of protein and lipid found in the leaves. In *A. Marina*, the leaf part showed maximum proximate composition with higher accumulation of carbohydrate and the protein accumulated more in the flowers respectively.

Table 1: Phytochemical analysis of artificial mangroves along vellar estuary

S. No	Phytochemicals	*R. mucronata*			*R. apiculata*			*A. officinalis*			*A. marina*		
		Leaf	Flower	Bark	Leaf	Flower	Bark	Leaf	Flower	Bark	Leaf	Flower	Bark
1.	Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
2.	Tannins	+	+	-	+	+	-	+	+	-	+	+	-
3.	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
4.	Anthroquinone	-	-	-	-	-	-	+	-	-	+	-	-
5.	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
6.	Steroids	+	-	-	+	-	-	+	-	-	+	-	-
7.	Phenolics	+	+	-	+	+	-	+	+	-	+	+	-
8.	Catachin	+	-	+	+	-	+	+	-	+	+	-	+
9.	Reducing sugar	+	+	+	+	+	+	+	+	+	+	+	+
10	Glycosides	+	+	-	+	+	-	+	+	-	+	+	-
11.	Saponins	+	-	-	+	-	-	+	-	-	+	-	-
12.	Phlobatannins	+	-	-	+	-	-	+	-	-	+	-	-
13.	Ketoses	+	+	+	+	+	+	+	+	+	+	+	+
14.	Starch	+	+	+	+	+	+	+	+	+	+	+	+
15.	Arginine	+	-	-	+	-	-	+	-	-	+	-	-
16.	Cysteine	+	-	-	+	-	-	+	-	-	+	-	-
17.	Aromartic amino acids	+	+	+	+	+	+	+	+	+	+	+	+
18.	Phenolic aminoacids	+	-	+	+	-	+	+	-	+	+	-	+

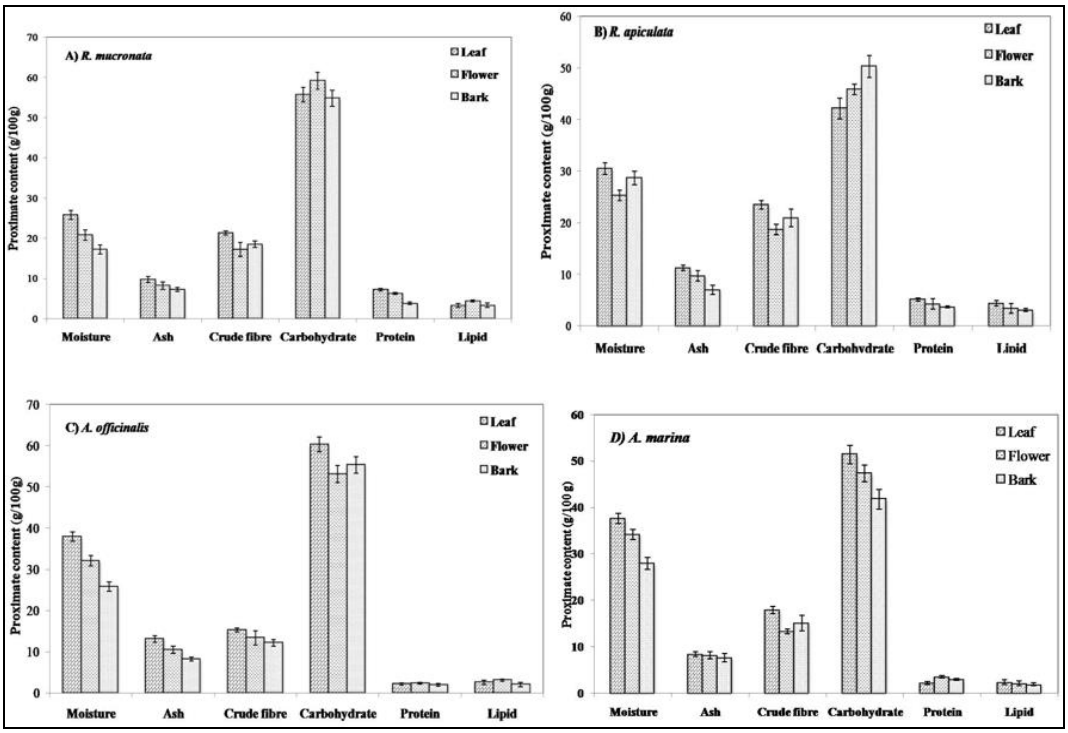

Fig 3: Proximate composition of various parts of different mangroves

The caloric values of the different parts of mangrove extracts were represented in Figure. 4. It was found that, the gross energy and caloric values was found to be higher in the leaves of all mangrove species and the bark exhibited lower energy content (Figure 4. A-D). Among the species, highest gross energy and caloric values were found in *R. mucronata* (86.16 kJ/g; 9.74 kcal/g), followed by *A. marina* (82.19 kJ/g; 8.73 kcal/g), whereas the energy values were found to be lower in *A. officinalis* (8.19 kcal/g).

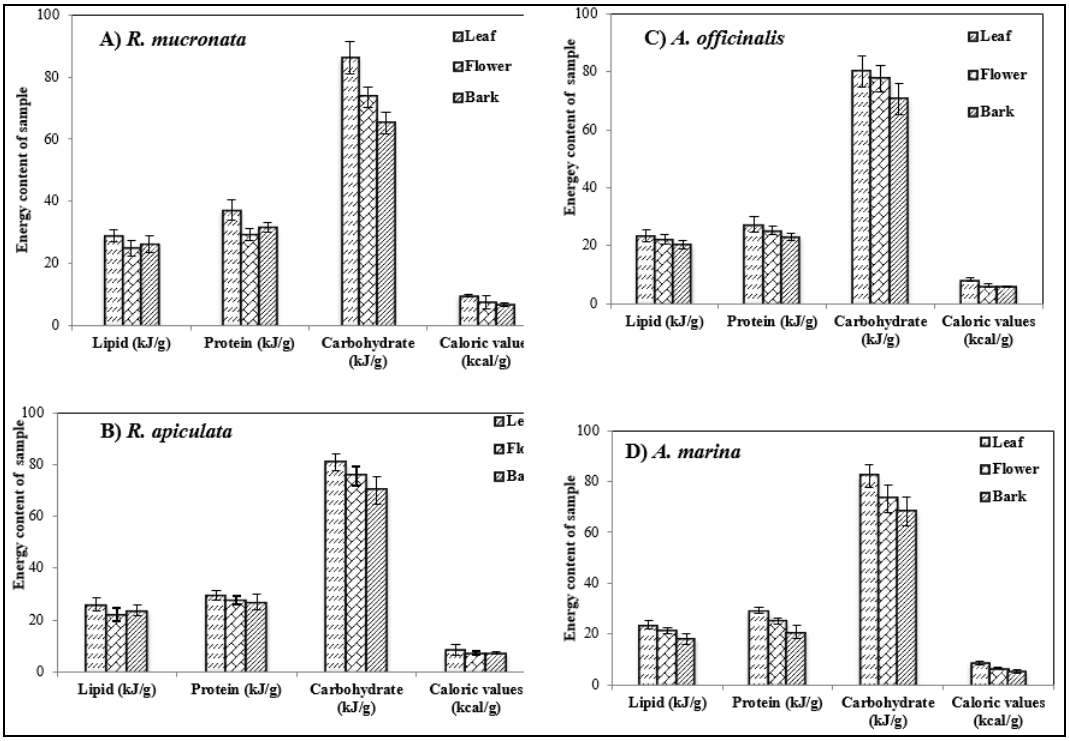

Fig 5: Gross energy values (kilojoules per gram) and caloric values (kilocalories per gram) of the various parts of artificial mangroves

4. Discussion

Mangroves have been reported to have rich phytoconstituents and other photochemicals which plays a vital role in the pharmaceutical sectors (Gupta *et al*., 2011) [9]. Research on the various parts of artificial mangroves in the vellar estuary has not yet been studied and hence the study delineated to analyse the proximate composition and phytochemical constituents of artificial mangroves. The study exhibited the presence of potent phytochemicals which might act as a potent source in the pharmaceutical and industrial sectors. The phytochemicals analysed in the present study found to be present in different parts of all the

species studied except phlobatannins and anthroquinones. Present findings coincide with the earlier report of George *et al*. (2012) [7] and Suganthi and Pandima (2016) [8]. However, their presence was found in *A. mucronata* and *A. apiculata*, which has not been reported so far in the artificial mangroves. The proximate composition of various parts of different mangroves showed that leaf has accumulated highest moisture content, ash, crude fibres in all the species studied. The higher moisture content is due to the high fluid content and the proximity of the leaf acts as source for transpiration. Moisture content in *R. mucronata* found to be lesser than the leafy vegetables and fruits of Indian origin (Singh *et al*., 2001; Ramula and Rao, 2003) [16, 14] and higher when compared to the cereals and pulses (Srikumar, 1993) [17]. Accumulation of protein and lipids found to be higher in the flower of *R. mucronata, A. officinalis* and *A. marina.*

Among the species, higher proximate composition found to be accumulated in *R. mucronata* and lowered in *A. marina*. The content of protein in R. mucronata found to be higher than the other species such as *Suaeda maritime, Lumnitizera racemosa, Avicennia marina, Bruguiera gymnorrhiza, Sonneratia apetala* and *Derris trifoliata* respectively (Bunyapraphatsara *et al*., 2002) [5]. The highest crude fibre was found in the leaves of *R. mucronata* followed by the bark and the flower. The leaf section of the plant play a major role in the plant metabolism, storage of metabolites and biomolecules. Similarly, the lipid contentment was found to be higher in the leaf of *R. mucronata* and the flower of *A. officinalis*. The presence of higher amount of essential oils has been reported in the seeds of mangroves and the crude lipid content of *R. mucronata* found to be more than the reported values (Singh *et al*., 2001) [16]. The soluble dietary fibre found to reduce the blood sugar level and cholesterol level, while insoluble fibre increases fecal bulk and decreases intestinal transit time. The carbohydrate content reported to be higher in the leaves of *R. mucronata*, followed by *A. marina* which coincides with the report of Agu and Okolie (2017) [2].

5. Acknowledgement

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6. Conflict of Interest

The authors declare that there is no conflict of interest

7. References

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