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SCREENING FOR THE TOTAL PHENOLIC CONTENT OF SELECTED MANGROVE SPECIES COLLECTED FROM SUNDARBAN MANGROVE FOREST

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ABSTRACT

In this present research, acetone extracts of *Avicennia alba and Aegialitis rotundifolia* were investigated for the estimation of their total phenolic content. The amount of total phenol present was calculated by assaying it with Folin's Ciocalteau Reagent and spectrophotometric analysis. Gallic acid was taken as the standard compound and the total phenolic content of each of the plant sample was expressed as mg/g Gallic acid equivalent (GAE). The standard curve equation was found out to be y=0.0597x+0.0198; R^2 = 0.9536. The results showed that *A. rotundifolia* had higher total phenolic content (53.23 mg/g GAE) than that of *A. alba* (42.147 mg/g GAE).Since antioxidant property and total phenol content are directly related, these plant leaf extracts could be used for various medicinal and agroeconomic development.

KEYWORDS: Total phenolic content, Folin's Ciocalteau Reagent, Gallic Acid Equivalent, *Avicennia alba, Aegialitis rotundifolia,* Sundarbans.

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INTRODUCTION

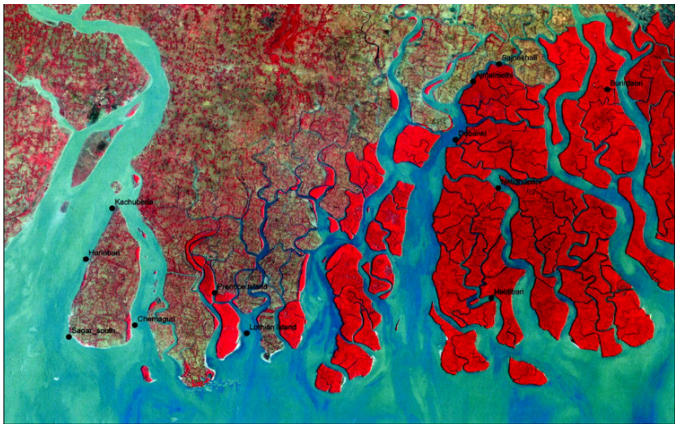
From the earliest days, plants have been looked towards as the source of various anti-pathological compounds that can be efficiently extracted and put to use for the benefit of mankind. They are considered to be the storehouse of various alkaloids, steroids, phenols, tannins etc. which has profound influence in the treatment of various common diseases afflicting other living organisms. There have been many benefits of using plants as the source various drugs. They are relatively safer and the ease of extracting it is more than their synthetic counterpart. The combination of various useful secondary metabolites adds up to the reason why plant extract were chosen as the source of various medicines in the late 1990s. [1] Mangroves are an unique set of trees flourishing in the salty regions especially in the delta region or places near the shore. The uniqueness lies in their pneumatophores or breathing roots which enables them to respire when the soil is too clogged with salty water. Besides this, the mangroves act as a storehouse of a plethora of secondary metabolites which they have to produce in large amounts provided the conditions that they are living in. Little work has been done with the Sunderban forests in India. This is an attempt to look into the total phenolic content of the extracts of both the plants and to correlate the results with some previously found properties of the plant. *A. alba* belonging to the family Avicenniaceae is a tropical mangrove species inhabiting the coastal and estuarine regions of India (Sunderbans), South East Asia, Australia, islands of the Pacific Ocean and Oceania. The leaves are green above and white underneath. They are lanceolated with a pointed tip. The antimicrobial activity of *A. alba* has been investigated upon and was found out to be effective against a wide range of bacteria and fungi including *Aeromonas hydrophila, Streptococcus mutans, Pseudomonas marginales, Aspergillus flavus* etc. [1,2] *A. rotundifolia* belongs to the family Plumbaginaceae. They are native to Bangladesh, India (Sundarbans), Indonesia, Myanmar and Thailand. The leaves are simple, alternate and petiolated. The leaves are characterized by their unique salt excretory mechanism. [3] Organic solvent extracts of the plant was found to have effective antibacterial activity against six virulent strains of bacteria pathogenic to fish. [4] Secondary phenolic metabolite has been an integral part of the plant defense mechanism, involved in a variety of cell processes. They are involved in various responses to abiotic[5,6] and biotic stresses[7,8]. Many of such phenolic compounds are gaining importance regarding human health and agricultural yield.[9] The total phenolic content of the acetone extracts prepared from both the plants were estimated.

MATERIALS AND METHODS

(i) Site Selection

The Indian Sundarbans (between $21^0$13'N and $22^0$40' N latitude and $88^0$03'E and $89^0$07'E longitude) has Bangladesh in the east, the Hooghly River (a continuation of the River Ganga) in the west, the Dampier and Hodges line in the north, and the Bay of Bengal in the south. The important landforms and distinguishable features of deltaic Sundarbans include beaches, mudflats, coastal dunes, sand flats, estuaries, creeks, inlets and mangrove swamps [10].

Map of Indian Sundarbans



(ii) Collection and authentication of Plant Sample

Fresh leaves of *A. alba* (SA) and *A. rotundifolia* (SG) were collected directly from the healthy trees growing in Bali islands of Sundarbans in the month of March,2013. The species were identified and authenticated by Dr. A. Mitra of Dept. Of Marine Science, University of Calcutta.

(iii) Preparation of plant extracts

Freshly collected leaves of *A. alba* and *A. rotundifolia* were surface sterilized by dipping them in 1% $HgCl_2$ for 30 seconds and then transferring them to sterilized water. The samples were then transferred to a hot air oven for drying. Dried-up leaves of each sample were crushed and the appropriate weight of the powder was mixed with measured volume of acetone in a conical flask. (The crushed powder should be completely submerged in acetone.) The entire set was kept undisturbed for one day. Extract was collected and the powders were resuspended in measured volume of acetone. This entire process was repeated three times to collect a sufficient volume of the extract.

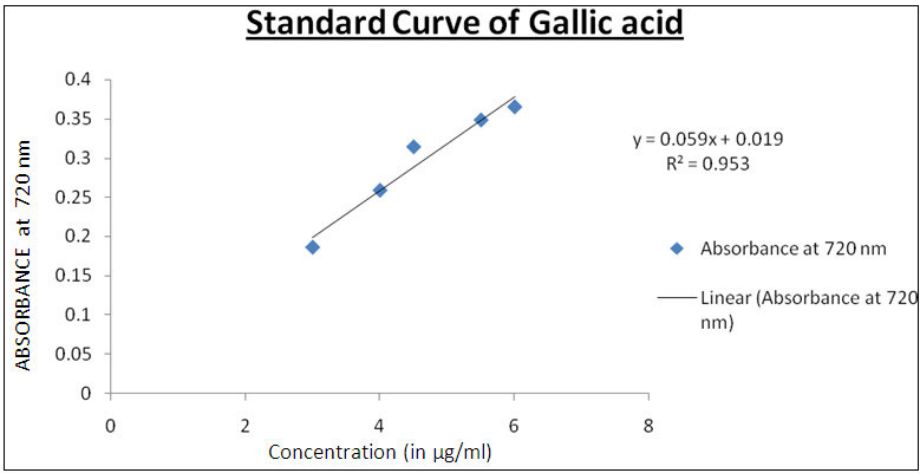
(iv) Total Phenolic Content Estimation

The total phenolic content[11] of the acetone extract of *A. alba* and *A. rotundifolia* were assayed using Folin's Ciocalteau Reagent [11]. Gallic acid stock solution (1 mg/ml) was prepared and various dilutions were obtained from it. Different volumes of both the plant samples and gallic acid were mixed with measured volumes of FCR and the volumes were made up to required amount using distilled water. The mixtures were incubated for about 30 minutes and their absorbance was measured at 720 nm. The calibration curve (Fig. 1) was plotted from the absorbance values of different concentrations of Gallic acid (3, 4, 4.5, 5.5, 6 µg/ml). The Gallic acid equivalent for both the plant sample was then calculated.

RESULTS AND DISCUSSIONS

The total phenolic content of both the plant samples were assayed using Folin's Ciocalteau reagent with Gallic acid as the standard.[11] The concentration of the plant extracts were expressed in terms of Gallic acid equivalent. The absorbance of both the plant samples as well as different concentration of Gallic acid was measured at 720 nm. The absorbance values for *A. alba* (3.398 ppm) was found out to be lower than that of *A. rotundifolia* (4.000 ppm). The total phenolic content of *A. alba* and *A. rotundifolia* was found out to be 42.147 mg/g GAE and 52.23 mg/g GAE respectively (Table. 2). The standard curve for gallic acid was plotted. The standard curve equation was found out to be $y=0.0597x + 0.0198$; $R^2= 0.9536$. This measure is later used in the calculation of mg/g GAE of individual plant extracts.

Graph 1
Absorbance Curve for Gallic Acid used as a Standard.



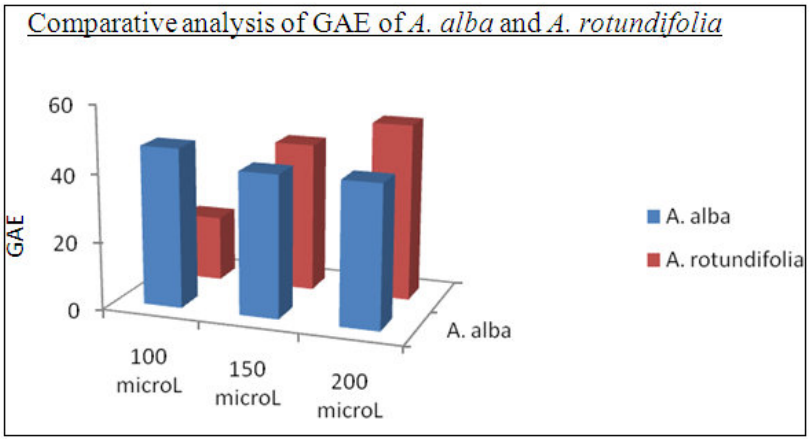
Calibration curve for Gallic acid The standard curve is mostly used as a reference to the unknown samples that is being tested for.

Table 2.
Absorbance Results for different concentrations of Plant Extract

# Sample Name	Absorbance	Stock Concentration (in µg/ml)	GAE (mg/g)
Only extract with ethanol	0.862		
Water	0.000		
SA/2 100 microL	3.699	1000	47.19
SA/2 150 microL	3.398	1000	42.147
SA/2 200 microL	3.398	1000	42.147
SG/2 100 microL	2.036	1000	19.33
SG/2 150 microL	3.523	1000	44.24
SG/2 200 microL	4.000	1000	52.23

From the table (Table 2.), It was found that at comparable concentration of both the plant extracts, *A. rotundifolia* has higher phenolic content than *A. alba*. A comparative analysis of the total phenolic content (GAE) of *A. alba* and *A. rotundifolia* is being demonstrated in the following figure (Figure 2).

Graph 2
Comparative analysis of GAE values of the two plant extracts



Comparative analysis of GAE of *A. alba* and *A. rotundifolia* The total phenolic content of *A. rotundifolia* was compared with the results from other studies on other varieties of mangrove species. *A.rotundifolia* was found to have comparable amounts of phenolics with *Sonneratia apetala* (47.52 GAE)[12], *Ipomoea pes-caprae* (39.12 GAE)[13], *Polygonum minus* (55.5 GAE, aqueous extract)[14], *Sesuvium portulacastrum* (59.04 GAE)[15,] much higher than *Avicennia marina* (24.412 GAE)[16]and much lower than *Excoecaria agallocha* (80.87 GAE)[17]. A comparative analysis of the total phenolic content[18] of the different mangrove species was given below.

Graph 3
A Comparative account of GAE of our plants of interest with results from other papers with different plant species.

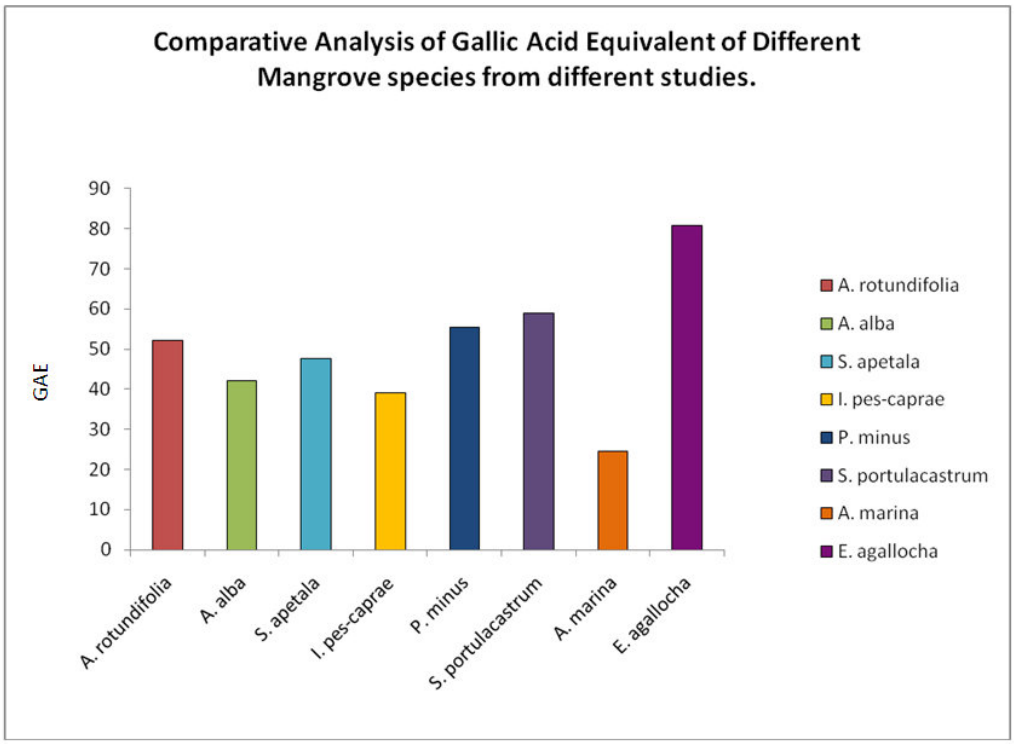


Figure 3.
A comparative analysis of the total phenolic content (Gallic acid equivalent) between A. rotundifolia and other mangrove species.

- Results Obtained
- Results Obtained.
- Banerjee et. al. (2008)
- Banerjee et. al. (2013)
- Qader et. al. (2011)
- Chinnappan et. al. (2013)
- Bharathi et. al. (2011)
- Thirunavukkarasu et. al. (2013)

From this analysis it can be concluded that *A. rotundifolia* and *A. alba* have relatively high level of phenolics. This might result due to the dynamic nature of the coastal environment which imposes a stress on these plant species. Since phenolics are a class of defense compounds being produced in the plant to combat the stress, this can be

considered to be an effective reasons for the abnormally high level of phenolics in these plants. Phenolics are secondary metabolites required in our body. Increase in the total phenolic content is an indication of the total antioxidant property of the plant sample which will play a pivotal role in treating a wide variety of diseases through their radical scavenging activity. Phenolics also have several beneficial biological properties like anti-tumour, anti-inflammatory and anti-microbial properties. As a result, additional works can be done to look into the antimicrobial properties of the plant extracts and to check the other metabolites present in the extract. Stress normally induces a defense response. Elevated level of stress is an indication of a strong level of response to the cause. Phenolics can be used by the plant as a barrier to pathogen infection. Other components of the extract can be profiled to get a list of other metabolites present in the extract.

CONCLUSION

The quantitative phytochemical screening for calculating total phenolic content of *A. rotundifolia* and *A. alba* was performed using Folin-Ciocalteau Reagent. The results of the experiment reveals that the plant extracts (SA/2 and SG/2) contain adequate amount of phenolic contents that can be correlated with the various protective mechanisms in-vivo. It was also found from the results that *A. rotundifolia* has higher phenolic content than *A. alba*. Hence it can be inferred that the plants have upregulated their defence mechanism in response to the dynamic environmental conditions prevalent in the Sundarbans. Further pharmacological activities have to be performed to establish the effectiveness of these secondary metabolites derived from these plants in the field of agroecology and agroeconomy.

Conflict of Interest

Conflict of interest declared none.

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