Research Article

COMPARATIVE STUDIES ON ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACTS OF TRUE MANGROVE AND MANGROVE ASSOCIATE LOCATED IN BHATYE BEACH AREAS OF MAHARASHTRA, INDIA

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ABSTRACT

The present study showed that among the phytochemicals both the mangroves revealed presence of flavonoids, tannins, triterpenes, alkaloids and saponins. Antioxidant and Anti-inflammatory activity of leaves, stems, pods and fruits of ethanolic extract of *Derris trifoliata* and *Sonneratia alba* mangrove species were studied. Total flavonoid content (TFC) was found to be maximum in *S.alba* fruits of ethanolic extract (792.6 mg quercetin equivalent QE/g) as well as the total phenolic content (TPC) was found to be highest (630.39 mg gallic acid equivalent GAE/g). Ethanolic extract at a concentration range (20-100 µg/mL) showed antioxidant and anti-inflammatory assay where free radical and hydrogen peroxide scavenging potentials of *S. alba* fruits IC₅₀ value (62.62 and 66.73 µg/mL) was greater as compared to other extracts respectively. The reducing power of all the extract of both the plant increased dose-dependently in which *S.alba* fruits showed maximum reducing power. Anti-inflammatory activity protects membrane stabilization, protein denaturation and also protease inhibition. In membrane stabilization, *S.alba* fruits revealed higher activity which represented $64.67 \pm 0.05\%$ protection and lower in *D.trifoliata* pods $46.97 \pm 0.06\%$ protection. All the ethanolic extracts displayed inhibition where *S.alba* fruits exhibited $80.07 \pm 0.02\%$ inhibition of proteinase activity. A concentration dependent inhibition of protein (albumin) denaturation where maximum amount of inhibition was found in *S.alba* fruits ($84.67 \pm 0.04\%$ inhibition). Present results confirmed that among the selected parts of mangroves ethanolic fruits extracts of *S.alba* showed more potent antioxidant and anti-inflammatory activities.

Keywords: *Derris trifoliata*, *Sonneratia alba*, Antioxidant, Anti-inflammatory

INTRODUCTION

The oxidative stress is originated by reactive oxygen species (ROS) responsible for majority of the diseases. Antioxidants interrupt the production of ROS and also play a key role to inactivate them. Ascorbic acid very effectively scavenges a wide array of ROS and free radicals. Inflammation is a very important facet in membrane injury. The mechanism of inflammation injury is attributed in part to release reactive oxygen species (ROS) from activated neutrophil and macrophages. This over release leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. Neutralization by radical scavenger antioxidants and anti-inflammation can attenuate inflammation¹.

Medicinal plants have been used for centuries as remedies for human diseases since they have chemical components of therapeutic value². According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The Indian mangroves consist of approximately 65 species belonging to 31 families, throughout the world there are 77 mangrove species. Mumbai Ratnagiri coast has 21 species belonging to 20 genera in which most dominating genera include *Avicennia*, *Sonneratia*, *Rhizophora* and *Acanthus*³. Mangroves adapt their fruits, leaves, stems, roots and their reproductive methods in order to survive in a harsh, dynamic environment of soft, low oxygen soils and varying salinity⁴. True mangroves, *Sonneratia alba* J Smith where in Latin; "*Sonneratia*", after Pierre

Sonnerat, French botanist and explorer; Latin "*alba*" is white, referring to the white stamens of the flowers, it has many commercial applications. *S.alba* sepals shows antioxidant³ and antimicrobial activity⁵. Triterpenes and sterols are found in *S.alba*⁶ Perennial climbing shrub a mangrove associate, *Derris trifoliata* Lour. Where in Greek "*derris*", is a leather covering possibly referring to the tough seed pods, Latin "*trifoliata*" with 3 leaves referring to the species leaflets these are reported to have various medicinal properties like bactericidal^{7, 8}, antidiarrheal⁹, antioxidant¹⁰, antimicrobial¹¹.

The present study deals with phytochemical composition, antioxidant and anti-inflammatory activities of ethanolic extracts of true mangrove *S.alba* and mangrove associate *D.trifoliata*.

MATERIALS AND METHODS

Sample collection Area

D.trifoliata leaves (DL), stem (DS) and pods (DP) and *S.alba* leaves (SL), stem (SS) and fruits (SF) of the mangroves as shown in Fig. (1 and 2) were identified, authenticated by taxonomist and collected from Bhatye beach area located at 16°58'44.0691''N and 73°17'38.7499''E. Ratnagiri District, Maharashtra, India. Fresh plant parts as in Table 1 were separated, washed thoroughly, chopped, dried at 40° C and pulverized.

Extract preparation

Ethanol Extract (EE): 10g plant powder was added in 200mL ethanol which was exhaustively extracted with ethanol by percolation method using the soxhlet apparatus. EE was concentrated by using rotary flash evaporator (Buchi, Japan) to get the residues and lyophilized (Benchtop 2K) to remove the excess organic residues. The residual extract was dissolved in 20mL ethanol for further use. The percentage of the extract was calculated and reported. For ethanolic extract, mangrove selected parts *D.trifoliata* was designated as DL-EE, DS-EE, and DP-EE; for *S.alba* SL-EE, SS-EE, and SF-EE.

Qualitative phytochemical Analysis

Phytochemical analysis of all the extracts were studied as follows^{12, 13}.

Alkaloids: Few drops of Meyer's reagent (Potassium Mercuric chloride solution) were added in 1mL extract. A creamish white precipitate was formed indicating the presence of alkaloids.

Phenol Compounds: 0.5mL 1% Lead acetate and 1% ferric chloride was added to the 1mL extract. A blue black precipitate with lead acetate and green brown with ferric chloride was obtained for both the extracts.

Flavonoids: 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.

Saponins: 5 mL extract was mixed with 20 mL distilled water and then agitated in a graduated cylinder for 15 min. Formation of foam indicates the presence of saponins.

Steroids: 1 mL extract was dissolved in 10 mL chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Terpenoids: 2 mL extract was added to 2 mL acetic anhydride and concentration of H₂SO₄. Formation of blue, green rings indicate the presence of terpenoids.

Tannins: 0.5 g of the extract was dissolved in distilled water and about 10 mL of bromine water added. Decolourization of bromine water indicates presence of tannins.

Reducing sugars: 0.5 mL of ethanol extracts was mixed with Fehling's I and II solutions and boiled for half an hour in water bath. Formation of red precipitation indicates presence of reducing sugars.

Proteins: 0.5 mL of ethanol extract was mixed with equal volume of 5% sodium hydroxide and copper sulphate. Formation of violet color indicates the presence of proteins.

Total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method¹⁴ with slight modification as follows. 10mg/mL extracts were used, the diluted solutions (0.5 mL) were separately mixed with 1.5 mL 95% ethanol, 0.1 mL 10% aluminum chloride, 0.1 mL 1M potassium acetate and 2.8 mL distilled water. After incubation at room temperature for 30 min,

the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-visible spectrophotometer (UV-1800 resolution: 1 nm). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Total phenolic content

Total phenolic content was determined with modification as follows¹⁵. 10mg/mL extracts were used, where 0.125 mL extracts was mixed with 0.5 mL deionized water. 0.125 mL folin-ciocalteau reagent was added and incubated for 5 min at RT followed by addition of 1.25 mL 7% Na₂CO₃ solution. The volume was made up to 4 mL with MilliQ water then incubated for 90 min at RT. Absorbance was recorded at 760 nm and total phenolic content was estimated from a standard gallic acid curve. The concentrations of phenolic content were calculated from the calibration point and expressed mg gallic acid equivalent per gram of sample.

In-vitro antioxidant and anti-inflammatory assays

1mg/mL ethanolic test extracts stock solution, 100µg/mL standard Ascorbic acid-AA for antioxidant assay and Diclofenac sodium -DfS for anti-inflammatory assay were prepared and carried out in triplicate with different concentrations (20, 40, 60, 80, and 100 µg/mL).

DPPH free radical scavenging assay

1, 1-Diphenyl-2-picrylhydrazyl-DPPH (Sigma Aldrich) free radical scavenging potential was determined¹⁶. In the reaction mixture, 1mL of freshly prepared DPPH (200µM dissolved in ethanol) was added and vortexes thoroughly. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. Ascorbic acid as a standard, control everything except sample and measured by UV visible spectrometer Shimadzu, UV-1800.

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Hydrogen peroxide (H₂O₂) assay

The ability to scavenge hydrogen peroxide was estimated with minor modification as follows¹⁷. A solution of hydrogen peroxide (30% w/v Thomas Baker SD Fine Chem, Mumbai) was prepared in 1M phosphate buffer (pH 7.4). In the reaction mixture 60 µL hydrogen peroxide was added. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The control contained all the reagents except sample. Ascorbic acid was used as standard. The percentage of inhibition for H₂O₂ scavenging activity was calculated using the following formula as in eq (1).

Reducing power assay

The reducing power was determined by Oyaizu's method with slight modifications different concentrations, reaction mixture were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL 1 % potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then cooled. Subsequently, 2.5 mL 10 % Trichloroacetic acid (TCA) was added with the above-

mentioned solution and centrifuged at 3000 rpm for 8 min. The collected supernatant was mixed with equal amount of Millipore Milli-Q water. Finally, 1 mL 0.1 % ferric chloride was added with the upper layer and the absorbance was measured at 700 nm¹⁸. The attained results were compared with ascorbic acid which was a positive control.

Human RBC membrane stabilization

Human RBC membrane stabilization bioassay¹⁹ was standardized using diclofenac sodium injection IP (I.M/intragluteal/I.V) 25mg/mL as standard. 4.5mL reaction mixture consisted with 2mL hyposaline (0.25% NaCl), 1mL 0.15M phosphate buffer (pH 7.4) and 1 mL test solution in normal saline, 0.5 mL 10% human RBC in normal saline was added. For control tests, 1mL isosaline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56°C for 30min. Then tubes were cooled under tap water for 20 min, centrifuged and the absorbance of the supernatants was read at 560nm. % protection was calculated as per eq (2).

$$\% \text{ Protection} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

Proteinase inhibitory bioassays

Proteinase inhibitory bioassay is followed as per with modifications²⁰. Initially, concentration of enzyme trypsin 0.06mg/mL, 2% casein and 5% trichloro acetic acid at pH 7.6 were optimized. 3 mL reaction mixture contained 100µL trypsin, 350µL 25mM Tris-HCl buffer (pH 7.4) and 50µL diclofenac sodium were added incubated at 37°C for 5 min. After this 500µL 2% w/v casein were incubated at 37°C for 20 min. 2mL 5% trichloro acetic acid (TCA) was added to terminate the reaction. Cloudy suspension was centrifuged at 5000 rpm for 5 min. Absorbance of the supernatant was observed at 280nm. A control where buffer compensated the volume of standard is measured²⁰. Percentage inhibition at different concentration of the different extracts was calculated as per eq (1).

Protein Denaturation Inhibition bioassays

In-vitro protein denaturation inhibition bioassay was optimized using diclofenac sodium as standard according to Sakat *et al* 2010. In 3mL reaction mixture 50µL samples and 450µL 5% w/v BSA was added. Test tubes were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the test tubes, 2.5mL phosphate buffer saline (pH 6.3) was added to each tube and absorbance was read at 660nm. A control where no drug was added²¹. Percentage protein denaturation inhibition at different concentration of the different extracts was calculated as per eq (1).

RESULTS

The present results have shown rich quantity of flavonoids and phenolic compounds in both the selected mangrove species. These mangrove species also recorded saponins, terpenoids, reducing sugars and proteins. *S.alba* showed great amount of flavonoids and phenols (Table 2). This could be related to more extraction of phytoconstituents from *S.alba* fruits (SF: 20%) followed by (SL: 10%, SS: 18%) as compared to *D.trifoliata* (DL: 11%, DS: 10.2%, DP: 10%) as presented in Table 3. The

biosynthesis capability of flavonoid and phenolic compound in this plant as shown in Table 4, SF-EE total flavonoid content was found to be 792.6 mg QE/g followed by (SS-EE: 486.4; SL-EE: 178.2; DS-EE: 609.9; DL-EE: 119.9 and DP-EE: 124 mg QE/g). The SF-EE showed highest total phenolic content (630.39 mg GAE/g) as compared to other mangrove extracts DS-EE (225.37 mg GAE/g) and DP-EE (254.05 mg GAE/g).

1, 1-Diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H₂O₂) scavenging and reducing power in-vitro antioxidant assay were quantified spectrophotometrically using ascorbic acid as a standard and % inhibition was calculated according to eq (1). Table 5 showed the antioxidant results as per % scavenging activity, In DPPH assay the SF-EE was found to exert the highest inhibition capacity with 74.16 ± 0.03 % inhibition as standard AA exhibited (93.99 ± 0.02%) followed by DS-EE (73.20 ± 0.02 %), SS-EE (50.74 ± 0.03 %), DL-EE (44.44 ± 0.06%), DP-EE (39.77 ± 0.05%) and SL-EE (29.58 ± 0.02%) extracts. Similarly in the hydrogen peroxide H₂O₂ inhibition assay, when compared to standard AA (95.80 ± 0.01%) inhibition the SF-EE extract had shown the greatest inhibition capacity with 68.72 ± 0.02 % inhibition followed by DS-EE (67.79 ± 0.01%), DP-EE (59.26 ± 0.10%), DL-EE (55.56 ± 0.00%), SS-EE (52.38 ± 0.05%) and SL-EE (46.41 ± 0.03%). In DPPH and H₂O₂ assay, IC₅₀ analysis for the both SF-EE was found to have the highest IC₅₀ (62.62 µg/mL and 66.73 µg/mL) when compared with AA (30.08 and 39.66) respectively. Lower the IC₅₀ higher is the activity as represented in Table 5. Reducing power activity of standard ascorbic acid and both the parts of the selected mangrove species was estimated and presented in Fig. (3); where it was found to be directly proportional to the concentration of the samples. As the concentration of the extracts increased reducing power increased. The highest reducing power activity was noted in SF-EE. All these results suggest dose dependent antioxidant potential in both the mangrove species.

The *in vitro* membrane stabilization potential of both the mangroves and its % protection was reported as per eq (2) as shown in Table 6. In this study, the maximum activity was found in the SF-EE at a concentration of 100 µg/mL (64.67 ± 0.05%) and IC₅₀ showed 73.61µg/mL, whereas the standard drug DfS showed (93.79 ± 0.01% protection) (IC₅₀ was 52.65 µg/mL). The other ethanolic extracts showed moderate activity, while DP-EE registered the lowest activity at 46.97 ± 0.06%. In determining trypsin's inhibitory activity, first it is necessary to identify trypsin's activity using trypsin enzyme and casein as substrate. Accurate concentration of both enzyme (0.06mg/mL) and (2%) casein substrate has shown the optimum concentration dependent proteolytic activity. Mangrove extracts exhibited proteinase anti-inflammatory activity at different concentrations as displayed in Table 6 and calculated as per eq (1). SF-EE showed maximum inhibition of 80.07 ± 0.02% at 100µg/mL followed by DS-EE (70.26 ± 0.05%), DP-EE (61.25 ± 0.02%), DL-EE (60.83 ± 0.02%), SL-EE (55.56 ± 0.00%) and SS-EE (51.69 ± 0.05%); DfS presented the maximum inhibition 92.48 ± 0.03% at 100µg/mL. Mangrove extract's ability to inhibit protein denaturation was studied where it was effective in inhibiting heat induced albumin denaturation. SF-EE illustrated maximum inhibition (84.67 ± 0.04%) at 100 µg/mL as compared to other extracts. DfS, a standard anti- inflammation drug showed the maximum inhibition 92.11 ± 0.01% at the concentration of 100 µg/mL as revealed in Table 6.

Figure 1: *D.trifoliata* (a) leaves, (b) stems and (c) pods

Figure 2: *S.alba* (a) leaves, (b) stems and (c) fruits

Figure 3: Reducing power activity

Table 1: Selected mangrove plant details

Sr.No	Mangrove plant name	Family	Common name	Parts used
1.	<i>D.trifoliata</i>	Fabaceae (Leguminosae)	Common Derris, Panlata, Karanj vel, Ketui, Salang	Leaves, Stems, Pods
2.	<i>S.alba</i>	Lythraceae	Perepat, Mangrove apple, Pedada	Leaves, Stems, Fruits

Table 2: Secondary metabolites classes in Mangroves

Sr.No.	Class of Secondary Metabolites	<i>D.trifoliata</i>			<i>S.alba</i>		
		Leaves (DL)	Stem (DS)	Pods (DP)	Leaves (SL)	Stem (SS)	Fruits (SF)
1.	Alkaloids	+	+	-	+	+	+
2.	Phenolic compounds	+	+	+	+	+	+
3.	Flavonoids	+	+	+	+	+	+
4.	Saponins	+	+	-	+	-	-
5.	Steroids	+	+	-	+	+	+
6.	Terpenoids	+	+	+	+	+	+
7.	Tannins	+	+	+	+	+	+
8.	Reducing sugars	+	+	+	+	+	+

+ = present; - = absent

Table 3: Percentage of Extracts

Sr.No.	Plant Samples	Plant parts	Percentage of the ethanolic Soxhlet extract
1.	<i>D. trifoliata</i>	DL	11%
2.		DS	10.2%
3.		DP	10%
4.	<i>S.alba</i>	SL	10%
5.		SS	18%
6.		SF	20%

Table 4: Total flavonoid and phenolic content

Sr.No.	Samples	Total Flavonoid Content mg QE/g	Total Gallic acid Content mg GAE/g
1.	DL	119.9	225.37
2.	DS	609.9	422.5
3.	DP	124	254.05
4.	SL	178.2	318.56
5.	SS	486.4	444.01
6.	SF	792.6	630.39

Table 5: Antioxidant Activities

Sr.No. Samples	Concentration in µg/mL	% Inhibition DPPH Assay	IC ₅₀ Values (µg/mL)	% Inhibition Hydrogen peroxide Assay	IC ₅₀ Values (µg/mL)
1. AA	20	30.08 ± 0.05	30.08	29.73 ± 0.03	39.66
	40	50.45 ± 0.06		40.24 ± 0.01	
	60	67.57 ± 0.06		61.86 ± 0.03	
	80	87.69 ± 0.06		87.09 ± 0.03	
	100	93.99 ± 0.02		95.80 ± 0.01	
2. DL-EE	20	19.61 ± 0.03	122.12	15.19 ± 0.05	83.38
	40	24.51 ± 0.06		33.33 ± 0.01	
	60	29.74 ± 0.03		40.74 ± 0.04	
	80	36.27 ± 0.05		48.89 ± 0.03	
	100	44.44 ± 0.06		55.56 ± 0.00	
3. DS-EE	20	15.69 ± 0.01	70.90	19.10 ± 0.03	69.12
	40	28.10 ± 0.03		34.83 ± 0.03	
	60	36.93 ± 0.01		44.57 ± 0.01	
	80	56.86 ± 0.02		56.55 ± 0.03	
	100	73.20 ± 0.02		67.79 ± 0.01	
4. DP-EE	20	9.09 ± 0.05	130.34	21.11 ± 0.02	78.55
	40	16.67 ± 0.03		33.70 ± 0.01	
	60	21.21 ± 0.03		42.96 ± 0.02	
	80	30.68 ± 0.03		50.00 ± 0.07	
	100	39.77 ± 0.05		59.26 ± 0.10	
5. SL-EE	20	7.08 ± 0.04	174.81	8.02 ± 0.04	114.16
	40	10.00 ± 0.02		18.99 ± 0.02	
	60	14.17 ± 0.02		19.83 ± 0.04	
	80	22.92 ± 0.03		33.33 ± 0.03	
	100	29.58 ± 0.02		46.41 ± 0.03	
6. SS-EE	20	11.85 ± 0.01	105.52	18.57 ± 0.06	87.70
	40	15.56 ± 0.01		26.19 ± 0.02	
	60	27.41 ± 0.04		39.52 ± 0.02	
	80	34.44 ± 0.01		50.00 ± 0.04	
	100	50.74 ± 0.03		52.38 ± 0.05	
7. SF-EE	20	19.85 ± 0.02	62.62	19.23 ± 0.05	66.73
	40	35.58 ± 0.02		36.41 ± 0.05	
	60	50.56 ± 0.02		45.90 ± 0.02	
	80	61.05 ± 0.02		59.23 ± 0.05	
	100	74.16 ± 0.03		68.72 ± 0.02	

Table 6: Anti-inflammatory Activities

Sr. No. Sample	Concentration in µg/mL	% Protection Membrane Stabilization	IC ₅₀ Values (µg/mL)	% Inhibition Proteinase Inhibitory	IC ₅₀ Values (µg/mL)	% Inhibition Protein denaturation	IC ₅₀ Values (µg/mL)
1. Df-S	20	23.33 ± 0.01	52.65	16.34 ± 0.04	50.42	28.32 ± 0.02	43.85
	40	41.48 ± 0.03		29.41 ± 0.01		50.18 ± 0.02	
	60	52.96 ± 0.03		75.82 ± 0.02		67.03 ± 0.01	
	80	69.63 ± 0.03		85.95 ± 0.01		73.12 ± 0.03	
	100	93.70 ± 0.01		92.48 ± 0.03		92.11 ± 0.01	
2.DL-EE	20	10.37 ± 0.01	85.99	3.33 ± 0.05	84.47	7.10 ± 0.02	121.87
	40	19.63 ± 0.03		11.67 ± 0.01		9.63 ± 0.03	
	60	40.74 ± 0.06		25.00 ± 0.04		21.82 ± 0.03	
	80	48.15 ± 0.06		53.33 ± 0.04		29.80 ± 0.02	
	100	54.81 ± 0.01		60.83 ± 0.02		41.57 ± 0.06	
3. DS-EE	20	11.59 ± 0.02	79.11	19.28 ± 0.02	66.19	19.00 ± 0.02	66.45
	40	22.10 ± 0.01		34.31 ± 0.05		26.16 ± 0.02	
	60	33.33 ± 0.02		47.71 ± 0.04		44.80 ± 0.04	
	80	47.83 ± 0.01		58.82 ± 0.03		57.71 ± 0.01	
	100	68.48 ± 0.02		70.26 ± 0.05		78.14 ± 0.01	
4. DP-EE	20	10.98 ± 0.08	110.81	9.17 ± 0.03	78.89	13.25 ± 0.01	103.13
	40	17.80 ± 0.07		19.58 ± 0.04		10.44 ± 0.01	
	60	28.03 ± 0.06		44.58 ± 0.05		23.29 ± 0.02	
	80	34.09 ± 0.07		51.25 ± 0.01		36.95 ± 0.03	
	100	46.97 ± 0.06		61.25 ± 0.02		52.61 ± 0.01	
5. SL-EE	20	8.10 ± 0.04	89.34	11.48 ± 0.02	94.51	18.33 ± 0.02	74.18
	40	12.38 ± 0.01		18.15 ± 0.06		24.67 ± 0.03	
	60	24.29 ± 0.04		28.52 ± 0.11		36.33 ± 0.04	
	80	42.38 ± 0.02		40.74 ± 0.12		55.00 ± 0.03	
	100	61.90 ± 0.06		55.56 ± 0.00		69.00 ± 0.01	
6. SS-EE	20	12.32 ± 0.01	74.88	10.49 ± 0.02	95.65	19.00 ± 0.03	65.54
	40	21.74 ± 0.03		14.23 ± 0.10		29.03 ± 0.01	
	60	38.41 ± 0.02		29.96 ± 0.08		46.24 ± 0.01	
	80	53.99 ± 0.01		43.82 ± 0.07		64.16 ± 0.02	
	100	69.20 ± 0.02		51.69 ± 0.05		80.65 ± 0.01	
7. SF-EE	20	17.33 ± 0.05	73.61	11.44 ± 0.02	66.06	16.67 ± 0.03	58.80
	40	27.33 ± 0.03		24.84 ± 0.02		30.00 ± 0.04	
	60	40.00 ± 0.02		40.20 ± 0.01		52.33 ± 0.03	
	80	58.00 ± 0.02		66.34 ± 0.02		71.67 ± 0.08	
	100	64.67 ± 0.05		80.07 ± 0.02		84.67 ± 0.04	

DISCUSSION

Mangroves plays a vital role in stabilizing, the degree of resilience of mangrove forests to large, infrequent disturbance (tsunamis) and their role in coastal protection, and to chronic disturbance events (climate change), they also provide habitat for a wide range of species, some of which occur only in the mangroves²². Due to the survival in harsh conditions, mangroves synthesizes very diverse group of secondary metabolites. These secondary metabolites are needed for the plants to interact with their environment for the protection against biotic or abiotic stresses such as infections, wounding, UV irradiation, exposure to ozone, pollutants, and herbivores. The medicinal potential of plant species is related to the presence of quality and quantity of the phytoconstituents. The phenolic compounds such as flavonoids, isoflavones, flavones, anthocyanins, coumarins, served as source of antioxidant. The phytochemical analysis have shown presence of most of these principles in the selected mangrove species, it differentiate in quality and quantity. In this study we examined total phenolics and flavonoids which were in accordance with other species of mangroves like *Ceriops decandra* and *Bruguiera cylindrica* L²³. The antioxidant potential of these selected mangrove species are in accordance with the presence of phytoconstituents responsible for antioxidant properties²⁴. A few mangroves species have shown antioxidant²⁵ and in-vivo anti-inflammatory properties²⁶. The extracts of ethyl acetate and water showed antioxidant activity in leaves, trunk and bark of *S.alba* species²⁷. A few other species

have shown anti-inflammatory activities²⁸. All these reports are in agreement with the present findings. Therefore amongst all the parts of selected mangrove species ethanolic extracts of *S.alba* fruits showed highest potential than stem and leaves and also amongst *D.trifoliata* stem, leaves and pods and can be used as potential source for antioxidant and anti-inflammatory activity. Also there is a need to study the different plant parts of both the mangroves in other organic solvents apart from the solvent employed in our study.

CONCLUSION

The phytochemical analysis of mangroves *D.trifoliata* and *S.alba* have shown that both the species are rich in flavonoids and phenolic compounds. The results of this study demonstrated that the true mangrove *S.alba* edible fruits contain considerable amount of phytoconstituents, antioxidant and anti-inflammatory properties as compared with mangrove associate *D.trifoliata*. The fraction of potent extracts served as a free radical inhibitors and stabilized the red blood cell membrane as well as inhibited the heat induced albumin denaturation and proteinase activity. However, further work in this way could lead to the discovery of potent bioactive principles from mangrove associate *D.trifoliata* and true mangrove *S.alba*. Thus, the above mentioned promising species deserved to be potent nutritional antioxidant and anti-inflammatory drug which can be sources based on various diseases.

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