

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *PTEROCARPUS SANTALINUS* L.F. PLANT PARTS

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ABSTRACT

Objective: Oxidation is a normal process in many biological metabolisms in living beings, but in this technology era radiation, pollution, irregular life style, and unhealthy diet leads to excessive oxidation resulting in imbalance between antioxidants and free radicals in body called oxidative stress, which may result in cardiac problems, diabetes, and carcinogenic diseases. Therefore, exploration of natural antioxidants in flora and fauna is becoming important. In this motive a phytochemical study was taken up on various parts of *Pterocarpus santalinus* L.f. plant belonging to Leguminosae family to check the presence of antioxidant activity (AA).

Methods: The leaves, flowers, fruits, and roots of this plant were extracted with six different organic solvents from polar to non-polar. To estimate the AA of these extracts, four types of antioxidant tests were done on them, namely, α , α - Diphenyl - β - Picryl - Hydrazyl free radical scavenging activity (DPPH RSA) assay, relative reducing power (RRP) assay, Ferric thiocyanate (FTC) assay, and β - carotene bleaching assay with Butylated Hydroxy Toluene (BHT) as synthetic standard.

Results: In extraction, Me extracts showed highest yields. Qualitative phytochemical screening has revealed many bioactive components in the Me and Et extracts of plant parts majorly. In DPPH RSA assay, Me extracts of leaves showed RSA activity almost equal to reference standard. In RRP assay, Me floral extracts showed AA higher than standard. FTC and β - Carotene assays showed that Me sample extracts possess good AA than BHT. In FTC assay, lower absorption values specify high AA, whereas in β - Carotene assay, high absorption values indicate high AA of the sample extracts.

Conclusion: Among six solvents methanol showed highest extraction efficiency and also revealed highest number of phytochemicals in its extracts. These four AA assays revealed that the methanol extracts of all plant parts, that is., leaves, roots, fruits, and flowers possess better AA compared other solvent extracts.

Keywords: *Pterocarpus santalinus* L.f., Phytochemical screening, Antioxidant activity, α , α - Diphenyl - β - picryl - hydrazyl radical scavenging activity assay, Relative reducing power assay, Ferric thiocyanate assay, β - carotene bleaching assay, Butylated Hydroxy Toluene.

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INTRODUCTION

With many limitations, the advanced contemporary sciences are now in retrospection of ancient conventional medicines and methods. Even people in sophisticated countries are changing their life style toward traditional compartment for hale and hearty longevity. Due to these changes, our Indian ayurvedic medicines and yogic practices are becoming prominent currently. Phytochemicals present in plants acts as natural curers for many diseases and health ailments [1]. These phytochemicals have antioxidant [2], antifungal [3], and antimicrobial [4] potentials naturally. The secondary metabolites which show antioxidant activity (AA) include polyphenols [5], ascorbic acid [6], anthocyanins [7], and flavonoids [8]. *Pterocarpus santalinus* L.f. is an ancient traditional medicinal plant in India, with many medicinal values. A preliminary phytochemical analysis was done on leaves, fruits, flowers, and roots extracts of this plant with six different solvents, namely, methanol (Me), ethanol (Et), ethyl acetate (EA), chloroform (Ch), carbon tetrachloride (CC), and n - hexane (He), which revealed the presence of several phytochemicals. Four different antioxidant assays were done on these extracts in different concentrations to assess their AA.

METHODS

Collection

P. santalinus L.f plant leaves, flowers, fruits, and roots were collected from Seshachalam hills, Tirupati, Andhra Pradesh, India, which were shown in Fig. 1. Authentication of the plant was done by Prof B. Ravi

Prasad Rao, the plant sample was deposited in herbarium of Botany Dept. possessing a serial no-48799 in Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh, India.



Fig. 1: Collected plant parts of *Pterocarpus santalinus* L.f. (a) Leaves (b) flowers (c) fruits (d) roots

Extraction

P. santalinus L.f. plant parts were cleaned with fresh water thoroughly and dried up in shade completely, then pulverized into powder. Five hundred grams of powder of each plant part were extracted with 1500 ml of solvent for 24 h with intermittent stirring. It was filtered with Whatman filter paper and the remainder was again extracted with the same solvent till the solution becomes pale in color. The solution of same solvent was pooled up and the solvent was recycled on a Heidolph Rota-evaporator below 40°C to give extract. Likewise each plant part was extracted with six different solvents, namely, Me, Et, Ea, Ch, CC, and He. The yields and extraction efficiencies of all solvents were shown in Table 1.

Qualitative phytochemical screening [9]

A qualitative phytochemical study was done on all solvent extracts of plant parts to identify the phytochemicals consisting and the results were presented in Table 2.

AA assessments

α , α - Diphenyl - β - picryl - hydrazyl (DPPH) free radical scavenging assay

DPPH assay was proposed by Von Gadov *et al.* [10] to assess the radical scavenging activity (RSA) of an extract. One milliliter of various concentrations (5, 10, 15, 20, and 25 mg/ml) of extract was prepared in Me. To 1 ml of the sample solution, 1 ml of 0.2 mM DPPH Me solution was added and absorption was read at 517 nm immediately. The absorption of 2 ml Me, which acts as a control was also read at 517 nm. The mixture was then vortexed and incubated for 16 min. The decrease in absorption was measured at 517 nm after 16 min. Butylated Hydroxy Toluene (BHT) Me solution was used as standard reference. Likewise this assay was performed for six solvent extracts of all plant parts and inhibition percentages of extracts were measured and shown as a graphical representation in Fig. 2. The percentage of DPPH RSA of extract was calculated [11] as follows:

$$\% \text{ Inhibition} = [(A_{c(0)} - A_{A(t)}) / A_{c(0)}] \times 100 \quad \text{Eq (i)}$$

Where $A_{c(0)}$ is the absorbance of control at $t = 0$ min, $A_{A(t)}$ is the absorbance of antioxidant at $t = 16$ min.

Relative reducing power (RRP) assay

RRP assay was proposed by Oyaizu [12], which was used to evaluate the RRP of the sample. Here five concentrations (25, 50, 100, 150, and 200 $\mu\text{g/ml}$) of extract were prepared using Me. 2.5 ml of phosphate buffer (0.2 M, pH - 6.6) and 2.5 ml of potassium ferric cyanide (1%) solution was mixed with 1 ml of extract, then incubated for 20 min at 50°C. By the end of incubation, 2.5 ml of 10% trichloroacetic acid was added and the above mixture was centrifuged at 5000 rpm for 10 min. One milliliter of the upper layer was added with 3 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance was read at 700 nm. BHT was used as standard antioxidant; the RRP of the six solvent extracts of all plant parts was assessed by this method and represented graphically in Fig. 3. The percentage of RRP of the extract was calculated using the below equation,

$$\% \text{ of RRP calculation} = [(A_A / A_C) - 1] \times 100 \quad \text{Eq (ii)}$$

Where A_A = the absorbance of antioxidant at 700 nm, A_C = the absorbance of control at 700 nm.

Ferric thiocyanate (FTC) assay

FTC assay [13] was used to estimate the ability of the sample to inhibit decomposition of peroxides. Four different concentrations (25, 50, 100, and 200 $\mu\text{g/ml}$) of the extract were prepared in Et. 4 ml of this extract was added with 4.1 ml of 2.5% ethanolic linoleic acid and 8 ml of 0.2 mM phosphate buffer and the above mixture was made up to 25 ml with distilled water. This mixed solution was kept in a closed vial at a constant temperature of 40°C in oven for incubation. To the 0.1 ml of the above solution, 9.7 ml of 75% Et and 0.1 ml of 30% ammonium thiocyanate were added. Exactly after 3 min, 0.1 ml of 20 mM ferrous chloride in 3.5% HCl was added. The resulted red color absorption was read at 500 nm successively for consecutive 7 days with 24 h of interval.

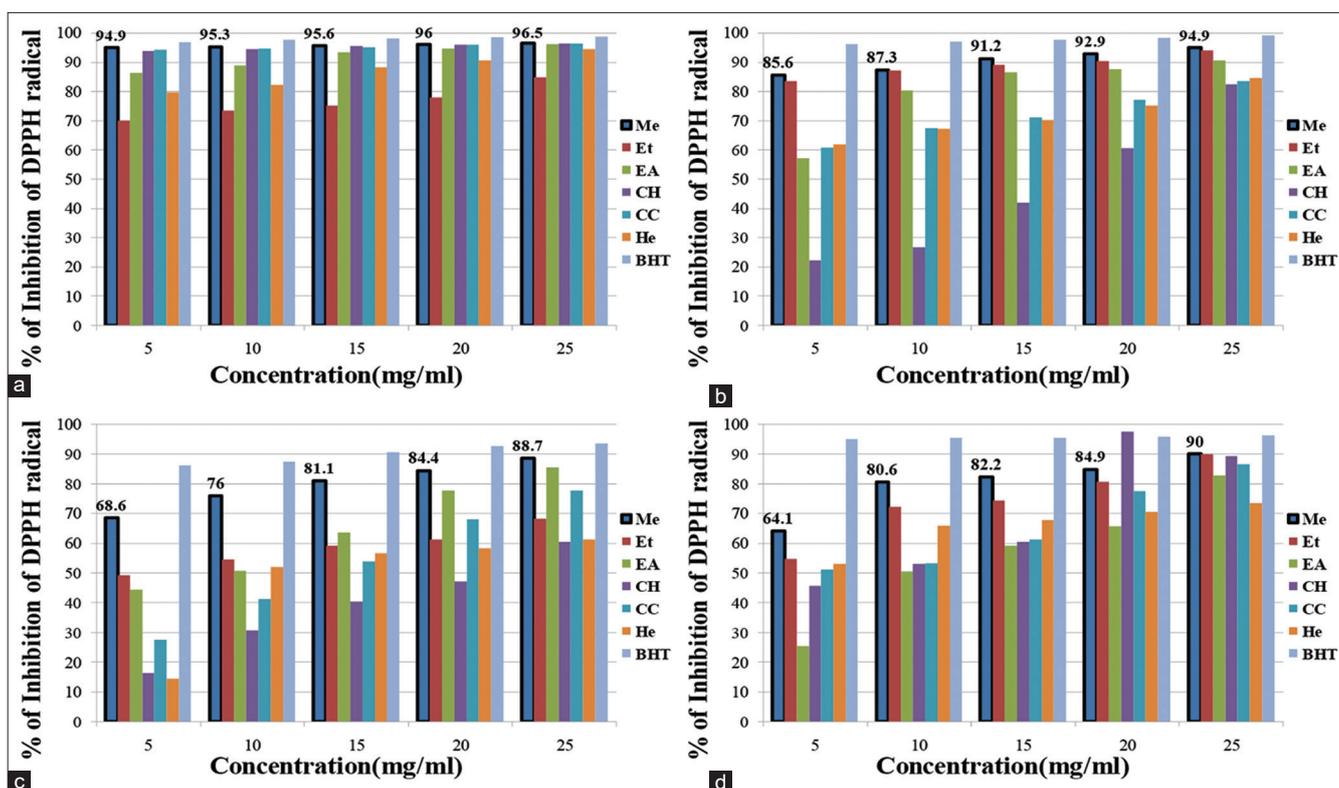


Fig. 2: DPPH assay of *Pterocarpus santalinus* L.f. Plant parts. *(a) Leaves (b) flowers (c) fruits (d) roots

Distilled water was used as control and BHT was used as standard antioxidant reference. All solvent extracts of plant parts were assessed in this method and showed in graphical form in following figures from Figs. 4-7.

β - Carotene bleaching assay

β - Carotene bleaching assay was proposed by Taga *et al.* [14]. Three milligrams of *β* - carotene were dissolved in 3 ml of Ch, 20 mg of linoleic acid, and 200 mg of tween - 40 were added. Then, Ch was removed by rota-evaporator at 50°C for few minutes and made up to 50 ml with oxygenated water by mixing well to form an emulsion. One immediate reading of emulsion absorption was measured at 470 nm. The emulsion prepared as above without *β* - carotene was acted as blank. One milliliter of the *β* - carotene linoleic acid emulsion was mixed with 1 ml of extract sample in different concentrations (50, 125, 250, and 500 µg/ml) and the above mixture was incubated at 50°C for 1 h. Absorbance of that mixture was read at standard intervals of 15 min at 470 nm in UV-visible spectrophotometer. Here distilled water was used as control and

BHT was used as standard reference. All plant part extracts were tested in this method and the absorption values were represented in graphs from Figs. 8-11.

RESULTS

Table 1 gives the results of extraction efficiencies and yields. Table 2 indicates the results of phytochemical screening. Fig. 2 represents the DPPH assay results graphically. Fig. 3 represents the Relative reducing power (RRP) assay results graphically. Figs. 4 - 7 represent the Ferric ThioCyanate (FTC) assay results graphically. Figs. 8 - 11 represent the *β* - Carotene bleaching assay results graphically.

DISCUSSION

Yields and extraction efficiencies

In Table 1, among six solvents Me and Et solvents showed high extraction efficiencies and yields. Me showed highest extraction efficiencies of 42%, 37%, and 41.3% of leaf, flower, and root extracts with yields

Table 2: Qualitative phytochemical screening of *Pterocarpus santalinus* L.f. plant parts

Phytochemical tests	Leaf Me extract						Flower Me extract						Fruit Me extract						Root Me extract					
	*1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Alkaloids																								
Mayer's reagent	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Wagner's reagent	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Hager's reagent	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Dragendorff's reagent	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Carbohydrates and glycosides																								
Molisch's test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-
Fehling's test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-
Barfoed's test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-
Benedict's test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-
Borntrager's test	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Legal's test	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Saponins																								
Foam test	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Proteins and amino acids																								
Millon's reagent	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Biuret reagent	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Ninhydrin reagent	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytosteroids																								
Liebermann's-Burchard's test	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
Fixed oils and fats																								
Spot test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponification test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Phenolic compounds and flavonoids																								
Ferric chloride test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Gelatin test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Lead acetate test	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Alkaline reagent	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-
Mg and HCl reduction	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
Gums and mucilages																								
Alcohol-95% test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*1 - Methanol, 2 - Ethanol, 3 - Ethyl acetate, 4 - Chloroform, 5 - Carbon tetrachloride, 6 - n- Hexane, (+) denotes positive, (-) denotes negative

Table 1: Yields of *Pterocarpus santalinus* L.f. plant parts and extraction efficiencies of the solvents

Solvent	Yield (g)				Extraction efficiency (%)			
	Leaf	Flower	Fruit	Root	Leaf	Flower	Fruit	Root
Me	210.0	185.0	140.0	206.5	42.0	37.0	28.0	41.3
Et	147.0	178.0	197.5	206.5	29.4	35.6	39.5	41.3
EA	44.5	53.5	40.5	23.5	8.9	10.7	8.1	4.7
Ch	42.5	34.0	40.5	29.5	8.5	6.8	8.1	5.9
CC	30.0	27.25	46.5	19.5	6.0	5.45	9.3	3.9
He	26.0	22.25	35.0	14.5	5.2	4.45	7.0	2.9

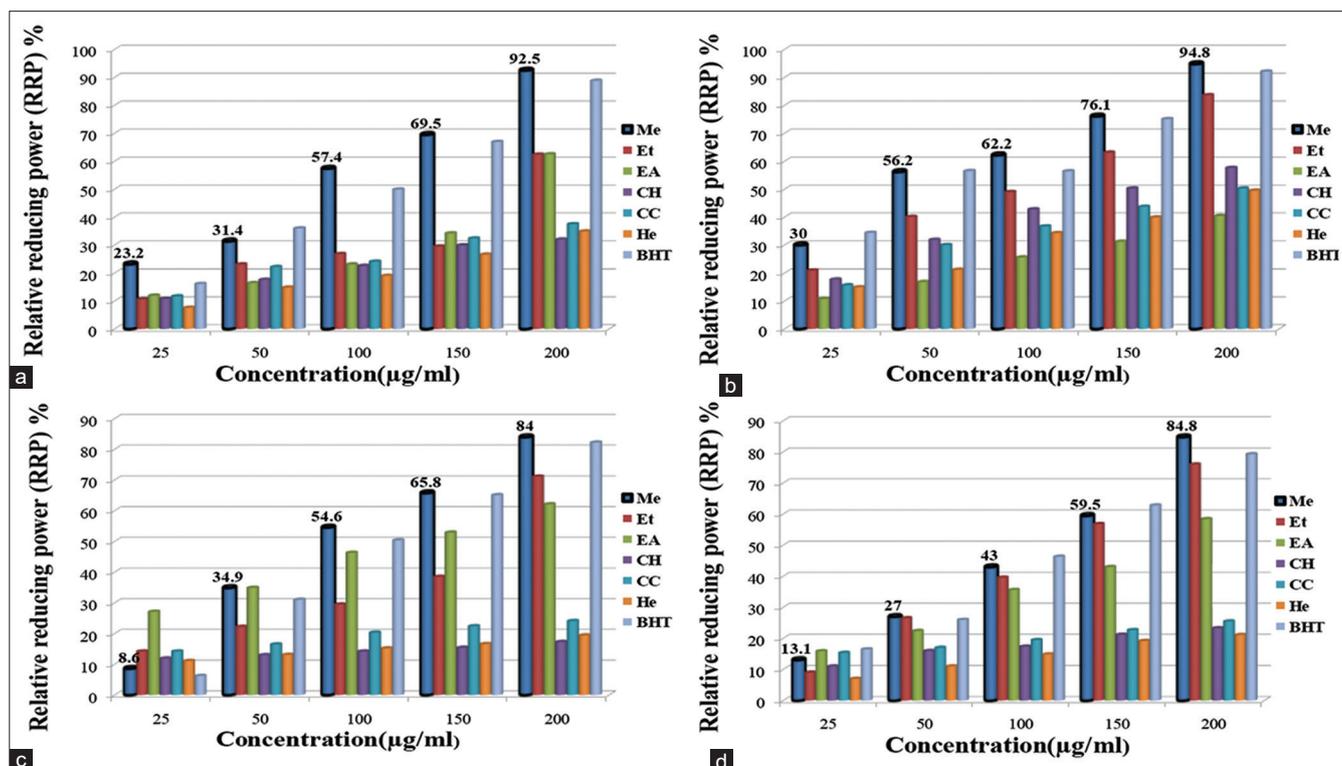


Fig. 3: Relative reducing power assay of *Pterocarpus santalinus* L.f. Plant parts. *(a) Leaves (b) flowers (c) fruits (d) roots

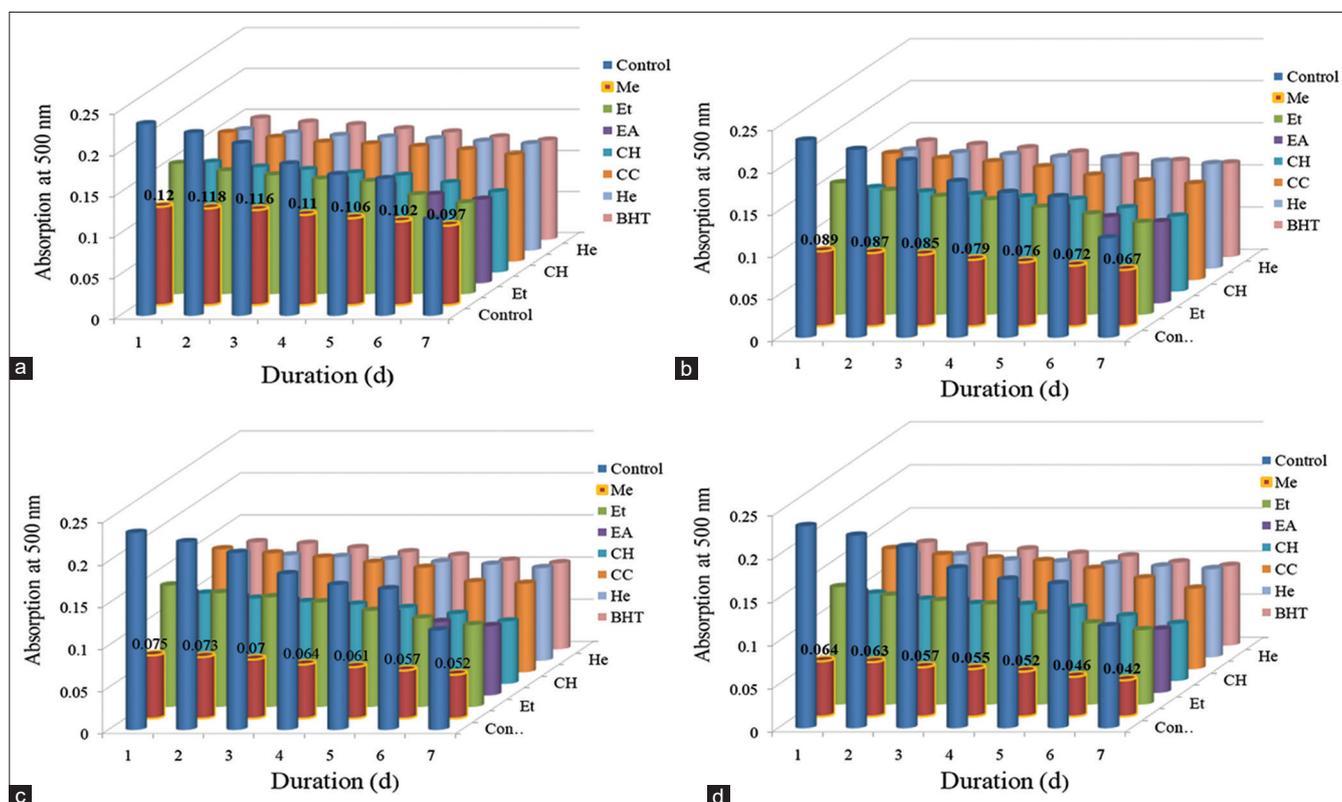


Fig. 4: Ferric thiocyanate assay of *Pterocarpus santalinus* L.f. leaves at different Conc. (a) 25 µg/ml (b) 50 µg/ml (c) 100 µg/ml (d) 200 µg/ml

210 g, 185 g, and 206.5 g, respectively, whereas Et showed extraction efficiencies of 39.5% and 41.3% of fruit and root extracts with yields 197.5 g and 206.5 g, respectively.

Qualitative phytochemical screening

In qualitative phytochemical screening, the Me and Et extracts of all plant parts showed the existence of all types of primary and secondary

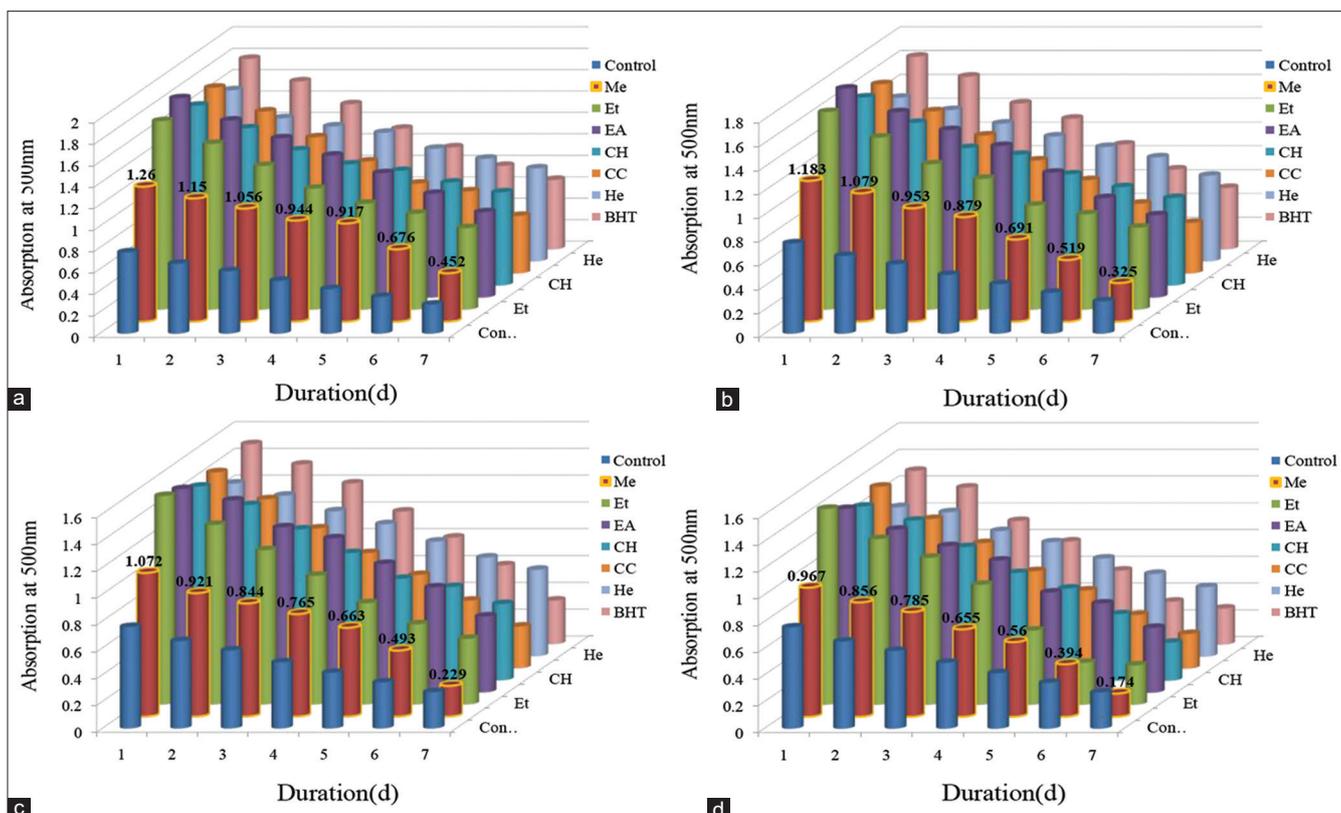


Fig. 5: Ferric thiocyanate assay of *Pterocarpus santalinus* L.f. flowers at different Conc. (a) 25 µg/ml (b) 50 µg/ml (c) 100 µg/ml (d) 200 µg/ml

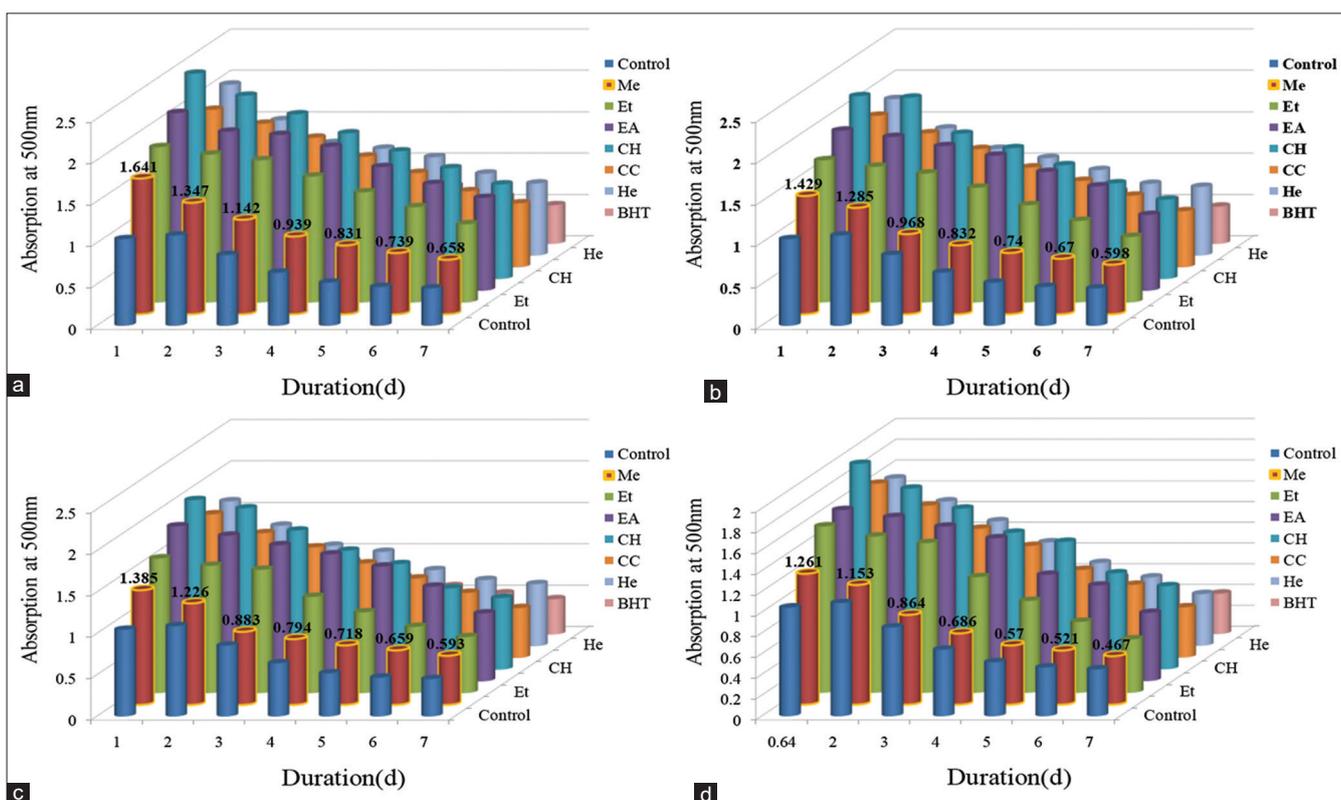


Fig. 6: Ferric thiocyanate assay of *Pterocarpus santalinus* L.f. fruits at different Conc. (a) 25 µg/ml (b) 50 µg/ml (c) 100 µg/ml (d) 200 µg/ml

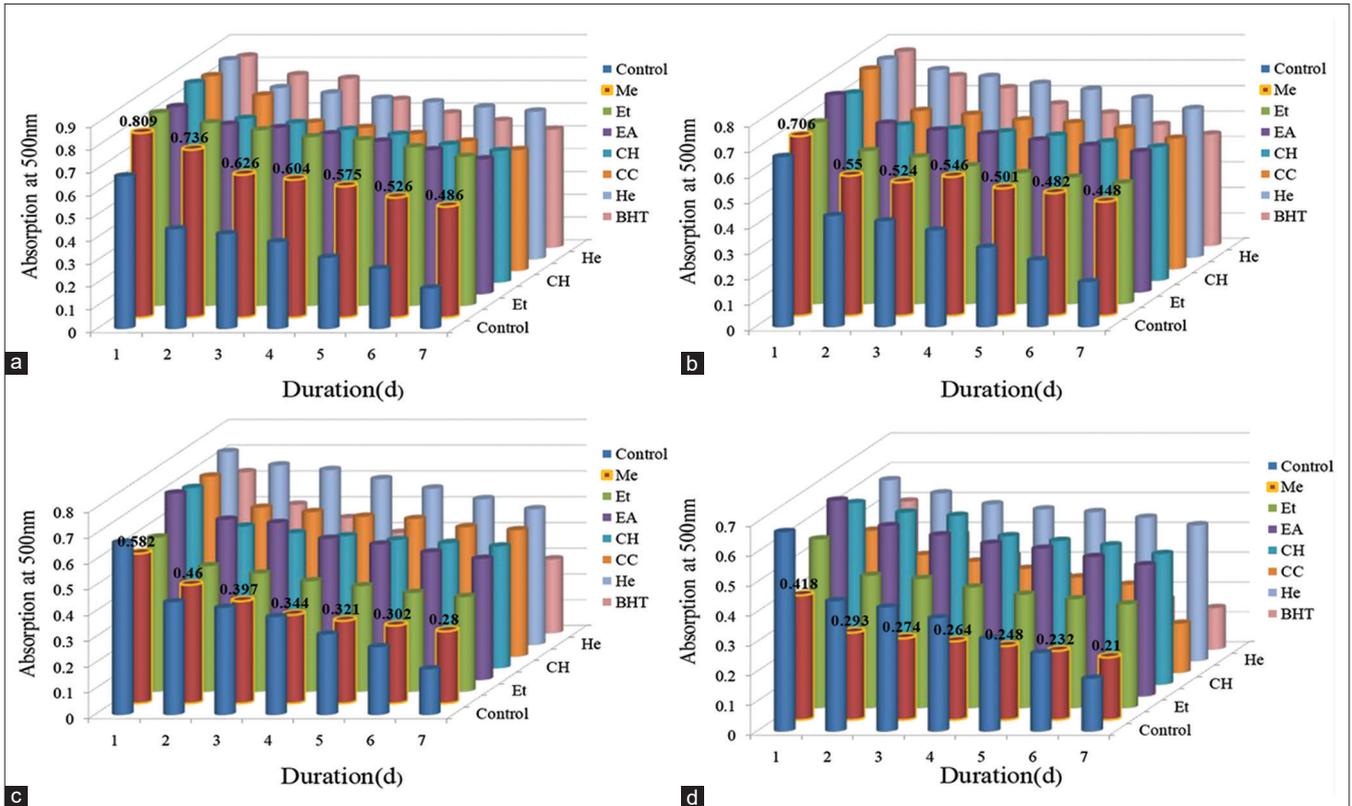


Fig. 7: Ferric thiocyanate assay of *Pterocarpus santalinus* L.f. roots at different Conc. (a) 25 µg/ml (b) 50 µg/ml (c) 100 µg/ml (d) 200 µg/ml

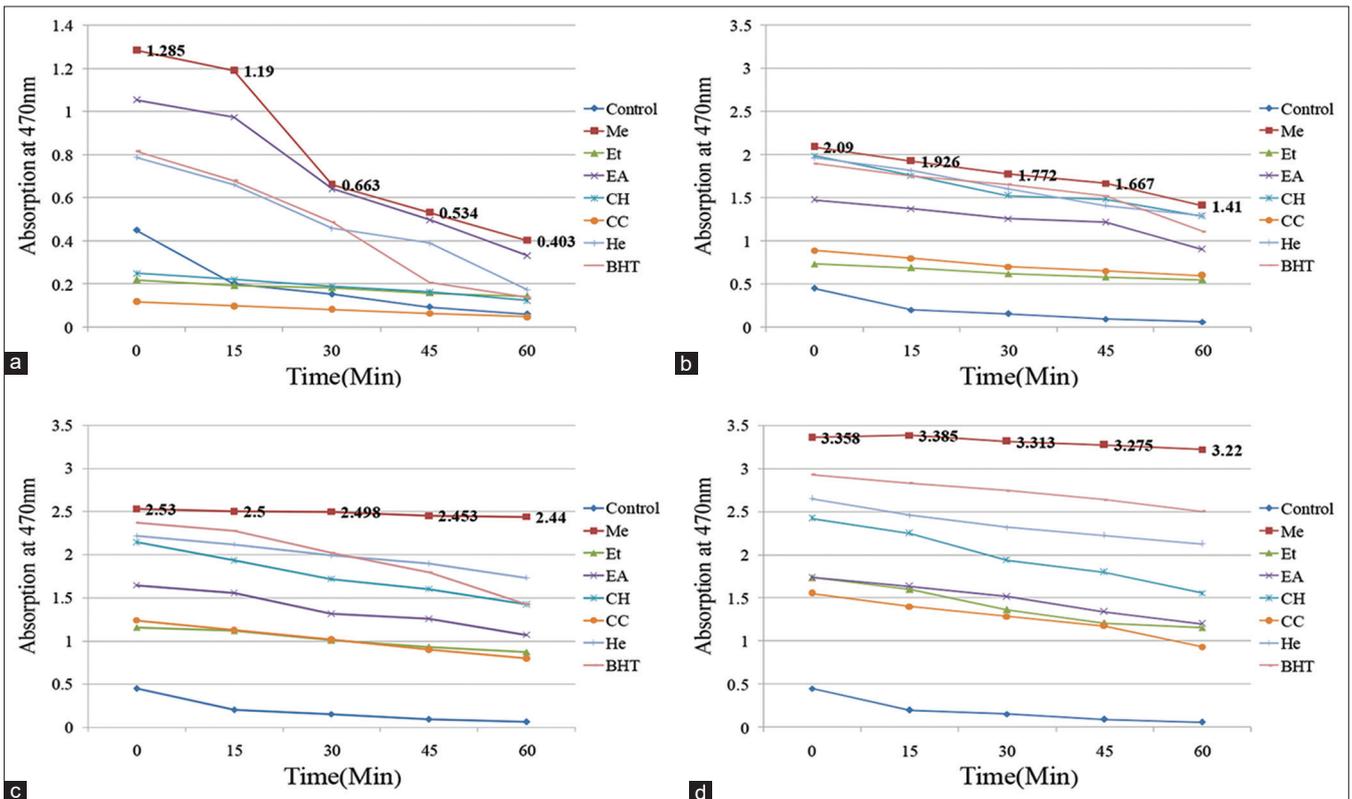


Fig. 8: β-carotene bleaching assay of *Pterocarpus santalinus* L.f. leaves. (a) 50 µg/ml (b) 125 µg/ml (c) 250 µg/ml (d) 500 µg/ml

metabolites, that is, alkaloids, carbohydrates, glucosides, saponins, proteins, phytosteroids, phenolics and flavonoids excluding fats, mucilages, gums, and fixed oils. All these phytochemicals were lacking

in EA, Ch, CC, and He extracts of fruits, roots, and leaves apart from floral extracts, whereas saponins, proteins, and phytosteroids were missing in fruit extracts of all solvents.

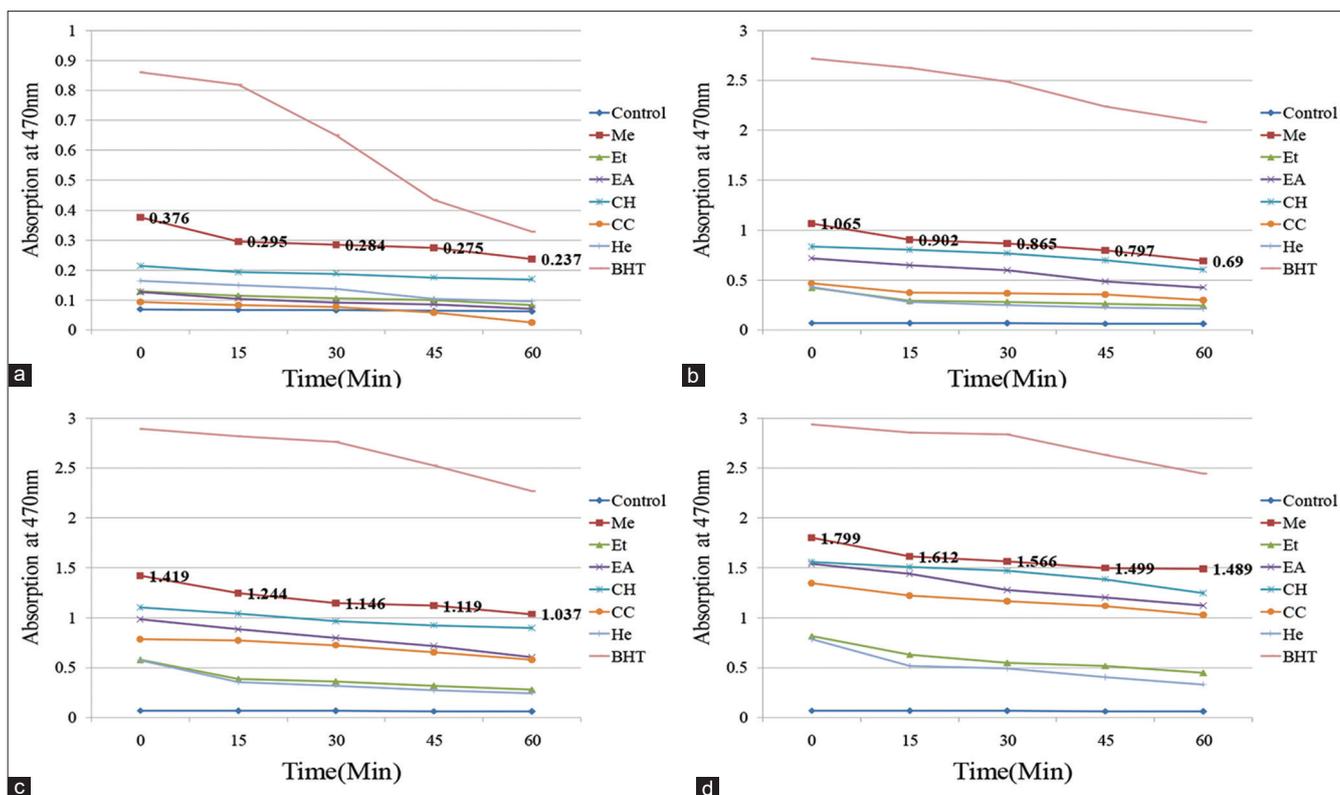


Fig. 9: β - carotene bleaching assay of *Pterocarpus santalinus* L.f. flowers. (a) 50 $\mu\text{g/ml}$ (b) 125 $\mu\text{g/ml}$ (c) 250 $\mu\text{g/ml}$ (d) 500 $\mu\text{g/ml}$

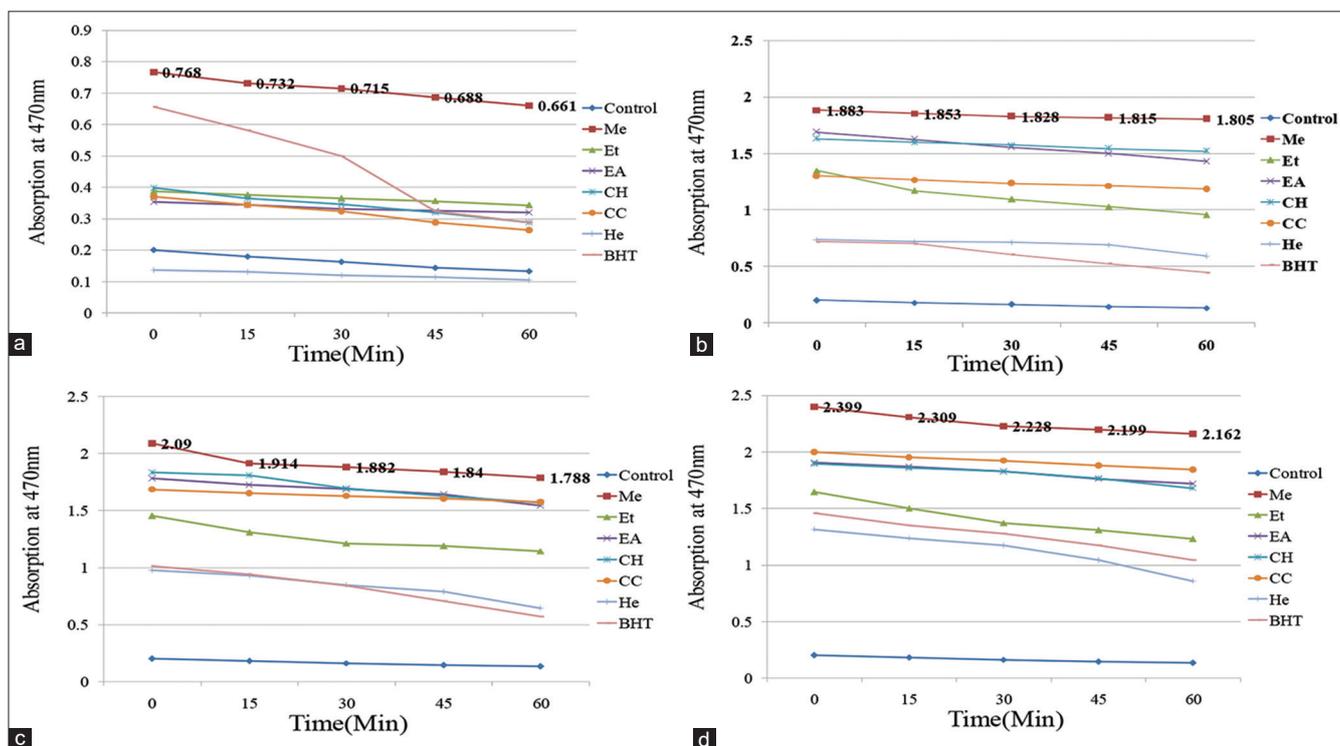


Fig. 10: β - carotene bleaching assay of *Pterocarpus santalinus* L.f. fruits. (a) 50 $\mu\text{g/ml}$ (b) 125 $\mu\text{g/ml}$ (c) 250 $\mu\text{g/ml}$ (d) 500 $\mu\text{g/ml}$

AA tests

DPPH RSA assay

The calculated percentages of DPPH RSA in formation of DPPH free radicals show that Me extract of all plant parts showed fair DPPH RSA amongst all six solvent extracts. At highest concentration (25 mg/ml),

Me extracts of leaf showed highest RSA (96.5%), almost equal to the RSA of the standard BHT (98.7%) in Fig. 2. As the concentration of extract increased, RSA of extract was also increased. DPPH assay showed that the AA of these extracts was probably due to the presence of phytochemicals which can acts as free radical scavengers.

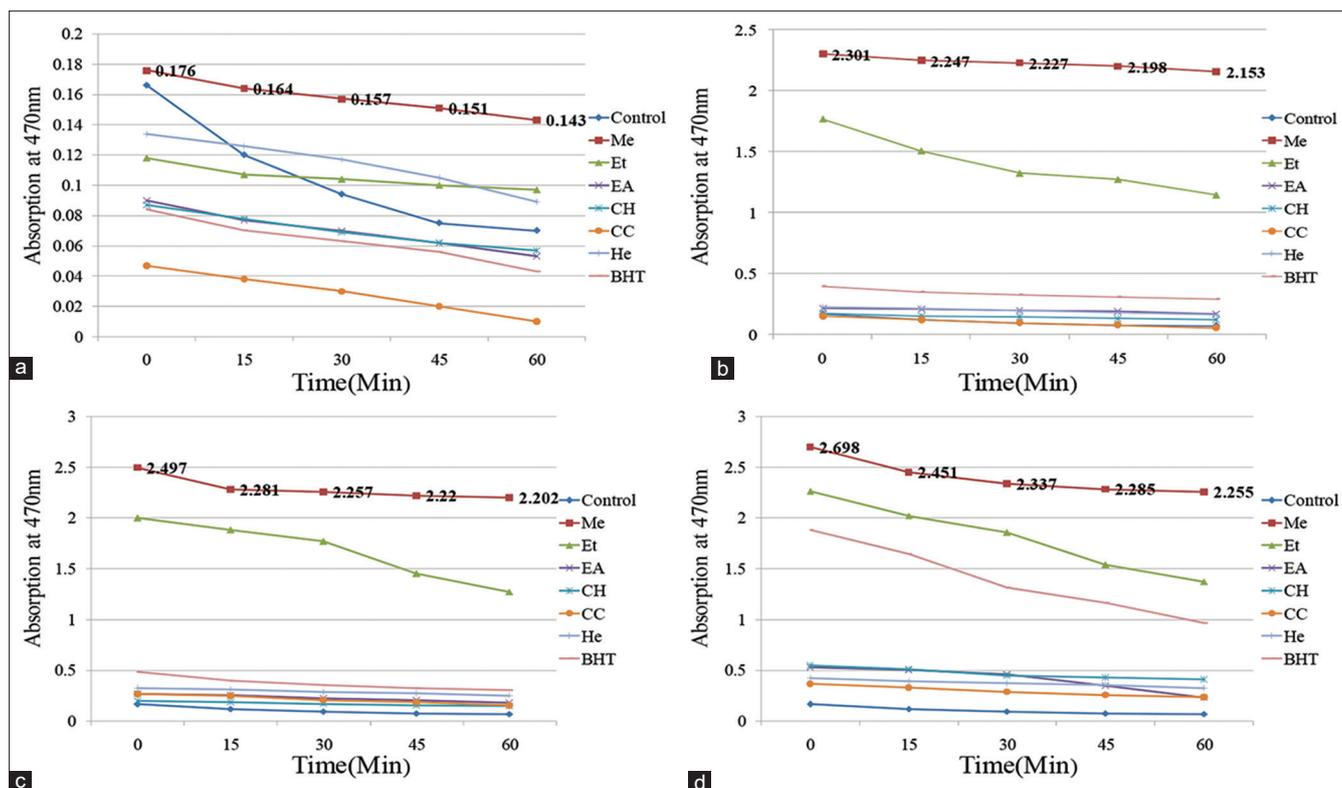


Fig. 11: β - carotene bleaching assay of *Pterocarpus santalinus* L.f. roots. (a) 50 $\mu\text{g/ml}$ (b) 125 $\mu\text{g/ml}$ (c) 250 $\mu\text{g/ml}$ (d) 500 $\mu\text{g/ml}$

RRP assay

RRP assay was carried out on all solvent extracts of *P. santalinus* L.f. and the percentages of RRP of all extracts were shown in graphs. At highest concentration of 200 $\mu\text{g/ml}$ concentration Me extracts of leaf and flower showed highest RRP of 92.5% and 94.8%, which was greater than RRP of BHT, that is, 88.8% in Fig. 3. Me extracts of all plant parts showed good RRP compared to other solvent extracts. The RRP of the extract was increased as the concentration of the extract was increased.

FTC assay

In FTC assay, the AA of extracts which inhibits the degradation of peroxides was estimated. In this test, Me solvent extracts of all the plant parts showed better FTC than all other solvent extracts. Here lower absorption values indicate the highest FTC potential of the sample. Me floral extracts showed lowest absorption values indicating the highest FTC potential than standard BHT. As the concentration of the extract was increased, the FTC of the extract was also increased.

β - Carotene bleaching assay

β - Carotene bleaching assay was used to evaluate the AA of the extracts against oxidation of β - Carotene. The Me extracts of all plant parts showed better activity than others solvent extracts. And also Me extracts of all plant parts except floral extract (Fig. 9) showed higher β -carotene bleaching activity than BHT. High absorption values specify high β -carotene bleaching activity. As the concentration of extract was raised, the β -Carotene bleaching activity of the extract was also increased.

CONCLUSION

The phytochemical investigation on *P. santalinus* L.f. leaves, flowers, fruits, and roots showed that Me and Et extracts of all plant parts showed the presence of almost all phytochemicals except oils, gums, mucilages, and fats. The Me extracts of all plant parts showed good AA in four types of antioxidant assays. By this we can conclude that the presence of different phytochemicals has attributed the natural

antioxidant potential to these extracts. Further studies on Me extracts of leaves and flowers of this plant are suggested for isolating these phytochemicals, which might be the reason for AA better than synthetic standards.

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AUTHORS' CONTRIBUTIONS

Dr. NBL Prasad sir has contributed in procuring the plant parts, also guided and supervised the entire research work with his valuable experience and suggestions.

Bharathi Bestha has done the extraction, qualitative phytochemical screening, and antioxidant assessments of *P. santalinus* L.f. plant parts.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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