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Total phenolic content, flavonoid content and antioxidant potential of *Ceiba Speciosa* and *Leucas cephalotes*

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***Abstract*---**Antioxidants are important to fight cellular stress-a primary cause of the majority of the chronic illness. Natural antioxidants are always preferable over synthetic due to the advantages such as safety. In the current investigation, two plants *Ceiba speciosa* and *Leucas Cephalotes* were selected, extracted using various solvents and also subjected to preliminary phytochemical analysis. The extracts were also screened for total phenolic content, total flavonoid content, total condensed tannin content by using standard models. These extracts were then investigated for the *Invitro* antioxidant activity evaluation using DPPH assay, nitric oxide free radical assay, reducing power assay and total antioxidant assay. The results suggest that the percentage yield is high for methanol extracts and also has better secondary metabolites. The *Invitro* antioxidant activity results for the extracts reveal that the methanol extract is considerably potent to neutralize the free radicals and produced better results when compared to the standard values. This declares the protective effect of the plants towards the diseases that are associated with cellular stress.

Keywords*---Ceiba speciosa, Leucas Cephalotes,* phytochemical, DPPH assay, nitric oxide free radical assay, reducing power assay and total antioxidant assay

Introduction

Ceiba speciosa (Syn: *Chorisia speciosa*) belongs to the family Malvaceae is commonly known as silk floss tree is famous for its silk of the pods (Benson L

1970). Traditionally, *Ceiba* species are used for treating diabetes, infections, pains, fever, diarrhoea, ulcers and arthritis (Hafez SS et al., 2003; Ashmawy AM et al., 2012; El-Alfy TS et al., 2012; Refaat J et al., 2015). Phytochemical investigation of *C. speciosa* revealed the presence of fatty acids, β-amyrin and verbascoside, p-hydroxy benzoic acid, β- sitosterol-3-O-β-dglucopyranoside, succinic acid, astragalin and cinaroside, in addition to stigmasterol, tiliroside and rhiofolin (Nasr et al., 2018; Rosselli S et al., 2020).

Leucas cephalotes (Syn: *L. Capitata*) famous as dronapushpi belongs to the family Labiatae is a famous ayurvedic herb; used in folklore medicine to treat diabetes, skin and respiratory infections, inflammations, fevers and urinary problems (Rahman Sm mushier et al., 2018). Various parts of the plants are reported to possess flavonoids, terpenoids, steroids, alkaloids, glycosides and other secondary metabolites (Sachin Chavan et al., 2013). Pharmacologically this plant is proved to exhibit analgesic, antidiabetic, anti-diarrhoeal (Baburao Bhukya et al., 2010; Surya Narayan Das et al., 2012), antiarthritic (Pathak Rajeev 2018)[12], antiepileptic (Chimbalkar AD et al., 2016), antibacterial, antifungal (Antariksh Katara et al., 2010; Jangra Pankaj et al., 2016), antiprotozoal (Dua Virendra et al., 2011), anticholinesterase (Shahwar Durre et al., 2012)[17], and hepatoprotective activities in various experimental models (Sailor GU et al., 2010; Sofi G et al., 2011).

The production of free radicals in the body is a defence mechanism to fight against the pathogens that skip the other immune system of the body and entered into the cellular environment (Nesy E et al., 2020)[20]. This production is balanced by the intrinsic antioxidant enzymes called free radical scavengers. When this delicate balance is disturbed by either overproduction of free radicals and underproduction of antioxidant enzymes, cellular stress will be generated, that targets own cells and chronic exposure of the body tissue to this harsh environment can damage the organ and disturb the homeostasis. External antioxidants either in the form of supplements or diet are necessary to support the free radical scavenging property of the body (Arathy R et al., 2020; Venkatesan S et al., 2020; Tejavathi DH et al., 2020; Shanmugavadivelu CM et al., 2020). The current work is aimed to investigate the detailed antioxidant potential of the selected plants *Invitro*.

Materials and Methods

Plant material

Aerial parts of *Ceiba speciosa* and aerial parts of *Leucas Cephalotes* were collected from the forest areas of Tirupati in February and authenticated by Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool and voucher specimen was (RU-BD-VSN-148) preserved in the herbarium.

Extraction

Two kilograms of the fresh plant parts were shade dried at temperature 25-30 ºC for 7 days. The dried leaves were powdered in a pulverizer. The powdered plant material was subjected to Soxhlet extraction using petroleum ether, chloroform, Ethyl acetate, Methanol and water to get the respective extracts.

The extracts were evaporated to dryness with rotary evaporator and lyophilized to get powder. The percentage of yield was calculated using the following formula (Dokuparthi SK et al., 2014): -

Yield (g/100 g) = $(W_1 \times 100)/W_2$

Where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the extractor

Phytochemical Screening

The preliminary phytochemical screening of all the extracts of *Ceiba speciosa* and *Leucas Cephalotes* was performed according to the standard procedures (Harborne JB 1973).

Total Phenolic content estimation

Preparation of Standard Gallic Acid for Calibration Curve

Concentrations ranging from 25-100 µg/mL of standard gallic acid solution was prepared by dissolving pure gallic acid in methanol; 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7% Sodium carbonate were also added to make a final volume of 10 mL followed by the 30 minutes incubation at 40°C. The coloured solution thus obtained was measured at 760 nm using UV-visible spectrophotometer and a calibration curve was plotted for the average values of the results obtained in triplicates.

Estimation of total Phenolic content

The total phenolic content of all the extracts of *C. speciosa* and *L. Cephalotes* was estimated by Folin Ciocalteu method as described by Singleton with slight modifications. Various concentrations of the extracts ranging from 25-100 µg/mL were prepared and the total phenolic content was estimated as described above. Total phenolic content of the extracts was expressed as mg of gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) (Singleton VL et al., 1965).

Total Flavonoid content estimation

The flavonoids content was determined by aluminium trichloride method using quercetin as reference compound. A volume of 125µL of extract is added to 75 µL of a 5% $NaNO_2$ solution. The mixture was allowed to stand for 6 min, then 150 µL of aluminium trichloride (10%) was added and incubated for 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500 µL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoid content was expressed as µg of quercetin equivalents per mg dry matter (µg QE/ mg dry weight) using calibration curve. All the experiments were run in triplicate. The mean values and standard deviations were calculated (Chang CC et al., 2002).

Total Tannin content estimation

The tannin content was determined by method of Broadhurst with slight modification, using tannic acid as a reference compound. A volume of 400µL of extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation, absorbance was read at 500 nm. The condensed tannin was expressed as µg of tannic acid equivalents per mg dry matter (µg TAE/ mg dry weight). All the experiments were run in triplicate. The mean values and standard deviations were calculated (Broadhurst RB et al., 1978).

In vitro antioxidant activity

DPPH assay:
In DPPH assay, 1ml of different concentrations (25-200 µg/ml) of extracts were added to the reference solution (0.004% in methanol) in test tubes. The tubes were incubated in dark for 30min at room temperature; absorbance of reaction mixture was measured in UV Visible spectrophotometer (Labindia 3000+) at 517nm. Ascorbic acid was used as standard. Methanol replacing the extract/ascorbic acid served as control (i.e., 1ml of methanol + 3ml of DPPH radical solution). Inhibition of DPPH radicals (%) was calculated and IC_{50} value was determined (Blois MS et al., 1958).

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined by the method reported by Garrat.[37] Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract of various concentrations and the mixture was incubated at 25°C for 180 min. From the above solution 0.5 ml was taken out and add 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid). The solution was incubated at room temperature for 5 min. finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) were added and incubated at room temperature for 30 min, the absorbance was measured at 540 nm using spectrophotometer. The nitric oxide radical scavenging activity was calculated (Rajasekaran SR et al., 2016).

Reducing power assay

Reducing power of the *C. speciosa* and *L. Cephalotes* were determined by Oyaizu method with slight modifications[38]. 0.75 mL plant extract of various concentrations (125,250,500,1000) were mixed with 0.75 mL of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium hexacyanoferrate (1%, w/v), the solution was incubated at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of 10% Trichloro Acetic Acid (TCA) and then centrifuged at 3000 rpm for 10 min. 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/*v*) for 10 min. The absorbance was measured at 700 nm using UV visible

Spectrophotometer. Higher the absorbance of the reaction mixture indicates greater the reducing power (Oyaizu M 1986).

Total antioxidant activity

Total antioxidant activity was determined by the phosphomolybdenum method. 0.1 ml of various concentrations of plant extracts (25-200µg/ml) were added to 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). After an incubation of 90 minutes at 95°C, samples were cooled to room temperature and absorbance of the mixture was measured at 695nm by using UV Visible spectrophotometer (Labindia 3000+). Ascorbic acid was used as standard. 0.1ml of methanol was used as blank (Prieto P et al., 1999).

Results and Discussions

Percentage yield

The percentage yield with all the extracts was calculated and depicted in the table1. The methanol yielded more quantity followed by extraction with water. *C. speciosa* is found to yield more extract than *L. Cephalotes.*

Phytochemical Screening

Preliminary phytochemical screening of the extracts revealed that the plants are rich in various phytochemicals listed in the table 2.

Total Phenolic content estimation

A Preparation of Standard Gallic Acid for Calibration Curve

A calibration curve was plotted by the absorbance and concentrations (mg/mL) using prepared dilutions. The regression analysis was performed and the resulting equation was Abs= $y = 0.0108x + 0.035$. The coefficient of determination for standard curves were greater than 0.99 ($R^2 = 0.9944$) (Figure 1). Thus, the calculated straight line could explain more than 99% of the experimental data. Total phenolic content of the extracts was calculated and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Where y is absorbance at 760 nm and x is total phenolic content in the extracts of *Ceiba speciosa* (182.47±1.21) and *Leucas Cephalotes* (172.34±2.16).

Estimation of total Phenolic content

The total phenolic content of all the extracts of *C. speciosa* and *L. Cephalotes* were estimated by Folin-Ciocalteu method as described by Singleton with slight modifications. Various concentrations of the extracts ranging from 25-100 µg/mL were prepared and the total phenolic content was estimated as described above. The total phenolic content of the extracts was expressed as mg of gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) (table 3a & Figure 2a).

% NO radical scavenging activity = [(Acontrol – Asample)/ Asample] × 100

Total Flavonoid content estimation

Total flavonoid content profile of the plant extracts was established through colorimetric method using $AlCl_3$. A calibration curve ($y = 0.0037x - 0.015$, $R^2 = 0.9958$) was plotted using various concentrations of standard quercetin (0–100

µg/mL) and expressed in quercetin equivalents (QE) per gram (Figure 2b). Total flavonoid content of the extracts was calculated and expressed as mg quercetin equivalents (QE) per gram of sample in dry weight (mg/g). Where y is absorbance at 760 nm and x is total flavonoid content in the extracts of *C. speciosa* and *L. Cephalotes* (table 3b& Figure 2c). Methanol extracts were found to have more flavonoids among others followed by ethyl acetate and chloroform extracts. *L. Cephalotes* (171±1.31) is identified as rich in flavonoids than *C. speciosa* (104.13±1.23)

Total Condensed tannins content

Total tannin content of the plant extracts was determined using Vanillin-HCl colorimetric method. A calibration curve ($y = 0.0016x + 0.01$, $R^2 = 0.9917$) was plotted using various concentrations of standard Tannic acid (0–100 µg/mL) (Figure 2d) and expressed in Tannic equivalents (TAE) per gram. Total tannin content of the extracts was calculated and expressed as mg Tannic acid equivalents (TAE) per gram of sample in dry weight (mg/g). Where y is absorbance at 760 nm and x is total tannin content in the extracts of *C. speciosa* and *L. Cephalotes* in µg TAE per mg dry weight of the extract (table 3c & Figure 2e). Total condensed tannins are found high in methanol extracts followed by aqueous extracts. From the results it is apparent that *C. speciosa* (139.1±1.13) is having more condensed tannins than *L. Cephalotes* (117±2.18).

***In vitro* antioxidant activity**

DPPH assay

Antioxidant potential of the extracts was evaluated using DPPH assay and significant free radical scavenging activity was determined with methanolic extracts of both the plants. The antioxidant activity was observed in a dose dependant manner activity in comparison with standard Ascorbic acid (table 4a & figure 3a). At higher concentration 75 µg/ml, methanol extract is showing comparatively better inhibition for both the plants (74.13±0.11&78.76±0.02) which are almost near to the percentage inhibition of standard ascorbic acid (81.02±0.01). IC_{50} values were calculated and depicted in table 4b & figure 3b. *C. speciosa* (34.13±0.94) leaves showed comparatively higher antioxidant activity than *L. Cephalotes* (29.14±1.32). The total phenolic content and flavonoid content in the methanol extract may be responsible for this potent antioxidant activity through DPPH assay.

NO free radical assay

All the leaf fractions of *C. speciosa* and *L. Cephalotes* were evaluated for nitrite free radical scavenging property. Percentage free radical scavenging was plotted against the concentration of the extracts as shown in Figure 4a. The plants exhibited antioxidant activity through competing with oxygen to scavenge for the nitrite radical which was generated from sodium nitroprusside at physiological pH in an aqueous environment. The antioxidant activity increased with an increased polarity up to methanol in a dose dependent manner. The maximum free radical scavenging activity was interpolated to give results as shown in Table 5a. Methanol extract of *C. speciosa* (75.32%±1.74) and *L. Cephalotes* (78.12%±0.64)

were exhibited better inhibition towards nitrite free radicals than other fractions compared with standard ascorbic acid (80.24%±0.21). Methanol extract of *L. Cephalotes* had a maximal scavenging activity with a potent IC_{50} value of 30.75±2.64 µg/mL followed by *C. speciosa* (34.58±2.18 µg/mL), while ascorbic acid is having 29.17±2.47 µg/mL (Table 4b).

Reducing Power assay

Ferric reducing power assay experiment determines the antioxidant potential of the plant. The plant extract with high reducing power reported to possess high antioxidant potential. Reducing power assay method is based on the principle that substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) converted to potassium ferrocyanide (Fe^{2+}), which in turn reacts with ferric chloride to form ferric–ferrous complex ions which is identified by change in color from yellow to bluish green and the absorbance is measured at 700 nm. The results for ferric reducing power activity of various extracts of *C. speciosa* and *L. Cephalotes* were compared with standard ascorbic acid are shown in (Table 6 &Figure 5). 1000µg/ml of *L. Cephalotes* methanolic extract has shown high reducing power (1.07±0.64) when compared with that of other extracts and also than *C. speciosa* (0.95±1.11). Reducing power potential of extracts increased with the increase in dose, however petroleum ether extracts of both the plants exhibited low reducing power when compared to that of ascorbic acid (1.47±0.45).

Total antioxidant activity

Phosphomolybdenum assay was used to determine the total antioxidant activity of the extracts. Ascorbic acid being as a reference standard for comparison (1.47±0.44) with the extracts of *C. speciosa* and *L. Cephalotes.* Methanolic extracts showed comparatively more total antioxidant activity than other extracts; methanolic extract of *L. Cephalotes* (1.07±0.92), exhibited higher antioxidant activity followed by methanolic leaf extract of *C. speciosa* (0.95±0.48) (Table 7 Figure 6).

From the results, *Ceiba speciosa* methanolic leaf extract demonstrated the highest antioxidant activity than other extracts. The antioxidant activity was assessed through DPPH assay, nitric oxide free radical assay, reducing power assay and total antioxidant assay by comparing with a reference standard and with the help of calibration curves. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, terpenoids, sterols etc. The testified antioxidant activity may have a firm relation with the polyphenolic components extant in them such as phenols, flavonoids and tannins; thus, provisions protective eminence of these plants in traditional medicine.

Conclusion

In conclusion, two plants *Ceiba speciosa* and *Leucas Cephalotes* were successfully subjected to extraction process and phytochemical investigations. Phytochamical analysis reveled the presence of diverse group of phytochemicals including flavonoids, alkaloids, steroids, terpenoids and tannins. These extracts were then

investigated for the *Invitro* antioxidant activity evaluation using DPPH assay, nitric oxide free radical assay, reducing power assay and total antioxidant assay. The results suggest that the percentage yield is high for methanol extracts and also has better secondary metabolites. The *Invitro* antioxidant activity results for the extracts reveal that the methanol extract is considerably potent to neutralize the free radicals and produced better results when compared to the standard values. This declares the protective effect of the plants towards the diseases that are associated with cellular stress.

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Tables

Table 1
Percentage yield of various extracts of *C. speciosa* and *L. Cephalotes*

Solvents	%yield	
	C. speciosa	*L. Cephalotes*
Petroleum ether	5.35	4.5
Chloroform	6.4	6.2
Ethyl acetate	4.6	5.52
Methanol	14.42	13.36
Aqueous	6.4	5.04

Table 2
Phytochemical analysis of various extracts of *C. speciosa* and *L. Cephalotes*

Phytochemicals	*C. speciosa*					*L. Cephalotes*				
	PE	CE	EE	ME	AE	PE	CE	EE	ME	AE
Alkaloids	+	+	+	+	+	-	+	+	+	+
Tannins	-	+	-	+	+	-	+	+	+	+
Steroids	+	-	-	-	+	+	+	-	-	-
Flavonoid s	-	-	+	+	-	-	+	+	+	+
Terpenoids	+	+	-	-	-	+	+	-	-	-
Proteins	-	-	-	-	-	-	-	-	+	+
Carbohydrates	-	-	-	+	+	-	-	-	+	+
Phenols	-	-	-	+	+	-	-	-	+	+
Saponins	-	+	+	+	+	-	-	-	-	-
Glycosides	+	+	-	+	+	-	-	-	+	+

PE- Petroleum ether extract, CE- Chloroform extract, EE- Ethyl acetate extract, ME- Methanolic extract, AE- Aqueous extract, '+'indicates presence,'-'indicates absence of phytochemical

Table 3a.
Total Phenolic content of *C. speciosa* and *L. Cephalotes*

Extracts	mg GAE/mg dry weight	
	C. speciosa	*L. Cephalotes*
Petroleum ether extract	75.4±1.24	82.3±0.35
Chloroform extract	127±0.51	143.4±1.34
Ethyl acetate extract	153±2.31	184.32±1.65
methanol extract	214±3.17	235.18±1.87
Aqueous extract	112±2.34	98.35±2.04

Table 3b.
Total flavonoid content of *C. speciosa* and *L. Cephalotes*

Extracts	mg QE/mg dry weight	
	C. speciosa	*L. Cephalotes*
Petroleum ether extract	11.3±0.31	17.4±1.11
Chloroform extract	82.1±0.17	101.13±1.16
Ethyl acetate extract	97.18±0.47	108±1.64
methanol extract	104.13±1.23	171±1.31
Aqueous extract	43.18±21.17	48.11±0.46

Table 3c.
Total condensed tannin content of *C. speciosa* and *L. Cephalotes*

Extracts	mg TAE/mg dry weight	
	C. speciosa	*L. Cephalotes*
Petroleum ether extract	2.3±1.26	5.4±1.35
Chloroform extract	41.4±1.22	57±0.43
Ethyl acetate extract	84.11±0.35	92±1.32
methanol extract	139.1±1.13	117±2.18
Aqueous extract	92.31±1.24	102±1.18

Table 4a.
DPPH assay of *C. speciosa* and *L. Cephalotes*

Conc µg/ml	% of inhibition										
	AA	CSP	CSC	CSE	CSM	CSA	LCP	LCC	LCE	LCM	LCA
75	81.02±0.01	22.17±0.11	25.09±0.14	26.08±0.17	74.13±0.11	34.12±0.07	23.08±0.14	27.39±0.06	28.11±0.08	78.76±0.02	30.05±0.12
50	62.13±0.04	17.34±0.12	20.08±0.04	20.14±0.24	61.14±0.21	27.23±0.12	19.55±0.24	22.38±0.18	22.33±0.24	66.53±0.06	26.84±0.18
25	42.31±0.05	15.12±0.08	17.15±0.03	18.27±0.16	53.17±0.14	20.64±0.18	16.1±0.11	18.23±0.34	20.17±0.31	55.08±0.04	24.21±0.14
15	39.3	12.3	15.1	14.9	45.3	14.2	13.1	15.4	16.0	47.0	14.0

	4±0.10	3±0.11	4±0.07	7±0.09	8±0.09	1±0.21	8±0.27	1±0.25	5±0.33	5±0.01	9±0.19
10	33.16±0.04	10.15±0.09	12.43±0.12	13.15±0.11	19.08±0.14	11.14±0.09	10.92±0.14	12.09±0.18	12.78±0.17	23.18±0.14	10.28±0.14

Table 4b.
IC_{50} values *C. speciosa* and *L. Cephalotes* extracts in DPPH assay

Extracts	IC_{50} values	
	C. speciosa	*L. Cephalotes*
Ascorbic acid	28.14±1.54	
Petroleum ether extract	128.26±1.02	225.07±0.07
Chloroform extract	216.73±1.23	177.71±1.07
Ethyl acetate extract	208.71±1.08	178.93±1.24
methanol extract	34.13±0.94	29.14±1.32
Aqueous extract	118.81±1.05	138.53±0.09

Table 5a.
NO free radical Scavenging activity of *C. speciosa* and *L. Cephalotes*

Conc µg/ml	% of inhibition										
	AA	CSP	CSC	CSE	CSM	CSA	LCP	LCC	LCE	LCM	LCA
75	80.24±0.21	15.11±0.94	21.06±2.37	24.57±1.32	75.32±1.74	33.17±2.74	17.28±1.24	26.74±0.99	24.14±2.17	78.12±0.64	34.84±0.88
50	61.13±0.08	12.19±0.36	15.67±0.89	16.24±1.27	59.54±2.64	28.27±1.95	12.97±1.06	20.41±3.2	22.34±2.65	62.74±1.33	28.14±2.13
25	42.37±0.14	10.22±0.64	13.44±1.37	14.27±1.54	51.24±2.65	21.67±1.46	11.37±2.11	16.87±2.66	18.94±1.94	54.37±1.49	25.11±2.62
15	38.12±0.17	9.14±0.72	12.34±1.29	12.97±1.96	43.12±2.87	13.26±0.67	10.41±1.55	14.22±0.78	15.64±2.16	45.17±1.63	15.23±2.99
10	32.24±0.06	8.23±0.61	10.1±1.74	10.34±.66	22.17±2.66	11.38±0.92	9.11±1.62	12.37±1.55	13.42±3.07	24.11±2.71	11.04±1.64
IC_{50} values	33.86±0.24	423.28±1.37	270.89±1.84	213.94±2.47	34.58±3.14	120.25±2.94	366.63±2.68	188.34±1.76	233.25±2.88	30.75±1.73	116.31±1.37

Table 5b.
IC_{50} values *C. speciosa* and *L. Cephalotes* in NO free radical Scavenging assay

Extracts	IC_{50} values	
	C. speciosa	*L. Cephalotes*
Ascorbic acid	29.17±2.47	
Petroleum ether extract	423.28±1.08	366.63±1.51
Chloroform extract	270.89±1.64	188.34±0.97
Ethyl acetate extract	213.94±0.87	233.25±3.59
methanol extract	34.58±2.18	30.75±2.64
Aqueous extract	120.25±1.05	116.31±2.09

Table 6.
Reducing Power assay of *C. speciosa* and *L. Cephalotes*

Conc µg/ml	Absorbance										
	AA	CSP	CSC	CSE	CSM	CSA	LCP	LCC	LCE	LCM	LCA
1000	1.47 ±0.45	0.4± 0.46	0.42 ±1.07	0.43 ±1.57	0.95 ±1.11	0.37 ±2.14	0.48 ±1.64	0.44 ±1.33	0.49 ±1.67	1.07 ±0.64	0.39 ±1.12
500	1.05 ±0.36	0.28 ±1.37	0.31 ±1.63	0.33 ±2.19	0.78 ±1.34	0.23 ±0.96	0.31 ±2.16	0.32 ±0.84	0.35 ±1.44	0.82 ±1.82	0.24 ±2.24
250	0.24 ±1.07	0.13 ±2.94	0.15 ±1.18	0.17 ±1.37	0.21 ±1.27	0.15 ±1.37	0.17 ±1.76	0.17 ±1.68	0.18 ±0.96	0.26 ±1.43	0.16 ±1.29
125	0.23 ±1.94	0.11 ±1.08	0.12 ±1.69	0.14 ±1.62	0.16 ±2.09	0.11 ±0.88	0.103±0.97	0.14 ±1.34	0.14 ±1.42	0.18 ±1.37	0.12 ±1.07

Table 7.
Total antioxidant activity of various extracts of *C. speciosa and L. Cephalotes*

Conc µg/ml	Absorbance										
	AA	CSP	CSC	CSE	CSM	CSA	LCP	LCC	LCE	LCM	LCA
1000	1.47 ±0.44	0.4± 0.36	0.42 ±1.54	0.43 ±1.07	0.95 ±0.48	0.37 ±1.33	0.48 ±0.66	0.44 ±1.31	0.49 ±2.18	1.07 ±0.92	0.39 ±1.25
500	1.05 ±1.67	0.28 ±0.42	0.31 ±1.65	0.33 ±2.71	0.78 ±1.34	0.23 ±2.49	0.31 ±0.42	0.32 ±1.66	0.35 ±2.37	0.82 ±1.63	0.24 ±0.52
250	0.24 ±0.46	0.13 ±1.37	0.15 ±1.57	0.17 ±0.92	0.21 ±0.64	0.15 ±2.46	0.17 ±1.37	0.17 ±0.94	0.18 ±1.93	0.26 ±1.48	0.16 ±1.63
125	0.23 ±0.69	0.11 ±1.66	0.12 ±2.05	0.14 ±2.33	0.16 ±0.95	0.11 ±1.63	0.103±1.62	0.14 ±1.65	0.14 ±1.46	0.18 ±1.41	0.12 ±1.99

Figures

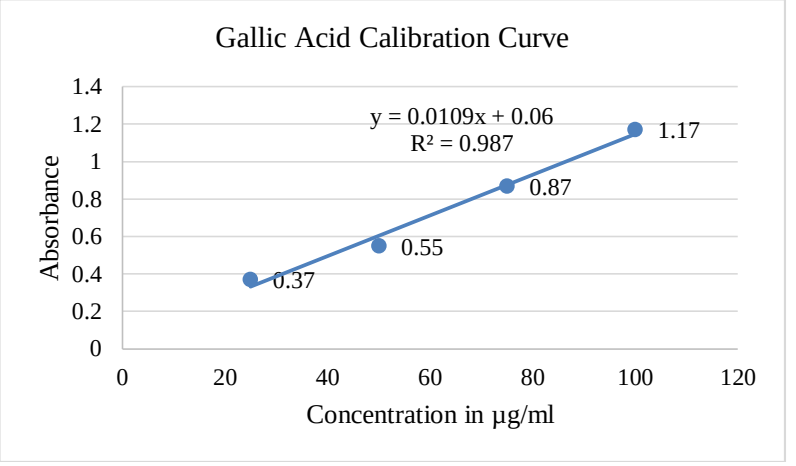

Figure 1. Calibration curve for Gallic acid

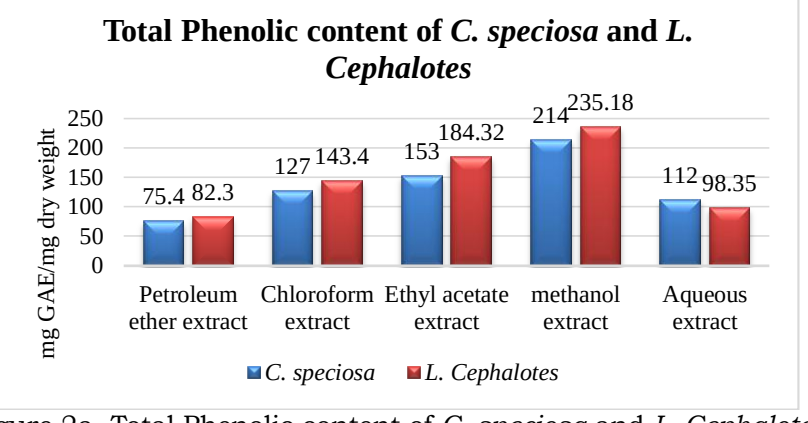

Figure 2a. Total Phenolic content of *C. speciosa* and *L. Cephalotes*

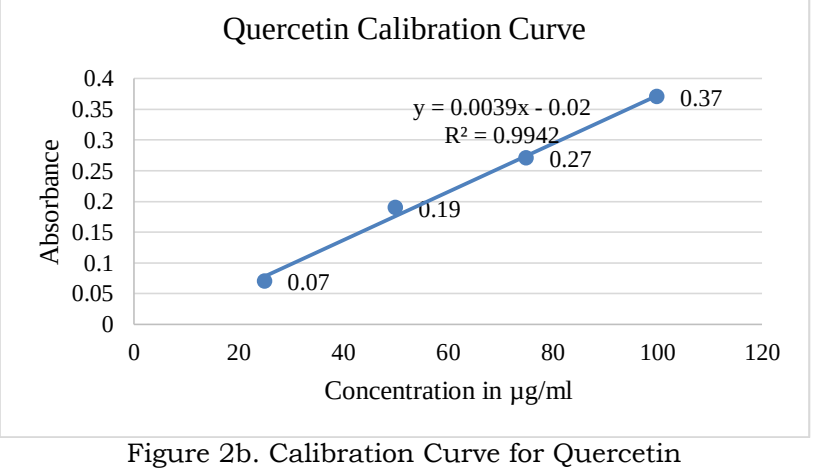

Figure 2b. Calibration Curve for Quercetin

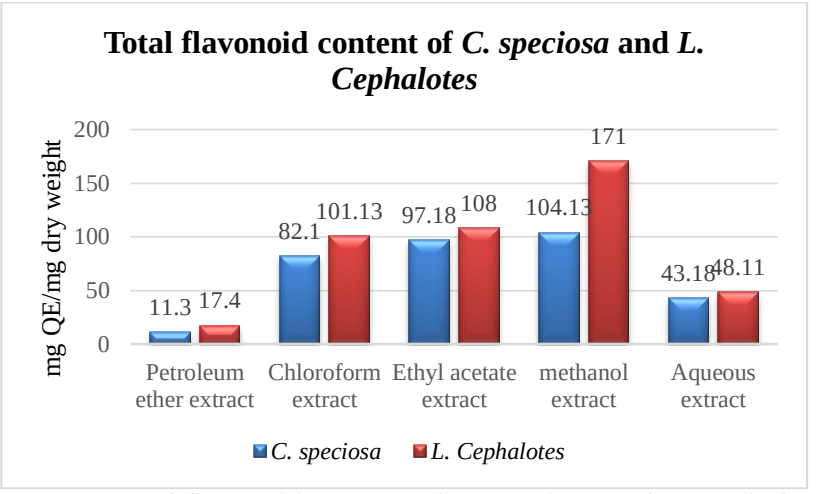

Figure 2c. Total flavonoid content of *C. speciosa* and *L. Cephalotes*

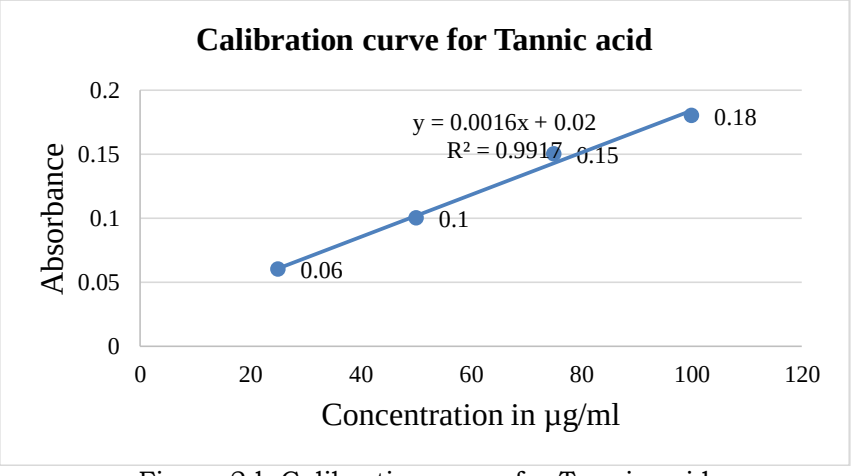

Figure 2d. Calibration curve for Tannic acid

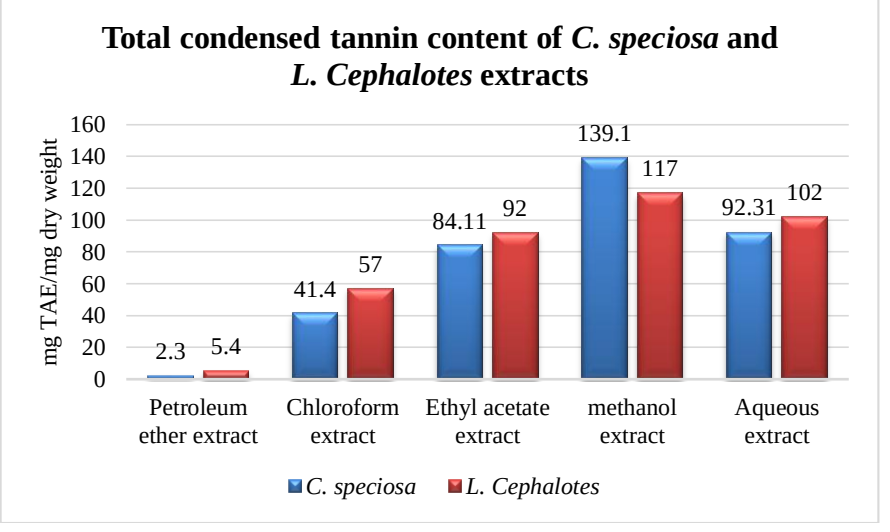

Figure 2e. Total condensed tannin content of *C. speciosa* and *L. Cephalotes*

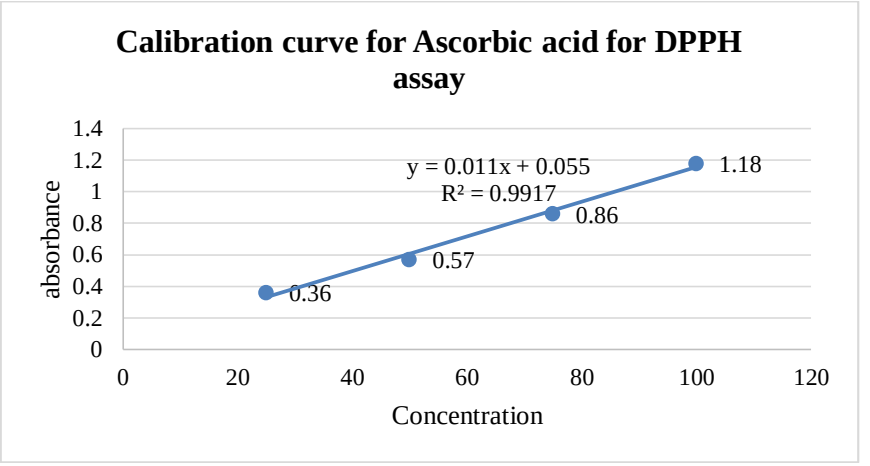

Figure 3a. Calibration curve for Ascorbic acid for DPPH assay

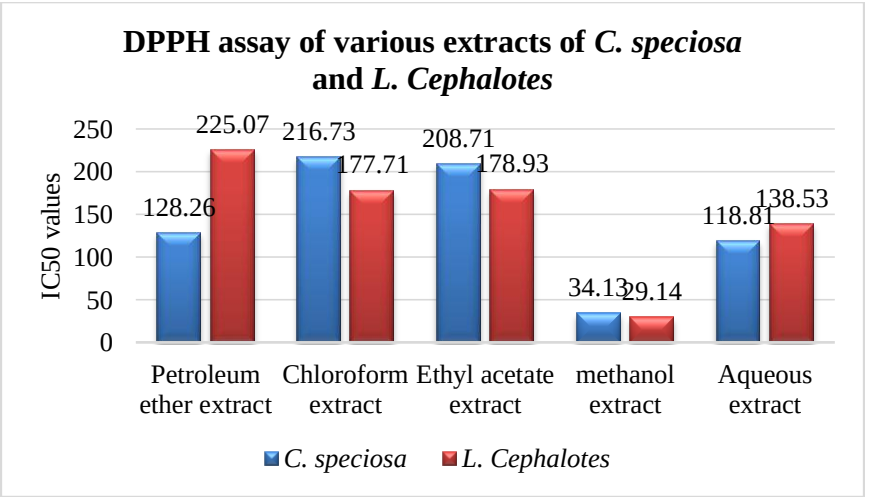

Figure 3b. DPPH assay of various extracts of *C. speciosa* and *L. Cephalotes*

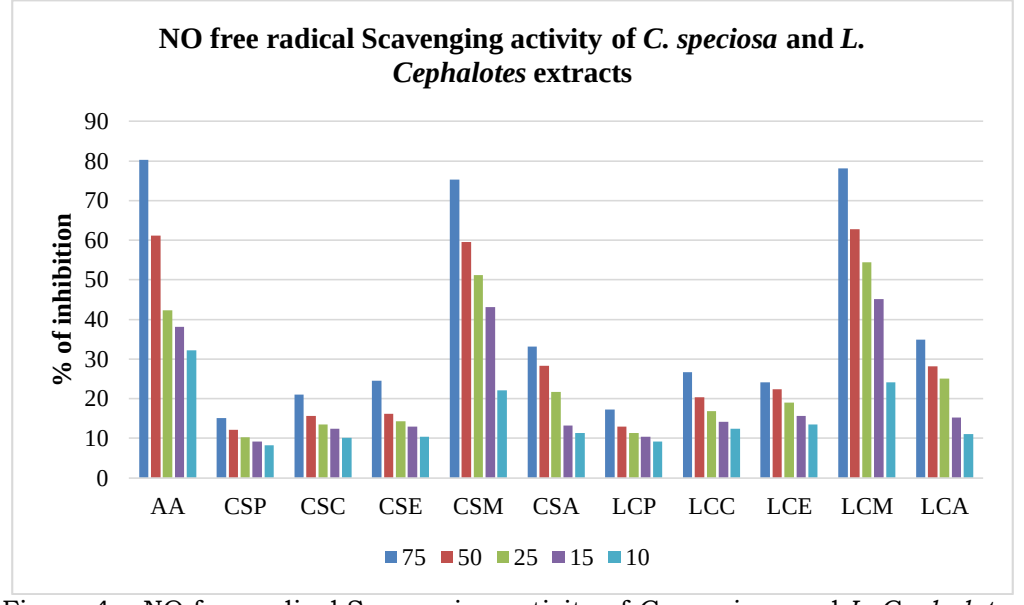

Figure 4a. NO free radical Scavenging activity of *C. speciosa* and *L. Cephalotes*

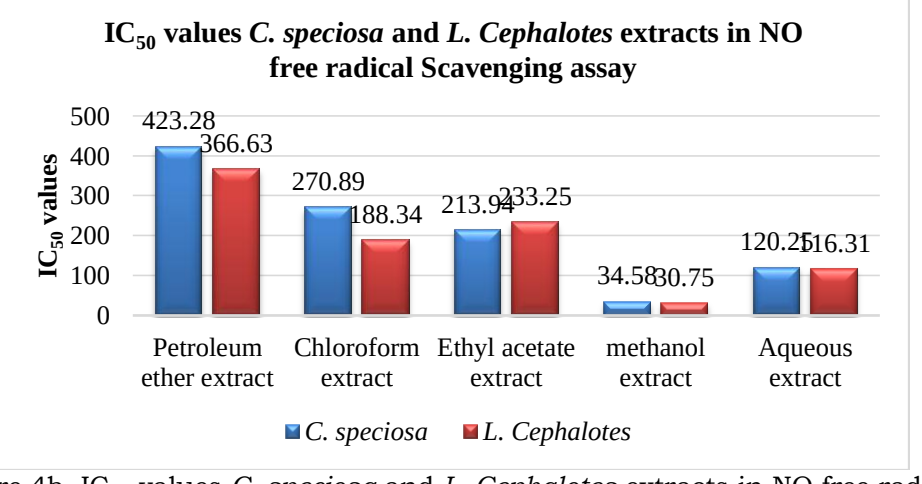

Figure 4b. IC_{50} values *C. speciosa* and *L. Cephalotes* extracts in NO free radical Scavenging assay

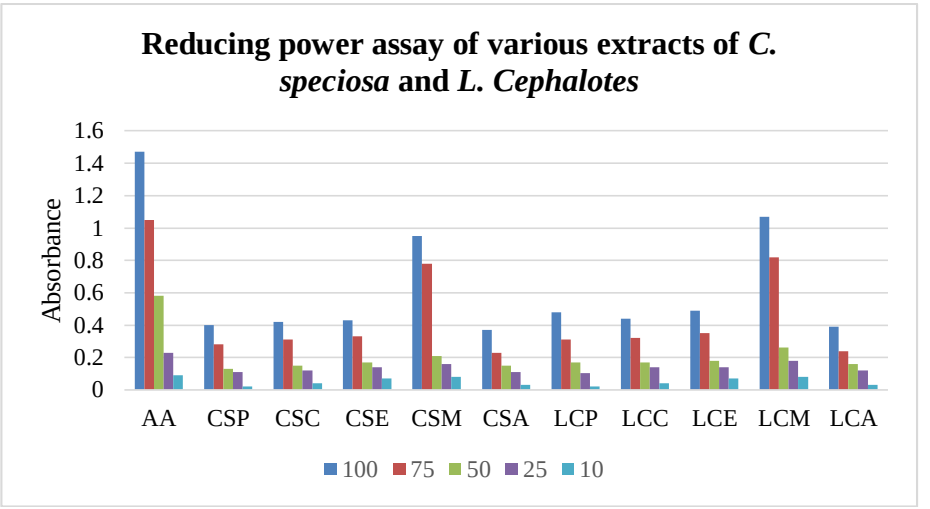

Figure 5. Reducing power assay of various extracts of *C. speciosa* and *L. Cephalotes*

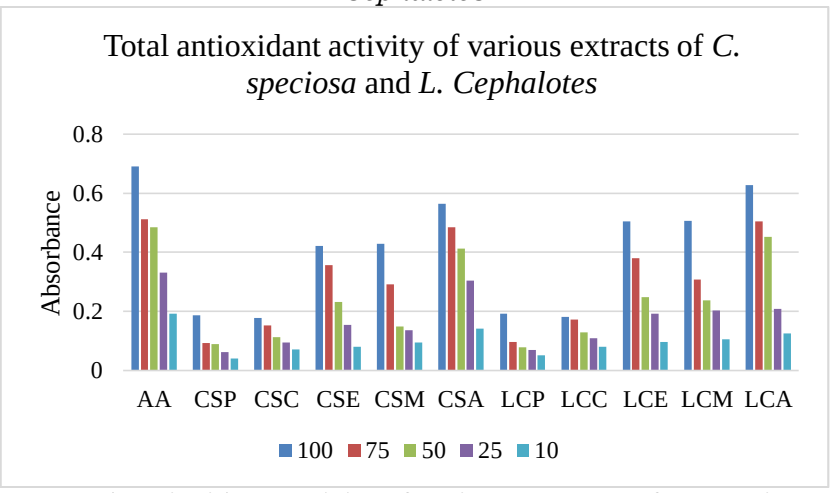

Figure 6. Total antioxidant activity of various extracts of *C. speciosa* and *L. Cephalotes*