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In vitro studies on antioxidants and free radical scavenging activities in the extracts of *Loranthus longiflorus* desr. bark samples obtained from two host trees

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Abstract

Antioxidant compounds and their free radical scavenging (FRS) activities in various solvent extracts of *Loranthus longiflorus* bark samples collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees were assessed. The results obtained confirm the presence of total flavonoids, total phenols and total tannins in all extracts at different proportions. Among the extracts tested, ethyl acetate extract shows maximum total phenols (301.25mg/g and 307.27mg/g) and total tannins (11.46mg/g and 204.83mg/g),while chloroform extract favours more amount of flavonoids (18.92mg/g and 26.13mg/g) in *Loranthus* bark samples collected from the host *Casuarina* and *Ficus*, respectively. Among the extracts of *Loranthus* bark samples, collected from *Casuarina* and *Ficus*, ethanol extract shows maximum scavenging activity on DPPH (4681.8% and 4890.6% at 1500µg), on Hydroxyl (49.37% and 55.58% at 250 µg), ethyl acetate (49.79%) and water extract (48.28%) on Nitric oxide (at 250µg) and ethanol (33.71%) chloroform (34.85%) on Superoxide (at 250 µg), respectively, as compared to other extracts. All the FRS activities, tested, were concentration dependent. The inhibitory concentration (IC_{50}) was determined in ethanol extracts as 5.70µg/ml and 5.32µg/ml for DPPH-FRS activity; as 34.34µg/ml and 38.35 µg/ml for HO-FRS activity, as 108.93µg/ml and 104.32µg/ml for SO-FRS activity and ethyl acetate extract as 188.5µg/ml and 116.1µg/ml for NO-FRS activity of *Loranthus* bark samples collected from *Casuarina* and *Ficus*, respectively, than other extracts, tested. The ferric reducing antioxidant power of *Loranthus* bark samples, from *Casuarina* and *Ficus* hosts, was maximum in ethanol extract (4053.53 and 4199.03mMol Fe (II)/mg extract, respectively) than other extracts tested. These results indicate that the host trees, on which the hemiparasite infested, influence the variations in antioxidant constituents and free radical scavenging activities of *L. longiflorus* bark extracts.

Keywords: Antioxidants, Bark extracts, *Casuarina equisetifolia* host tree, *Ficus religiosa* host tree, Ferric reducing antioxidant power, Free radical scavenging activities, Hemi-parasite, *Loranthus longiflorus.*

INTRODUCTION

The search for raw materials containing potent antioxidants continues to attract the attention of researches. Fruits, vegetables, seeds and spices are all known to be rich sources of natural antioxidants, and medicinal plants are another important source for a wide variety of natural antioxidants [1]. The antioxidant property of plant might be due to their phenolic compounds [2, 3] including tannins and flavonoids and they have been reported as promising antioxidants [4]. Antioxidants act as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation that delay or inhibit the oxidation process and increase shelf-life [5]. In recent years, interest in the study of antioxidant activity of plant extracts [6] and isolation of antioxidants from plants have grown due to the fact that the free radicals have been related to degenerative diseases [7, 8]. The discovery of 'taxol' in the bark of the Pacific Yew tree stimulated interest in antioxidants from woody plants and other medicinal plants as anticancer agents. Compared with wood or leaves, bark is the most economical and convenient resource for the extraction of possible antioxidant compounds. Previous studies have focused on the isolation and identification of chemical compounds from bark and have found polyphenol compounds [9]. Angiospermic hemiparasitic plant *Loranthus longiflorus* (Syn. –*Dendrophthoe falcata* (L.F.) Ettingsh) reported to contain biologically active substances [10, 11, 12]. *Loranthus parasiticus* reported to possess highest antioxidant capacities and total phenolic content among 50 plants tested, and could be rich potential source of natural antioxidants [1]. The present study aims to evaluate and compare the antioxidant compounds (total phenol, tannins and flavonoids) and free radical (DPPH, Nitricoxide, Superoxide, Hydroxyl) scavenging potential and Ferric reducing antioxidant power of *Loranthus longiflorus* bark collected from *Casuarina equisetifolia* and *Ficus religiosa* host trees.

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MATERIALS AND METHODS

Plant material used

The selected plant, *Loranthus longiflorus*, a hemiparasite, was collected from two host trees such as *Casuarina equisetifolia* and *Ficus religiosa*, during the month of October, around Nagercoil town, Kanyakumari District, Tamil Nadu and identified based on the characters of Gamble Flora. The herbarium of the plant was prepared and preserved in the department of Botany, S.T. Hindu

College, Nagercoil, Kanyakumai, District, Tamil Nadu, India.

Preparation of extract

The bark of *L. longiflorus* collected from both host trees were washed in freshwater to remove adhering dust and then dried under shade. The air dried, powdered bark of *Loranthus* was extracted at 20% (w/v) in Soxhlet extraction successively with chloroform, ethyl acetate, hexane, ethanol, and water. The successive extracts were evaporated to dryness and the stored residues were used for analysing antioxidants and free radical scavenging activities.

Determination of antioxidants and free radical scavenging activities

The antioxidants and free radical scavenging activities was determined in the extracts of *Loranthus* bark samples collected from *Casuarina* and *Ficus* host trees by using the following methods. Total flavonoid content was measured according to the method of Zhishen*et et al*. [13]. Total phenols and tannins were determined by using the method of Siddhuraju and Becker [14], and Siddhuraju and Manian [15], respectively. The free radical scavenging activities of DPPH [16], Nitric oxide [17] and Superoxide [18] and Hydroxyl [19] were analysed *in vitro*. Ferric reducing antioxidant power of extracts was performed as described by Pulido*et et al. [*20]. All the data obtained from three replicates were analysed statistically (standard deviation, Two-way and Three-way ANOVA) and presented in table 8.

RESULTS AND DISCUSSION

Total phenol

The total phenolic content of the five successive extracts of *L.longiflorus* bark sample collected from *Casuarina* and *Ficus* host trees were quantified and the data are shown in Table 1. The ethanol extract of *Loranthus* bark from both (*Casuarina* and *Ficus*) host trees showed maximum total phenolic content (301.25mg/g and 307.27mg/g, respectively) than other successive extracts tested. But higher level of total phenolic content was noted in the successive extracts of *Loranthus* bark sample obtained from *Ficus* host than the *Casuarina* host tree. The high concentration of phenolics appeared to be a general feature of parasitic organisms [21]. Phenols are very important plant constituents because of their radical scavenging ability due to the hydroxyl groups [22].

Total tannins

The total tannin content of the successive extracts of *L. longiflorus* bark samples obtained from *Casuarina* and *Ficus* host trees are shown in Table 1. Among the extracts tested, ethanol extracts of *Loranthus* bark samples from both host trees contain maximum amount of total tannins than other extracts. However, high level of tannin was recorded in the successive extracts of *Loranthus* bark samples from *Ficus* than from *Casuarina* hosts. Tannins, the high molecular weight phenols, act as a good scavenger of free radicals either by donating hydrogen atom or by reducing them. This property is attributed by the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution and specific functional groups present in the tannins [23, 24]. Thus, the successive extracts may have more polyhydroxyl phenols, which may be acting synergistically with other phytoconstituents to exhibit its antioxidant property as suggested by Thendral *et al*. [24].

Total Flavonoids

The chloroform extract of *L. longiflorus* bark samples obtained from *Casuarina* and *Ficus* host trees contain maximum amount of flavonoids (18.92mg/g and 26.13mg/g, respectively) followed by hexane and ethyl acetate, while other extracts (ethanol and water) shows trace (not detectable) amount only (Table 1). The flavonoid content in the bark sample of *Loranthus* from *Ficus* shows more amount of flavonoids than the extracts of *Loranthus* bark from *Casuarina*. Flavonoids and their relative compounds are effective in scavenging hydroxyl radicals [25] and in DPPH radical [26].

Table 1. Determination of total phenol, tannin and flavonoid content in the bark of *L.longiflorus* obtained from two host trees.

Solvent extracts used	Total phenolics (mg TAE/g extract)		Total tannins (mg TAE/g extract)		Total flavonoids (mg RE/g extract)	
	Ll –bark from Ce-host	Ll –bark from Fr-host	Ll –bark from Ce-host	Ll –bark from Fr-host	Ll –bark from Ce-host	Ll –bark from Fr-host
Chloroform	27.07 ± 3.16	57.35 ± 6.87	13.87 ± 1.67	21.55 ±14.41	18.92 ± 0.31	26.13 ± 0.55
Ethyl acetate	132.11 ±1.76	134.00 ±4.98	66.22 ± 1.01	67.05 ± 2.52	3.04 ± 0.22	6.35 ± 0.02
Hexane	14.53 ± 4.52	17.75 ± 2.27	5.08 ± 3.11	6.74 ± 4.53	6.27 ± 0.28	7.01 ± 0.29
Ethanol	301.25 ±19.51	307.27 ± 2.69	191.46 ±9.26	204.83 ±18.60	ND	ND
Water	100.83 ±2.08	183.92 ±7.94	50.86 ± 1.11	92.51 ± 3.71	ND	ND

Ll –*Loranthus longiflorus* Ce –*Casuarina equisetifolia* Fr –*Ficus religiosa* ± -Standard Deviation
Each value in the table is the mean of three replicates ND –Not detectable

Free Radical Scavenging Activities

Ferric reducing antioxidant power (FRAP)

It was recorded in the extracts of *L. longiflorus* bark samples collected from *Casuarina* and *Ficus host* trees. Among the extracts, ethyl acetate extract of *Loranthus* bark samples from the two host trees showed more activity (4053.53% and 4199.03%, respectively) than other extracts (Table 2). Among the host trees, *Ficus* favours higher FRAP in the bark samples of *Loranthus* than the *Casuarina* host tree. Many studies revealed that only polar extracts of plants

showed effective antioxidant activity and some researches further proved that moderate polarity extracts are more potent even if their total phenolic content did not include all the antioxidant [27]. Vinson *et al*. [28] suggested that the synergism among the antioxidant in the mixture made the antioxidant activity not only dependant on the concentration of antioxidant but also on the structure and interaction among the antioxidant.

Table 2. Estimation of ferric reducing antioxidant power of *L. longiflorus* bark obtained from two host trees.

Concentration of solvent extracts used	Ferric reducing antioxidant power (mmol Fe (II)/mg extract)	
	LI –bark from Ce-host	LI –bark from Fr-host
Chloroform (50 µg)	109.98 ± 13.25	4.71 ±0.18
Ethyl acetate (50 µg)	2493.43 ± 62.42	2129.93 ±237.69
Hexane (50 µg)	4.46 ±1.25	4.03 ±1.07
Ethanol(50 µg)	4053.53 ±259.86	4199.03 ±17.42
Water (50 µg)	1027.74 ± 78.40	1952.80 ±122.16

DPPH Free Radical Scavenging Activity

The DPPH scavenging activities in the extracts of *L. longiflorus* bark samples collected from *Casuarina* and *Ficus* host trees were summarized in Table 3 and Figure 1. All the extracts of *Loranthus* bark sample collected from *Ficus* favours more DPPH radical scavenging activities than the *Casuarina* host tree, except chloroform extract which shows vice verse. Maximum DPPH radical scavenging activity (4681.8% and 4890.6%) was noted at high concentration (1500µg) of ethanol extract of *Loranthus* bark collected from *Casuarina* and *Ficus*, respectively, while all other extracts of both samples shows low activity. At all concentrations (300µg to 1500 µg) tested, the extracts of *Loranthus* bark samples collected from the two hosts exhibited increasing scavenging activity with increase in concentration of extracts. The scavenging activity of all samples on the DPPH radicals was found to be strongly dependent on the extract concentration as reported by Motalleb *et al*. [3]. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [29]. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517nm which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate free radical scavenging activity of antioxidants [30] The use of DPPH radicals provides an easy, rapid and convenient method to evaluate the antioxidant and radical scavenging [31, 32, 33, 34]. This method is a sensitive way to survey the antioxidant activity of a specific compound or plant extracts [35].

Table 3. DPPH radical scavenging activity in the extracts of *L. longiflorus* bark collected from two host trees.

***Loranthus* infested Host trees (source of sample)**	**Concentration of solvent extracts used (µg)**	**DPPH radical scavenging activity (%) of *L. longiflorus* bark extracts**				
		Chloroform	**Ethyl acetate**	**Hexane**	**Ethanol**	**Water**
C. equisetifolia	300	52.92 ±2.01	517.50 ±32.40	9.39 ±0.34	2173.2 ±72.6	743.4 ±40.2
	600	89.67 ±2.22	720.60 ±00.00	17.92 ±0.94	2831.4 ±64.8	1072.2 ±04.2
	900	117.12 ±3.03	867.90 ±10.20	24.55 ±0.20	3466.2 ±16.2	1255.2 ±36.6
	1200	133.98 ±0.21	1038.00 ±16.20	29.79 ±0.20	4066.8 ±32.4	1581.6 ±44.4
	1500	155.73 ±1.41	1192.50 ±08.10	35.27 ±0.13	4681.8 ±36.6	1801.8 ±16.2
F. religiosa	300	46.42 ±3.38	564.90 ±42.60	17.87 ±0.20	2445.0 ±125.4	906.6 ±04.2
	600	65.58 ±0.80	730.80 ±14.10	24.31 ±0.54	2894.4 ±32.4	1258.2 ±40.2
	900	76.94 ±0.68	942.30 ±42.60	29.17 ±0.40	3817.8 ±12.0	1661.4 ±04.2
	1200	90.76 ±1.88	1113.90 ±02.10	32.60 ±0.13	4470.0 ±20.4	2076.0 ±00.0
	1500	103.06 ±0.68	1266.90 ±12.00	37.04 ±0.61	4890.6 ±7.8	2373.6 ±16.2

Nitricoxide free radical scavenging (NO-FRS) activity

The ability of NO-FRS activity was assessed in the extracts of *L. longiflorus* bark samples from *Casuarina* and *Ficus* host trees and the results show that the percentage of NO-FRS activity was concentration dependent in both samples. Among the extracts, ethyl acetate extracts of *Loranthus* bark samples from *Casuarina* host trees show higher activity than the other extracts at all

concentrations, tested (Table 4; Figure 2), while maximum activity was noted in water extract of *Loranthus* bark sample from the host of *Ficus*. Maximum NO-FRS activity (49.79% and 48.28%) was noted in the ethyl acetate extract and water extract (at 250µg) of *Loranthus* bark samples from *Casuarina* and *Ficus* hosts, respectively. Except ethyl acetate extract, all other extracts of *Loranthus* bark samples collected from *Ficus* favours more NO-FRS activities than from *Casuarina*. Nitricoxide is a potent diffusible free radical involved in a variety of biological functions [36]. This is due to the fact that nitric oxide can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH- and NO [37].

Table 4. Nitric oxide radical scavenging activity in the extracts of *L. longiflorus* bark collected from two host trees

***Loranthus* infested Host trees (source of sample)**	**Concentration of solvent extracts used (µg)**	**Nitric oxide radical scavenging activity (%) of *L. longiflorus* bark extracts**				
		Chloroform	**Ethyl acetate**	**Hexane**	**Ethanol**	**Water**
C. equisetifolia	50	5.35 ±0.82	12.23 ±1.52	6.00 ±0.65	7.04 ±0.91	6.65 ±0.91
	100	15.06 ±0.49	23.82 ±0.91	11.55 ±0.98	16.09 ±0.30	16.52 ±0.91
	150	19.40 ±0.33	33.05 ±1.21	14.78 ±0.33	24.03 ±0.61	25.97 ±0.91
	200	24.02 ±0.33	43.35 ±1.21	20.21 ±0.16	31.76 ±1.21	31.55 ±0.30
	250	28.64 ±0.33	49.79 ±0.61	24.02 ±0.33	39.06 ±1.21	39.70 ±2.12
F. religiosa	50	5.43 ±0.16	11.59 ±0.61	6.89 ±0.82	7.94 ±2.73	18.24 ±0.91
	100	15.24 ±0.98	17.60 ±0.61	13.16 ±0.00	16.89 ±0.91	28.11 ±0.91
	150	21.25 ±0.33	21.67 ±1.52	18.94 ±0.33	24.25 ±1.52	34.98 ±0.91
	200	26.21 ±0.16	27.25 ±0.30	23.33 ±0.65	35.19 ±2.43	41.63 ±0.61
	250	32.45 ±0.82	34.12 ±0.30	27.60 ±0.49	39.48 ±0.61	48.28 ±0.30

Superoxide Free Radical Scavenging (SO-FRS) Activity

From the results of present study (Table 5; Figure 3), it was found that the successive extracts of *L. longiflorus* leaf, collected from *Casuarina* and *Ficus host* trees, possess the SO-FRS activity and is concentration dependent. Maximum activity (33.71% and 34.85%) was recorded at high concentration (250µg/ml) of ethanol and chloroform extracts of *Loranthus* leaf collected from *Casuarina* and *Ficus* host trees, respectively, as compared to other extracts. Among the host trees, *Casuarina* influences more SO-FRS activity in ethanol and water extracts of *Loranthus* bark sample than other extracts, while *Ficus* host tree promote the higher SO-FRS activity in the chloroform, ethyl acetate and hexane extracts than the ethanol and water extracts. Superoxide radicals are known to be very harmful to the cellular components. It is formed by alkaline Dimethyl Sulphoxide (DMSO) which reacts with Nitrobluetetrazolium (NBT) to produce coloured diformazan. It is biologically important as it can form singlet oxygen and hydroxyl radical [38]. Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences [39]. The differences recorded in the scavenging activity between extracts of *Loranthus* bark samples collected from *Casuarina* and *Ficus* hosts might be due to their difference antioxidant mechanisms or variations in their ability to scavenge free radicals or due to the influence of host trees on *Loranthus*. The results of present study were in agreement with the report of Ravi Shankar *et al*. [40] and Mary *et al*. [41]. However, a large number of phytocompound groups are implicated for antioxidants activity [42]. They have reported varying levels of antioxidants and free radicals scavenging properties of plant extracts of *Acorus calamus* and *Hemidesmus indicus*. The antioxidant activity is affordable not only by phenolic compound but also has important contributions from other superoxide anion radical scavengers such as essential oils, carotenoids and vitamins [43]. Some variations in the extent of extract in antioxidant activity were observed for each type of assay used in this study.

Hydroxyl radical scavenging (HO-FRS) activity

Hydroxyl radical was generated in the presence of $Fe3^{+}$-EDTA, ascorbate and H_2O_2 (Fenton system) and monitored by evaluating hydroxyl radical-induced deoxyribose degradation [44]. The obtained results demonstrate that the successive extracts of *L. longiflorus* bark samples collected from *Casuarina* and *Ficus host* trees possess significant HO-FRS activity at all concentrations tested (Table 6; Figure 4). The HO-FRS activity was concentration dependent, i.e., the activity was increased with increasing concentration of extracts. Among the extracts tested, maximum activity (49.37% and 55.58%) was noted in the ethanol extract of *Loranthus* leaf samples from *Casuarina* and *Ficus* host trees, respectively. The host tree of *Casuarina* favours more HO-FRS activity in the water, chloroform, ethyl acetate and hexane extracts of *Loranthus* bark samples, except ethanol which shows less activity than the extracts of *Loranthus* from *Ficus* host tree (Table 6; Figure 4).

Table 5. Superoxide radical scavenging activity in the extracts of *L. longiflorus* bark collected from two host trees

***Loranthus* infested Host trees (source of sample)**	**Concentration of solvent extracts used (µg)**	**Superoxide radical scavenging activity (%) of *L. longiflorus* bark extracts**				
		Chloroform	**Ethyl acetate**	**Hexane**	**Ethanol**	**Water**
C. equisetifolia	50	2.85 ±0.10	3.41 ±0.50	2.13 ±0.45	15.45 ±0.54	5.39 ±0.26
	100	6.03 ±0.70	7.87 ±0.94	4.26 ±0.47	20.09 ±0.38	10.39 ±0.10
	150	9.51 ±0.25	11.77 ±0.15	7.93 ±0.15	25.20 ±0.63	17.55 ±0.76
	200	13.37 ±0.29	15.57 ±0.54	10.52 ±0.06	30.83 ±0.92	24.21 ±0.31
	250	16.26 ±0.65	19.60 ±0.50	12.89 ±0.20	33.71 ±0.41	30.27 ±0.36
F. religiosa	50	9.25 ±0.30	6.05 ±0.45	4.88 ±0.37	4.46 ±0.66	8.26 ±0.48
	100	14.35 ±0.77	9.76 ±0.55	11.13 ±0.39	9.75 ±0.45	14.09 ±0.27
	150	22.21 ±0.74	14.25 ±0.22	16.84 ±0.39	16.27 ±0.31	18.69 ±0.32
	200	28.84 ±0.82	19.08 ±1.22	24.16 ±0.39	21.03 ±0.64	24.99 ±0.48
	250	34.85 ±0.53	25.72 ±0.22	29.23 ±0.72	28.04 ±0.95	30.14 ±0.56

Table 6. Hydroxyl radical scavenging activity in the extracts of *L. longiflorus* bark collected from two host trees

***Loranthus* infested Host trees (source of sample)**	**Concentration of solvent extracts used (µg)**	**Hydroxyl radical scavenging activity (%) of *L. longiflorus* bark extracts**				
		Chloroform	**Ethyl acetate**	**Hexane**	**Ethanol**	**Water**
C. equisetifolia	50	16.60 ±1.12	14.73 ±1.01	9.60 ±0.67	17.45 ±0.25	17.36 ±0.38
	100	24.31 ±0.28	19.98 ±0.33	14.15 ±1.33	25.18 ±0.76	24.73 ±0.13
	150	31.42 ±0.84	28.07 ±1.00	20.05 ±0.33	32.91 ±0.51	31.38 ±0.13
	200	35.57 ±0.56	36.56 ±0.33	26.18 ±1.00	40.29 ±0.25	37.77 ±1.27
	250	40.71 ±0.56	46.22 ±1.33	31.84 ±1.01	49.37 ±0.38	42.81 ±0.51
F. religiosa	50	6.92 ±0.64	13.67 ±0.25	8.72 ±0.64	27.43 ±0.38	15.83 ±0.01
	100	12.77 ±0.76	19.78 ±0.51	13.49 ±0.25	34.98 ±0.64	22.21 ±1.14
	150	19.78 ±0.25	27.88 ±0.76	18.71 ±0.25	41.55 ±0.25	28.15 ±0.38
	200	26.62 ±0.25	33.09 ±0.51	22.21 ±0.38	48.65 ±1.40	34.17 ±0.25
	250	32.82 ±0.89	37.68 ±0.13	27.34 ±0.25	55.58 ±0.76	40.20 ±0.64

Table 7. Inhibitory concentration (IC_{50}) of extracts for radical scavenging activity of *L. longiflorus* bark collected from two host trees.

***Loranthus* infested host trees (source of sample)**	**Solvent extracts used**	**IC_{50} concentration of *L. longiflorus* bark extracts (µg/ml)**			
		DPPH-RSA	**NO-RSA**	**SO-RSA**	**HO-RSA**
C. equisetifolia	Chloroform	173.91	204.9	260.42	39.03
	Ethyl acetate	22.50	118.5	213.68	38.61
	Hexane	800.00	252.5	326.80	54.95
	Ethanol	5.70	158.2	108.93	34.34
	Water	15.02	156.3	138.89	37.39
F. religiosa	Chloroform	156.41	188.0	117.37	54.53
	Ethyl acetate	21.10	116.1	168.35	43.03
	Hexane	74.29	215.5	143.68	62.11
	Ethanol	5.32	152.4	104.32	38.35
	Water	11.55	117.4	134.41	40.82

linhibitory concentration (IC_{50})

The minimum inhibitory concentration of successive extracts of *L. longiflorus* bark samples, obtained from *Casuarina* and *Ficus* host trees, required for free radical scavenging activities of DPPH, Nitric oxide, Superoxide and Hydroxyl radicals was determined and the data are presented in Table 7; Figure 5. Among the extracts tested, ethanol extract of *Loranthus* bark obtained from *Casuarina* and *Ficus* hosts show free radical scavenging activity at lowest concentration, i.e., 5.70µg/ml and 5.32µg/ml for DPPH; and is followed by 34.34µg/ml and 38.35µg/ml for Hydroxyl; 108.93µg/ml and 104.32µg/ml for Super oxide respectively, while ethyl acetate extract show potent activity against Nitric oxide radicals at low concentration of 188.5µg/ml and 116.1µg/ml, respectively). Among the host trees, *Ficus* offer low IC_{50} for DPPH, NO and SO radical scavenging activities in all extracts except water extract of *Loranthus* bark samples than *Casuarina*, in which the HO-FRS activity noted at low IC_{50} of water extracts than other extracts.

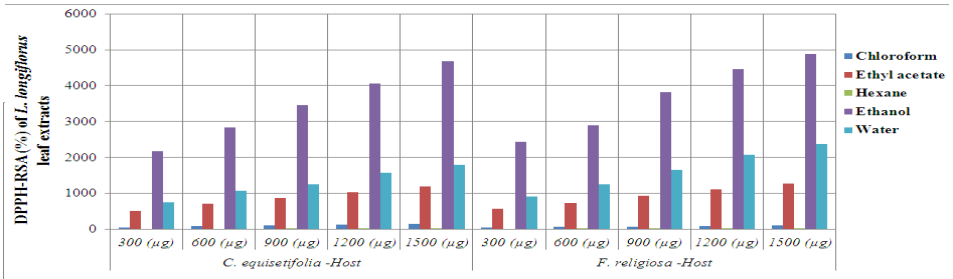

Fig. 1. Concentration of extracts of *L. longiflorus* bark from two host trees

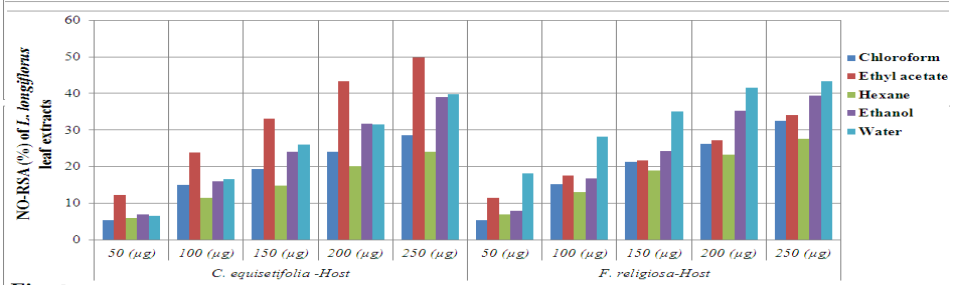

Fig. 2. Concentration of extracts of *L. longiflorus* bark from two host trees

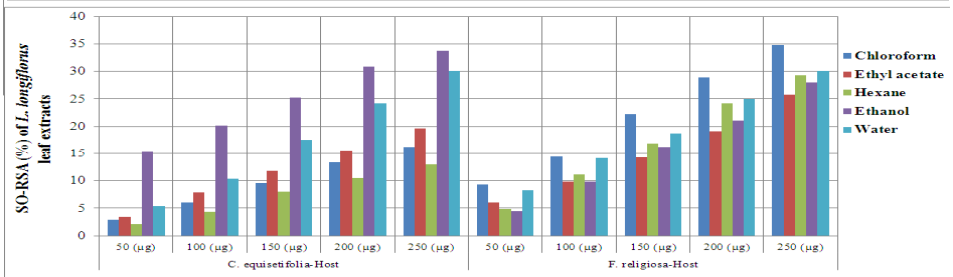

Fig. 3. Concentration of extracts of *L. longiflorus* bark from two host trees

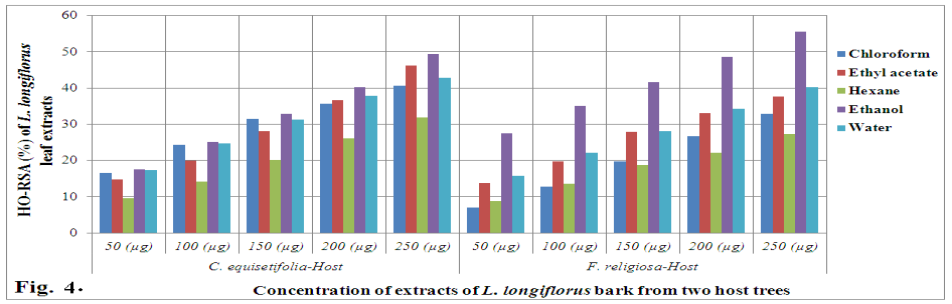

Fig. 4. Concentration of extracts of *L. longiflorus* bark from two host trees

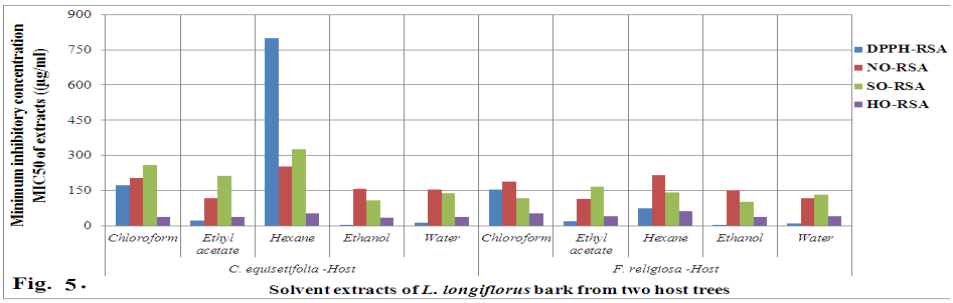

Fig. 5. Solvent extracts of *L. longiflorus* bark from two host trees

Figure 1-5. Antioxidant activities (Fig.1-4) and IC_{50} (Fig. 5) of various solvent extracts of *L. longiflorus* bark samples collected from two host trees.

Table 8. Analysis of variance (ANOVA) for the data of antioxidant and free radical scavenging activities of extracts of *L. longiflorus* bark samples collected from two host trees.

Parameters analyzed		H	E	C	HE	HC	EC	HEC
Two-way ANOVA								
Total Phenols	S Ed	2.18	3.45		4.88			
	CD (P=0.05)	4.58	7.25		10.25			
	F-value level	**	**		**			
Total Tannins	S Ed	2.23	3.53		4.99			
	CD (P=0.05)	4.68	7.41		10.47			
	F-value level	**	**		**			
Total Flavonoids	S Ed	0.09	0.15		0.21			
	CD (P=0.05)	0.19	0.31		0.43			
	F-value level	**	**		**			
FRAP	S Ed	35.85	56.69		80.17			
	CD (P=0.05)	75.33	119.11		168.44			
	F-value level	**	**		**			
Three-way ANOVA								
DPPH-FRSA	S Ed	1.41	10.12	5.19	12.88	6.71	14.50	19.47
	CD (P=0.05)	6.06	21.45	10.33	27.64	14.04	29.76	39.85
	F-value level	**	**	**	**	**	**	**
NO-FRSA	S Ed	0.02	0.23	0.12	0.29	0.16	0.34	0.45
	CD (P=0.05)	0.09	0.49	0.25	0.62	0.32	0.69	0.93
	F-value level	**	**	**	**	**	**	**
SO-FRSA	S Ed	0.02	0.07	0.09	0.09	0.11	0.19	0.27
	CD (P=0.05)	0.08	0.16	0.17	0.21	0.23	0.38	0.53
	F-value level	**	**	**	**	**	**	**
HO-FRSA	S Ed	0.07	0.09	0.23	0.14	0.30	0.47	0.67
	CD (P=0.05)	0.30	0.20	0.46	0.37	0.64	0.95	1.33
	F-value level	**	**	**	**	**	**	**

H –Between host; E –Between extract; C –Between concentration; ** -Significance at 1% level

CONCLUSION

The results indicate that *L. longiflorus* bark obtained from *C. equisetifolia* and *F. religiosa* host trees are rich source of natural total phenols and total tannin, while poor source for flavonoids. The ethanol extract of bark samples shows more content of total phenol and total tannin content, while chloroform contain maximum flavonoid content. Among the host trees, *Ficus* support more tannin content in the bark sample of *Loranthus*. The high content of antioxidants such as total phenols, tannins (in ethanol extracts) and flavonoids (in the chloroform extracts) of *Loranthus* bark samples obtained from *Casuarina* and *Ficus* host trees, respectively, may impart health benefits by combating free radicals in synergistic manner along with other compounds. This observation also suggests that the phytochemicals, necessary for free radical scavenging activity, are present abundantly in the polar fractions and is confirmed by several workers. The pronounced antioxidant activity of the extracts of *L. longiflorus* bark samples obtained from *C. equisetifolia* and *F. religiosa*, manifested as scavengers of DPPH, hydroxyl, nitricoxide, superoxide and ferric reducing power, was possibly due to the presence of high phenolic contents.

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