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Antioxidant Activities of *Saussurea lappa* (Root), *Ficus bengallensis* (Root), *Flacourtria romantchi* (Stem Bark and Root), and *Oroxyllum indicum* (Root) in Swiss Albino Mice

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Keywords: Root extract of *Saussurea lappa* (RESL), Root extract of *Ficus bengallensis* (REFB), Stem bark and root extract of *Flacourtria romantchi*(SBAREFR), Root extract of *Oroxyllumindicum* (REOI), OECD guideline 425

ABSTRACT

The present study was aimed to evaluate the *In-vitro* and *In-vivo* antioxidant activities of different solvent extracts of selected herbal medicinal plants sources namely *Saussurea lappa* (Root), *Ficus bengallensis* (Root), *Flacourtria romantchi* (stem bark and Root), and *Oroxyllum indicum* (Root). Free radical (DPPH and OH) scavenging potential of the extracts revealed that both extracts to be active radical scavengers. Reducing (Fe^{+3}-Fe^{+2}) power and lipid peroxidation inhibition efficiency of four extracts were also evaluated and all extracts showed promising activity in preventing lipid peroxidation and might prevent oxidative damages to the biomolecules. All extracts were able to protect DNA from oxidative damage. The results obtained suggest that extracts of *Saussurea lappa*, *Ficus bengallensis*, *Flacourtria romantchi*, and *Oroxyllum indicum* have promising therapeutic potential and could be considered as a potential source for drug development by pharmaceutical industries.

INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves.[1] As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. [Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals and hydrogen peroxide, which are generated by normal physiological processes and various exogenous factors initiate peroxidation of membrane lipids, as well as a wide range of other biological molecules through a process that is believed to be implicated in the etiology of several disease conditions, including coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer.[2] Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infections and degenerative diseases. However, during recent years, people have been more concerned about the safety of their food and the potential effect of synthetic additives on their health. The two most commonly used synthetic antioxidants; Butylated hydroxyanisole (BHA) and Butylated (BHT) have begun to be restricted because of their toxicity and DNA damage induction.[3,4] Therefore, natural antioxidants from plant extracts have attracted increasing interests due to their safety. Antioxidants can either directly scavenge or prevent generation of ROS.[5,6] Recent researches have been interested in finding novel antioxidants to combat and/or prevent ROS-mediated diseases.[7,8] However oxidative stress is directly related to necrosis and apoptosis which manifests several malfunctions in the body and may be attributed to high level of arsenic but more study is needed to establish a vital relationship for considering it as a cause for cervical cancer.[9,10]

In-Vitro Antioxidant Activity [11, 12, 13]

Antioxidants can be defined as any substance that reduces oxidative damage (damage due to oxygen) such as that caused by free radicals. Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Antioxidants can be divided as follows.

Naturally Occurring and Derived Anti-oxidants-

- Enzymatic – SOD, Catalase, Glutathione Peroxides, Transferases.
- Nonenzymatic – Ascorbic Acid, Tocopherols, Carotenoids, Lipoic Acid, Flavonoids, Albumin, Ceruloplasmin, Bilirubin, N-aceylcysteine, Lactoferine, Nicotinamide.
- From plants – Cucurmin, Chlorogenic Acid, Caffeine, Strychnine, Brucine, Spartine, β-Carotene, Phosphatidylethanolamine.

Synthetic Anti-oxidants

- Thiols-GSH Precursors (Cysteine, Cysteamine), Dimercaprol,
- Glutathione Peroxidase Mimic – Ebselen
- Xanthine Oxidase Inhibitors – Allopurinol, Oxyprino, Lodoxamide, Amflutizole
- Chelating Agents – Deferoxamine, Lazaroids
- Chain Breaking Agents - Probucol

Oxidative Stress

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells.[14]This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.[14]

In humans, oxidative stress is involved in many diseases, such as Atherosclerosis, Parkinson's disease, Heart Failure, Myocardial Infarction, Alzheimer's disease and Chronic fatigue syndrome, but it may also be important in the prevention of aging by induction of a process named mitohormesis.[15]Reactive oxygen species can be beneficial as they are used by the immune system as a way to attack and kill pathogens. Reactive oxygen species are also used in cell signaling. This is dubbed redox signaling.[16]

In chemical terms, oxidative stress is a large rise (becoming less negative) in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione. The effects of oxidative stress depend on the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis while more intense stresses may cause necrosis.[17,26]

A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by Oxido - reduction reactions with transition metals or other redox cycling compounds (including Quinones) into more aggressive radical species that can cause extensive cellular damage. The major portion of long term effects is inflicted by damage on DNA.[18]

A large number of plants have shown anti-oxidant activities.[19] The present study was undertaken to test four plant extracts of *(1) Saussurea lappa (Root) (2) Ficus bengallensis (Root) (3) Flacourtria romantchi (stem bark and Root)(4) Oroxyllum indicum (Root)* for their *in-vitro* anti-oxidant activity using following assays-

a. DPPH free radical scavenging activity assay
b. Phosphomolybdenum reduction assay
c. Nitric oxide radical-scavenging assay
d. Reducing power assay

MATERIAL AND METHODS

Plant Material:

The plant extract was used by initially making a stock solution of 1mg/ml and thereafter concentrations of 25, 50, 75, 100, 250 and 250µg/ml were made.

Methods:

DPPH radical scavenging activity [18, 19]

a. The free radical scavenging activity of the extract was measured by 1, 1 diphenyl-2-picryl hydrazyl (DPPH) using the method described by Shimada et al. Briefly, 0.1 Mm solution of DPPH in ethanol was prepared.

b. Plant extract (0.1 ml) at different concentrations (25-250µg/ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Distilled water (0.1 ml) in place of the plant extract was used as a control.

c. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min.

d. Absorbance at 517 nm was determined after 30 minutes by UV-Visible Spectrophotometer.

e. Calculation:

Free radical scavenging capacity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

I (% inhibition) = 100 × (1-Absorbance of sample/Absorbance of control)

$$\left(\frac{A_o - A_1}{A_o} \right) \times 100$$

where A_o=Absorbance of the control.
A_1=Absorbance of the standard or sample.

From the obtained values, the IC_{50} (defined as the concentration of extract at which 50% of maximum scavenging activity was recorded) was calculated for each extract.

Phosphomolybdenum Reduction Assay [20]

Chemicals:

- Ammonium molybdate
- Sulfuric acid
- Sodium phosphate
- Methanol
- Butylated hydroxy toluene (B.H.T.).

Method:

a. 0.3 ml plant extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate).

b. The tubes containing the reaction solution were incubated at 95°C for 90 min.

c. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of the extract was used as the blank.

d. The antioxidant activity is expressed as the number of equivalents of B.H.T. A standard curve of B.H.T. prepared to determine the gram equivalent activity of plant extract to B.H.T.

e. The absorbance of the test solution interpolated to determine its equivalent concentration to B.H.T.

Calculation:

The antioxidant activity is expressed as the number of equivalents of BHT. A standard curve of B.H.T. was prepared to determine the gram equivalent activity of plant extract with reference to B.H.T. Absorbance of the test solution intrapolated to determine its equivalent concentration to B.H.T.

Nitric Oxide Radical-scavenging Activity Assay [21]

Free radical scavenging activity was evaluated by studying the inhibition of the generation of Nitric Oxide from Sodium nitroprusside. An aqueous solution of sodium nitroprusside at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. The nitrite ions thus produced can be quantified using their reaction with Griess reagent that leads to the formation of a chromophore, the concentration of which is proportional to that of the generated nitrite ions. Scavengers of nitric oxide compete with oxygen leading to a reduced production of nitric oxide.

Chemicals: Sodium nitroprusside, Sodium phosphate, Griess reagent.

Griess reagent: This reagent is a mixture of following three solutions (1) 50 ml Sulfanilamide solution. (2) 50 ml of NED solution. (3) 1 ml of Nitritestandard solution (0.1 M sodium nitrite in water).

NED: 0.1% N-1- naphthylethylenediamine dihydro chloride in water.
Sulfanilamide solution: 1% sulphanilamide in 5% phosphoric acid.
Nitrite standard: 0.1 M sodium nitrite in water.

Method: The method of Sreejayan et al, 1997 was followed. In this assay, 1 ml of sodium nitroprusside (5mM) in phosphate buffered saline was mixed with 3 ml of different concentrations (50-250mcg/ml) of the plant extract dissolved in ethanol and incubated at room temperature for 150 minutes. The same reaction without the sample but an equivalent amount of ethanol served as control. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm and compared with the standard solution of ascorbic acid.

As a blank, the assay mixture similarly run in the absence of the extract or B.H.T was used.

Reducing power assay [22, 23, 24]

Chemicals: Potassium ferricyanide, Trichloro-acetic acid, Ferric-chloride.

Method:

1 ml of different concentrations of the plant extract solutions were added to 2.5 ml of 1 % potassium ferricyanide in different test tubes and the resultant mixture incubated at 50^0 C for 20 minutes. Then, 2.5 ml of 10% trichloroacetic acid was added to each tube. The tubes were centrifuged for 10 minutes at 3000 rpm. The supernatant from each tube (2.5 ml) was taken in a separate test tube and 2.5 ml of distilled water 0.5 ml (0.1%) ferric chloride solution were added to each test tube.

The absorbance of these assay mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

In parallel to this, the reducing power of ascorbic acid was also determined for comparison.

RESULTS

The crude extracts were screened for *in-vitro* anti-oxidant activity using DPPH radical scavenging method, Phosphomolybdenum reduction assay, Nitric oxide scavenging activity and reducing power assay and were compared with standard Butylated hydroxyl toluene (B.H.T.). The crude extracts exhibited anti-oxidant activity in the scavenging of DPPH, Phosphomolybdenum reduction assay, Nitric oxide scavenging assay and Reducing power assay. The anti-oxidant potency of a compound was inversely proportional to the IC_{50} value. It was observed that a dose response relationship is found in the DPPH radical scavenging activity; the activity was increased as the concentration increased.

The results obtained in all antioxidant assays showed statistically significant difference between Different solvent extracts at $P<0.05$.

Table: 1. DPPH Scavenging Activity of B.H.T and root extract of *Saussurea lappa (SLE)*.

S. No.	Groups	Concentration (µg/ml)	Log Conc.	% Scavenging activity (Mean ± S.E.M)	Probits of % Scavenging Activity
1.	B.H.T	25	1.39	39.22 ± 0.0321	4.72
		50	1.69	62.29 ± 0.0163	5.31
		100	2.00	65.75 ± 0.0216	5.41
		150	2.17	75.35 ± 0.0768	5.67
		200	2.30	78.24 ± 0.0435	5.77
		250	2.39	85.47 ± 0.0147	6.04
2.	SLE	25	1.39	20.36 ± 0.2471	4.16
		50	1.69	35.46 ± 0.5262	4.61
		100	2.00	42.38 ± 0.4326	4.80
		150	2.17	48.47 ± 0.3241	4.95
		200	2.30	65.26 ± 0.4475	5.39
		250	2.39	71.32 ± 0.5351	5.55

Fig: 09 Probits Vs Log conc. of B.H.T. to calculate the IC50 value of B.H.T.

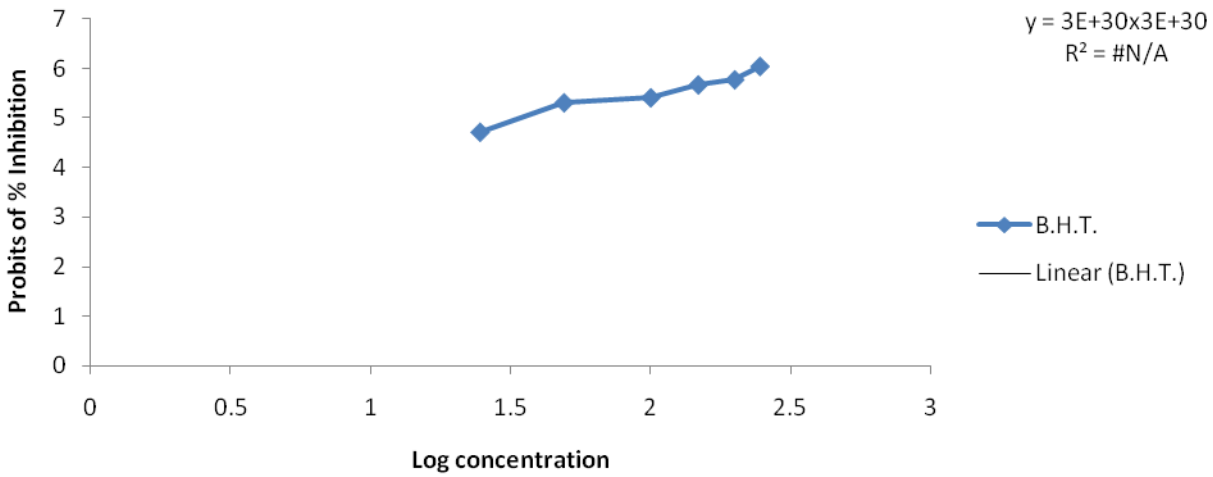


Fig: 10 Probits vs Log Conc. of root extract of SL to calculate the IC50 value of *Saussurea lappa* (SL).

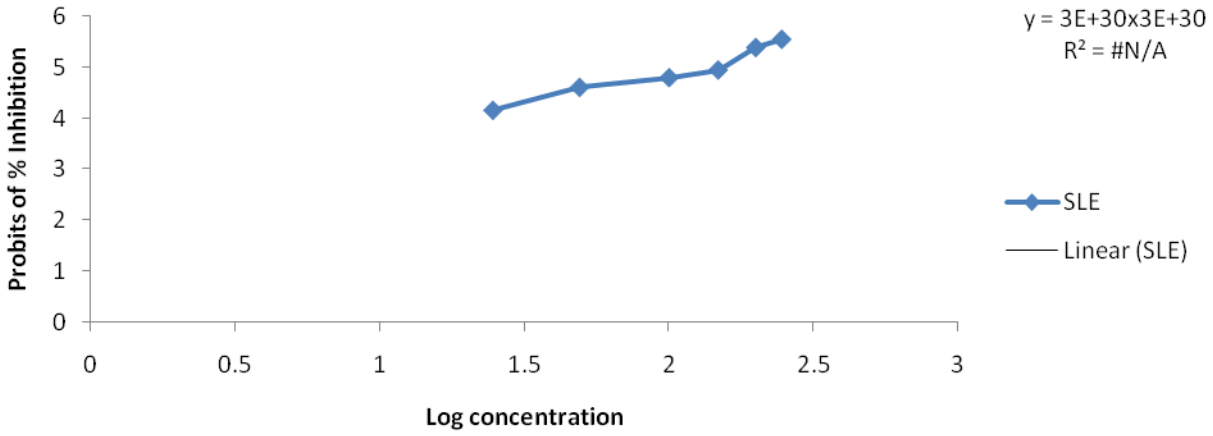


Table 2. DPPH Scavenging Activity of B.H.T and root extract of *Ficus bengallensis (FBE).*

S. No.	Groups	Concentration (µg/ml)	Log Conc.	% Scavenging activity (Mean ± S.E.M)	Probits of % Scavenging activity
1.	B.H.T	25	1.39	39.22 ± 0.0321	4.72
		50	1.69	62.29 ± 0.0163	5.31
		100	2.00	65.75 ± 0.0216	5.41
		150	2.17	75.35 ± 0.0768	5.67
		200	2.30	78.24 ± 0.0435	5.77
		250	2.39	85.47 ± 0.0147	6.04
2.	FBE	25	1.39	25.46 ± 0.3246	4.33
		50	1.69	37.49 ± 0.4405	4.67
		100	2.00	45.28 ± 0.2161	4.87
		150	2.17	53.62 ± 0.4177	5.10
		200	2.30	57.37 ± 0.4343	5.18
		250	2.39	65.51 ± 0.3341	5.39

Fig: 1 Probits vs Log Conc. of root extract of *FB* to calculate the IC50 value of *Ficus bengallensis extract(FBE)*

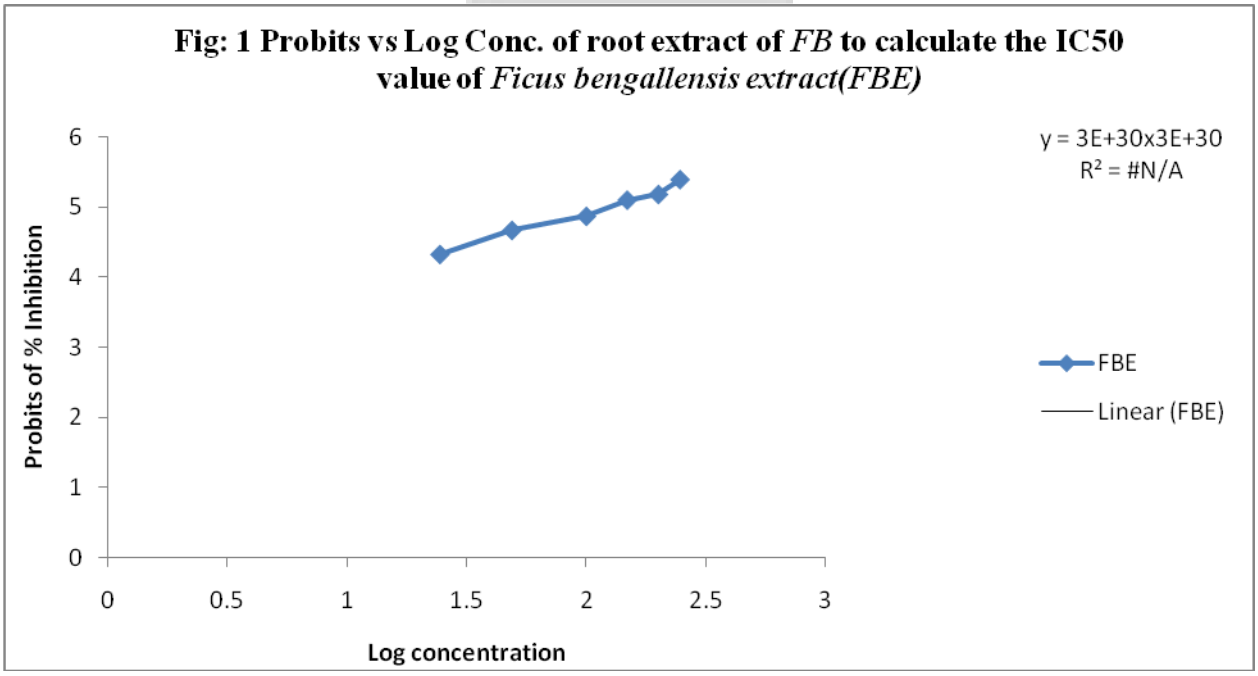


Table: 2 DPPH Scavenging Activity of B.H.T and stem bark and root extract of *Flacourtria romantchi.*

S. No.	Groups	Concentration (µg/ml)	Log Conc.	% Scavenging activity (Mean ± S.E.M)	Probits of % Scavenging activity
1.	B.H.T	25	1.39	39.22 ± 0.0321	4.72
		50	1.69	62.29 ± 0.0163	5.31
		100	2.00	65.75 ± 0.0216	5.41
		150	2.17	75.35 ± 0.0768	5.67
		200	2.30	78.24 ± 0.0435	5.77
		250	2.39	85.47 ± 0.0147	6.04
2.	FRE	25	1.39	18.22 ± 0.247	4.08
		50	1.69	45.36 ± 0.3412	4.87
		100	2.00	58.38 ± 0.4326	5.20
		150	2.17	66.37 ± 0.271	5.41
		200	2.30	77.22 ± 0.375	5.77
		250	2.39	88.12 ± 0.221	6.18

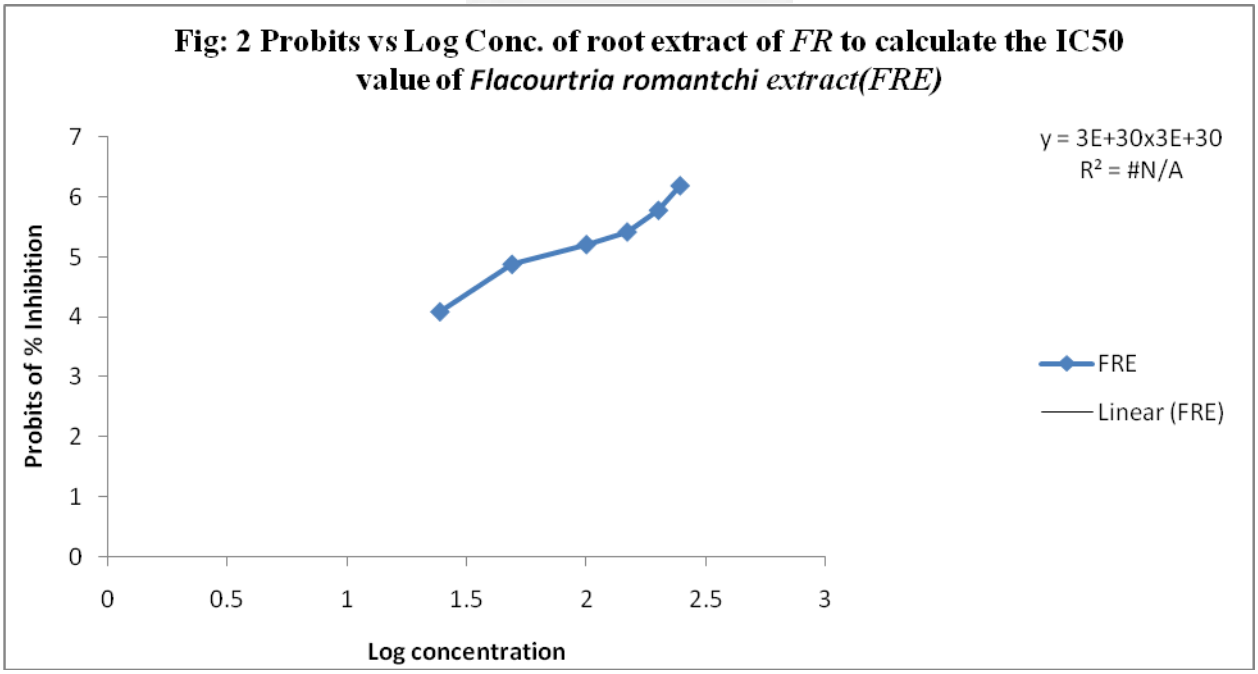


Fig: 2 Probits vs Log Conc. of root extract of *FR* to calculate the IC50 value of *Flacourtria romantchi extract(FRE)*

Table:3 DPPH Scavenging Activity of B.H.T and root extract of *Oroxyllum indicum*.

S. No.	Groups	Concentration (µg/ml)	Log Conc.	% Scavenging activity (Mean ± S.E.M)	Probits of % Scavenging activity
1.	B.H.T	25	1.39	39.22 ± 0.0321	4.72
		50	1.69	62.29 ± 0.0163	5.31
		100	2.00	65.75 ± 0.0216	5.41
		150	2.17	75.35 ± 0.0768	5.67
		200	2.30	78.24 ± 0.0435	5.77
		250	2.39	85.47 ± 0.0147	6.04
2.	OIE	25	1.39	35.22 ± 0.1331	4.61
		50	1.69	39.46 ± 0.3453	4.72
		100	2.00	42.38 ± 0.3135	4.77
		150	2.17	48.47 ± 0.5871	4.95
		200	2.30	65.26 ± 0.6753	5.39
		250	2.39	71.32 ± 0.1675	5.55

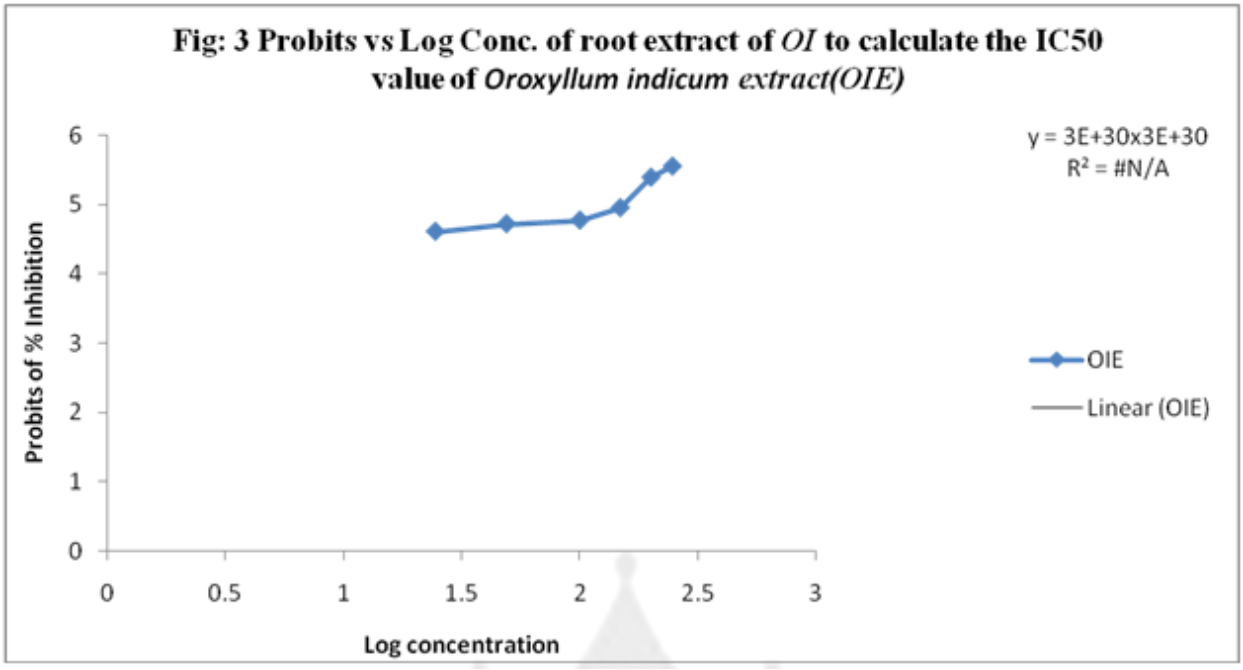


Fig: 3 Probits vs Log Conc. of root extract of *OI* to calculate the IC50 value of *Oroxyllum indicum extract(OIE)*

Phosphomolybdenum Reduction Assay

Total antioxidant activity of the extract evaluated by Phosphomolybdenum Reduction Assay in terms of microgram equivalent to B.H.T. was determined (Table-7). The concentration (100 mcg/ml) of plant extract was found to be equivalent to 81.52 ± 01.2 mcg/ml of B.H.T.

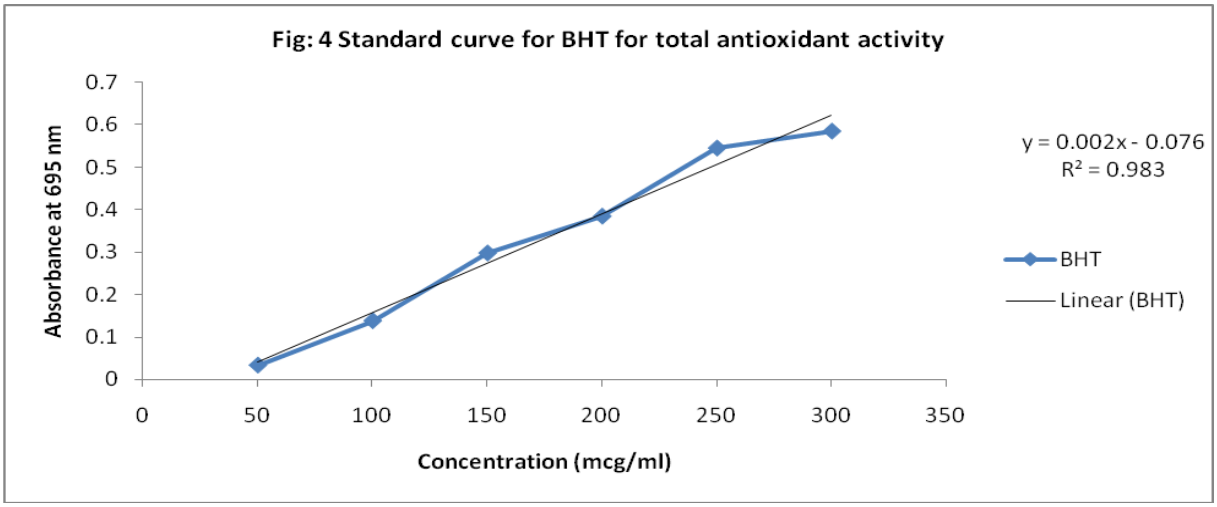
Calculation:

The antioxidant activity is expressed as the number of equivalents of BHT. A standard curve of BHT was prepared to determine the gram equivalent activity of plant extract with reference to BHT.

The absorbance of the test solution extrapolated to determine its equivalent concentration to B.H.T.

Table: 4 Total antioxidant activity of root extract of *Saussurea lappa* in terms of mcg Equivalent of BHT by Phospho-molybdenum reduction assay

Concentration (mcg/ml)	Total antioxidant activity of *Saussurea lappa* extract (mcg equivalent of B.H.T.)
50	35.4 ± 0.3634
100	81.52 ± 0.1267
150	120.54 ± 0.0843
200	178.54± 0.1512
250	230.59 ± 0.0423



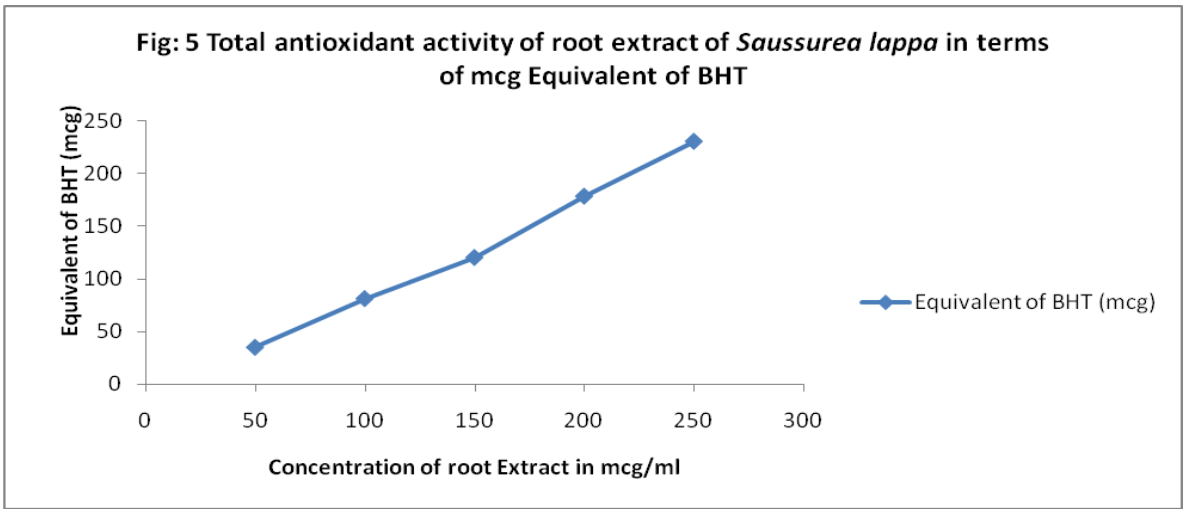


Table: 5 Total antioxidant activity of root extract of *Ficus bengallensis* in terms of mcg Equivalent of BHT by Phosphomolybdenum reduction assay

Concentration (mcg/ml)	Total antioxidant activity of *Ficus bengallensis* extract (mcg equivalent of B.H.T.)
50	14.95± 0.3606
100	36.75± 0.5107
150	71.73± 0.1386
200	118.35 ± 0.2309
250	174.93 ± 0.2079

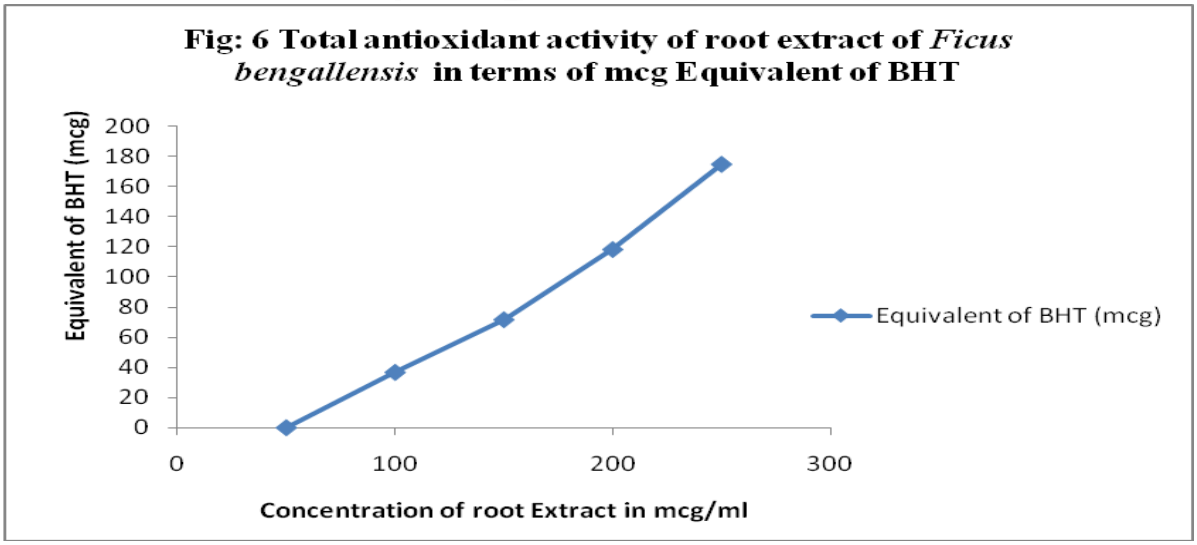


Table: 6 Total antioxidant activity of stem bark and root extract of *Flacourtria romantchi* in terms of mcg Equivalent of BHT by Phospho-molybdenum reduction assay

Concentration (mcg/ml)	Total antioxidant activity of *Flacourtria romantchi* extract (mcg equivalent of BHT)
50	32.45 ± 0.3214
100	71.43 ± 0.3876
150	110.24 ± 0.0987
200	125.54± 0.2365
250	166.76± 0.0897

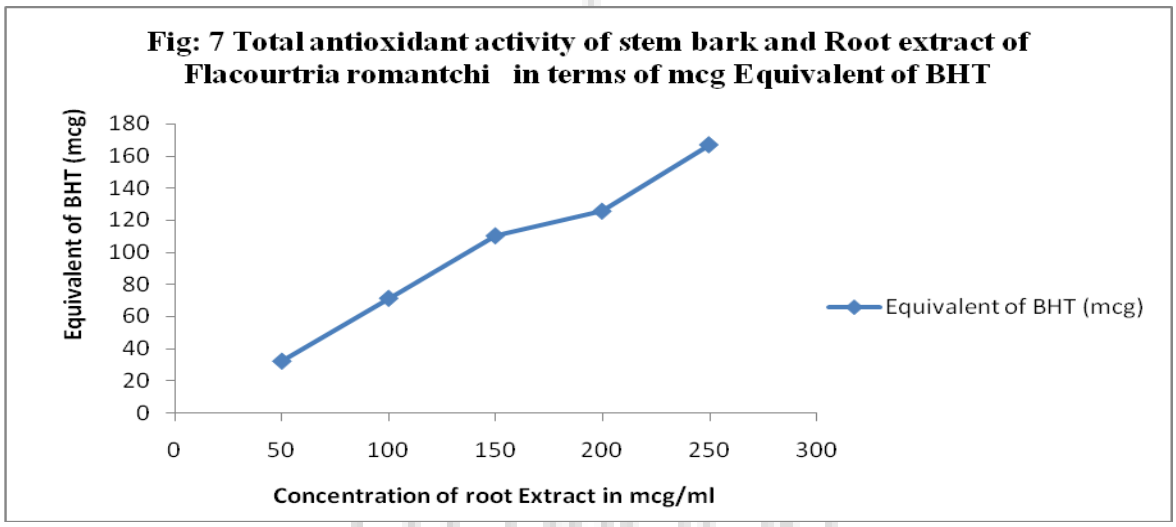
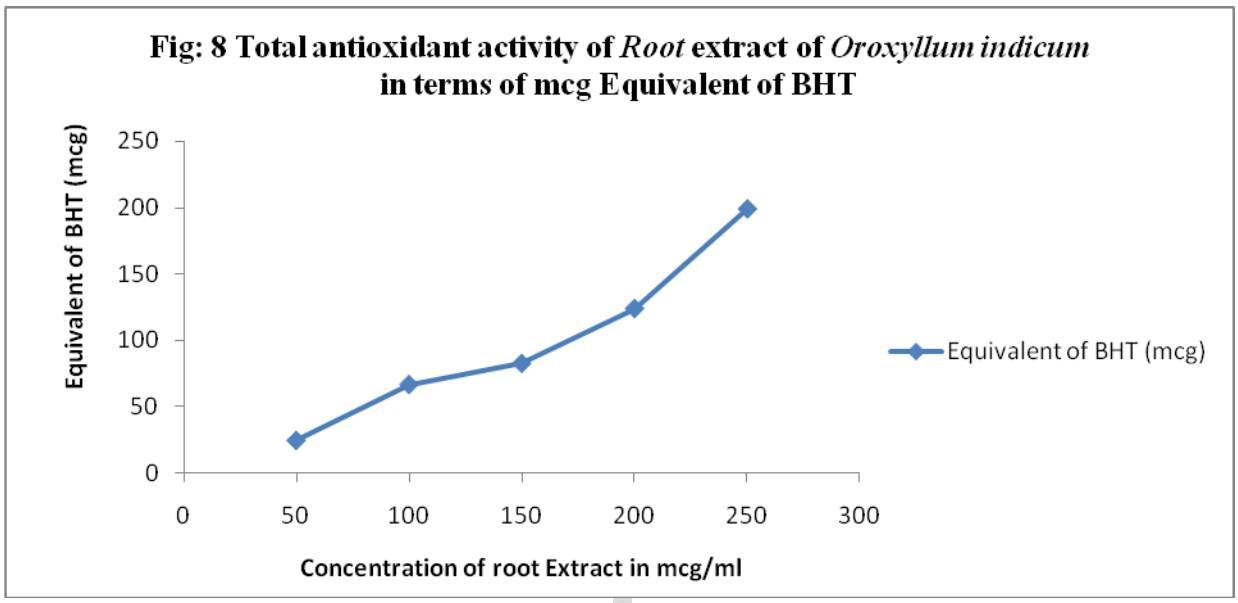


Table: 7 Total antioxidant activity of *Root* extract of *Oroxyllum indicum* in terms of mcg Equivalent of BHT by Phosphomolybdenum reduction assay

Concentration (mcg/ml)	Total antioxidant activity of *Oroxyllum indicum* extract (mcg equivalent of BHT)
50	24.77± 0.1549
100	66.76± 0.2437
150	82.66± 0.2458
200	123.78± 0.3652
250	198.66± 0.3653



Nitric Oxide radical scavenging activity

Nitric Oxide radical scavenging activity of plant extract with reference to B.H.T. has been tabulated in Table-8. The crude plant extract exhibited anti-oxidant activity in the scavenging of nitric oxide radicals. The IC_{50} value of B.H.T. in the scavenging of nitric oxide radicals was 71.302µg/ml which has been shown in fig.9

Table: 8 Nitric Oxide radical scavenging activity of root extract of *Saussurea lappa* with reference to BHT.

Concentration of solutions (mcg/ml)	Log conc.	% Inhibition by BHT	Probits of % Inhibition by BHT	% Inhibition by ***Saussurea lappa*** extract	Probits of % Inhibition by ***Saussurea lappa*** extract
50	1.39	32.4 ± 0.43	4.53	29.35 ± 0.38	4.45
100	1.69	55.18 ± 0.79	5.13	35.3 ± 0.29	4.61
150	2.00	61.72 ± 0.58	5.31	46.02 ± 0.68	4.9
200	2.17	71.45 ± 0.34	5.55	52.59 ± 0.42	5.08
250	2.30	83.5 ± 0.24	5.95	58.43 ± 0.17	5.20

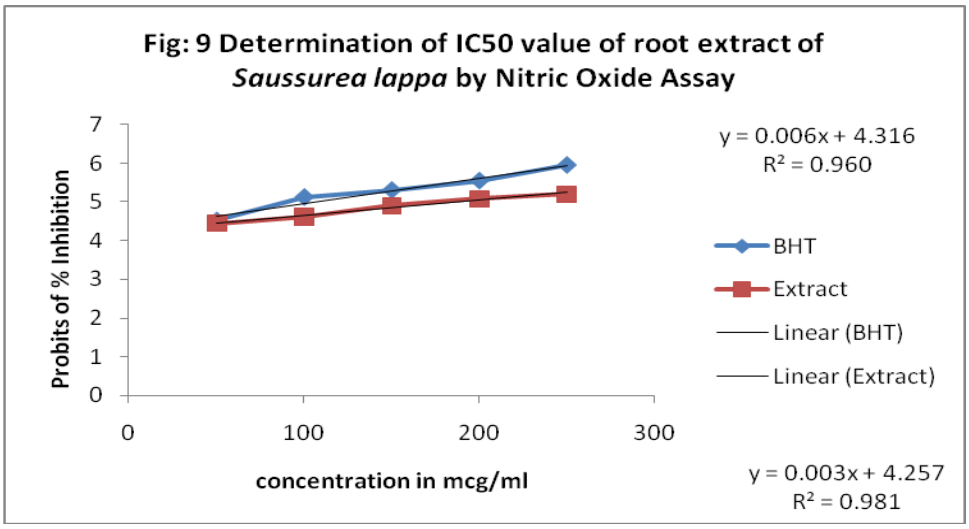


Table: 9 Nitric Oxide radical scavenging activity of root extract of *Ficus bengallensis* with reference to BHT

Concentration of solutions (mcg/ml)	Log conc.	% Inhibition by BHT	Probits of % Inhibition by BHT	% Inhibition by ***Ficus bengallensis*** extract	Probits of % Inhibition by ***Ficus bengallensis*** extract
50	1.39	32.4 ± 0.43	4.53	28.25 ± 0.28	4.42
100	1.69	55.18 ± 0.79	5.13	34.21 ± 0.19	4.59
150	2.00	61.72 ± 0.58	5.31	51.22 ± 0.38	5.03
200	2.17	71.45 ± 0.34	5.55	57.34 ± 0.52	5.18
250	2.30	83.5 ± 0.24	5.95	62.33 ± 0.16	5.31

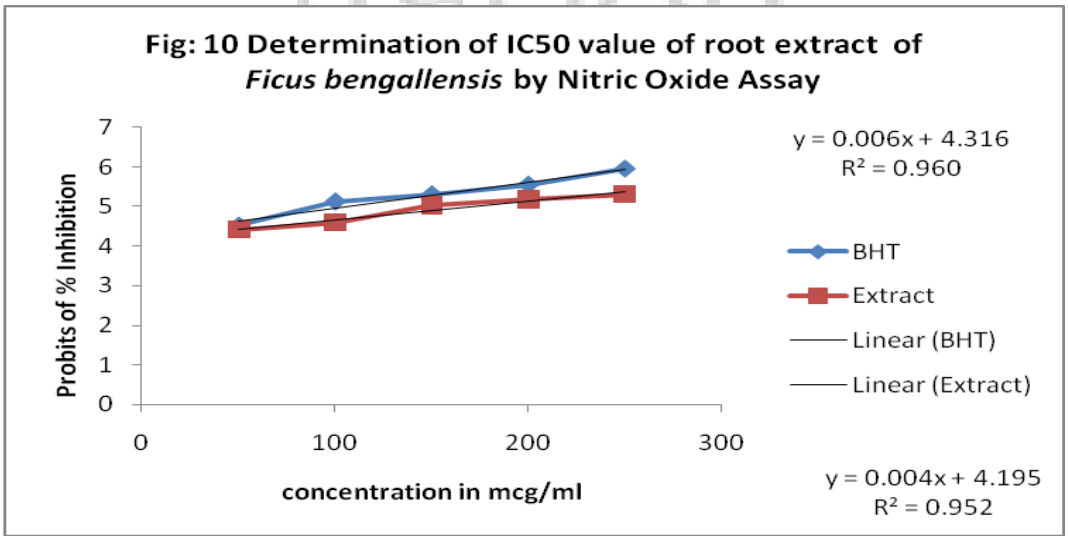


Table: 10 Nitric Oxide radical scavenging activity of stem and root extract of *Flacourtria romantchi* with reference to B.H.T.

Concentration of solutions (mcg/ml)	Log conc.	% Inhibition by BHT	Probits of % Inhibition by BHT	% Inhibition by ***Flacourtria romantchi*** extract	Probits of % Inhibition by ***Flacourtria romantchi*** extract
50	1.39	32.4 ± 0.43	4.53	27.21 ± 0.18	4.39
100	1.69	55.18 ± 0.79	5.13	39.4 ± 0.23	4.72
150	2.00	61.72 ± 0.58	5.31	49.14 ± 0.28	4.97
200	2.17	71.45 ± 0.34	5.55	62.23 ± 0.32	5.31
250	2.30	83.5 ± 0.24	5.95	68.28 ± 0.13	5.47

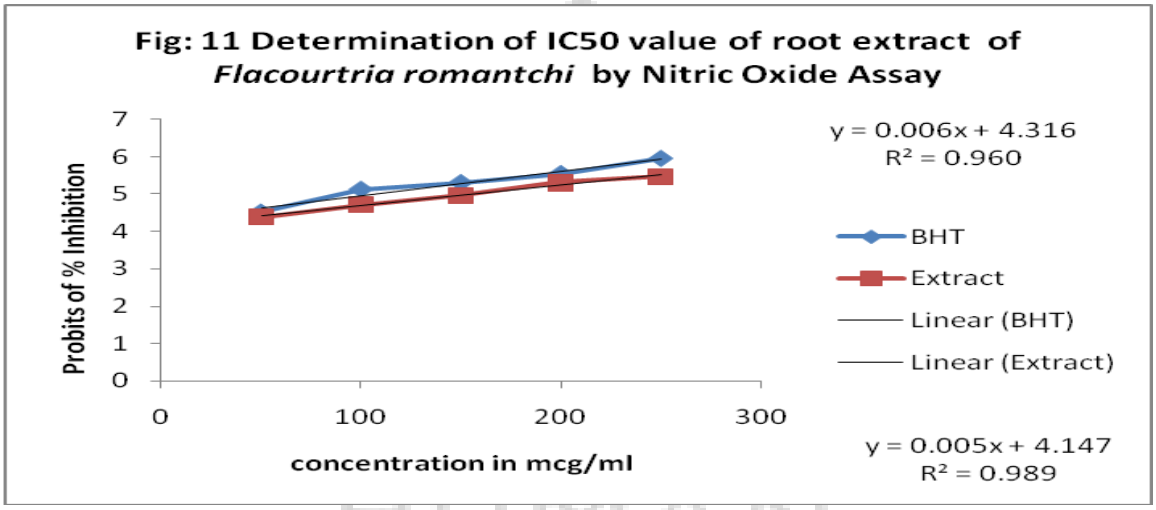
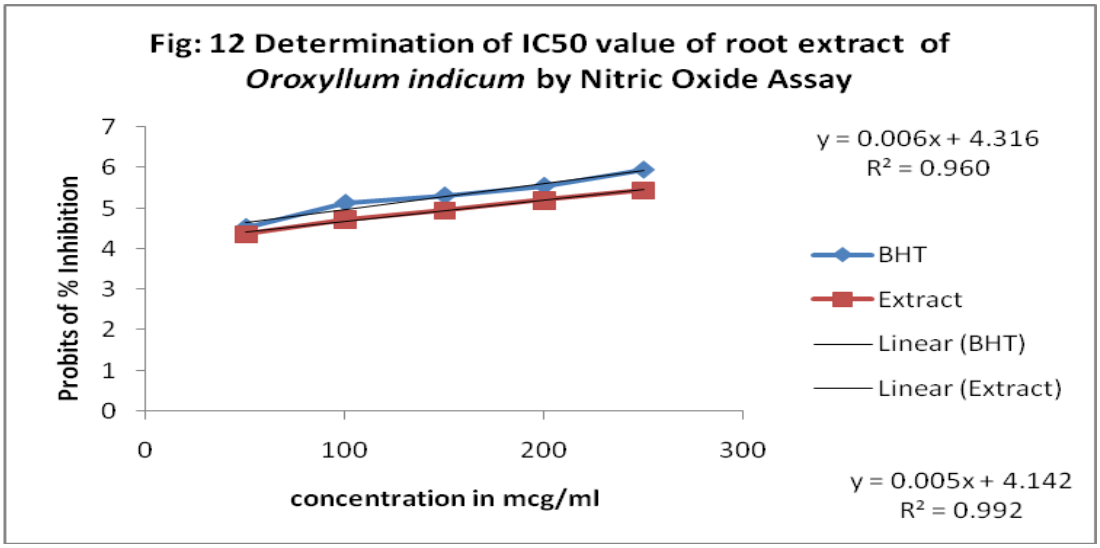


Table: 11 Nitric Oxide radical scavenging activity of root extract of *Oroxyllum indicum* with reference to BHT

Concentration of solutions (mcg/ml)	Log conc.	% Inhibition by BHT	Probits of % Inhibition by BHT	% Inhibition by ***Oroxyllum indicum*** extract	Probits of % Inhibition by ***Oroxyllum indicum*** extract
50	1.39	32.4 ± 0.43	4.53	26.22 ± 0.44	4.36
100	1.69	55.18 ± 0.79	5.13	39.2 ± 0.52	4.72
150	2.00	61.72 ± 0.58	5.31	48.22 ± 0.18	4.95
200	2.17	71.45 ± 0.34	5.55	58.49 ± 0.22	5.20
250	2.30	83.5 ± 0.24	5.95	67.33 ± 0.27	5.44



Reducing power assay:

Fig. shows the reducing capacity of plant extract as compared with B.H.T. The reducing power of plant extract was found to increase with the increasing concentration of the extract, which is comparable to the standard drug B.H.T.

The absorbance of these assay mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

In parallel to this, the reducing power of B.H.T. was also determined for comparison.

Table: 12 Reducing capacity of Plants extracts as compared with BHT.

Concentration of solutions (mcg/ml)	Absorbance of B.H.T.	Absorbance of ***Saussurea lappa***	Absorbance of ***Ficus bengallensis***	Absorbance of ***Flacourtria romantchi***	Absorbance of ***Oroxyllum indicum*** extract
50	0.171	0.226	0.127	0.098	0.067
100	0.348	0.337	0.298	0.312	0.156
150	0.628	0.387	0.556	0.456	0.387
200	0.918	0.484	0.654	0.675	0.478
250	0.987	0.609	0.865	0.765	0.654

Fig: 13 Reducing property of root extract of *Saussurea lappa* (SL).

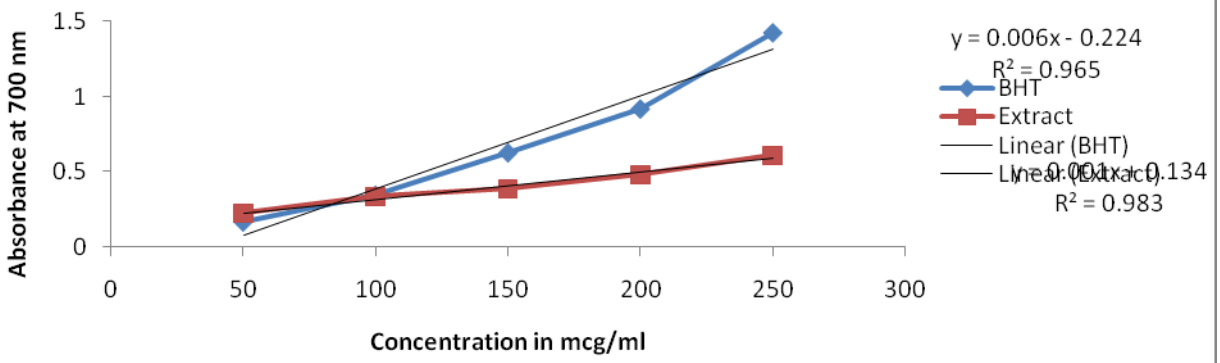


Fig: 14 Reducing property of root extract of *Ficus bengallensis (FBE)*

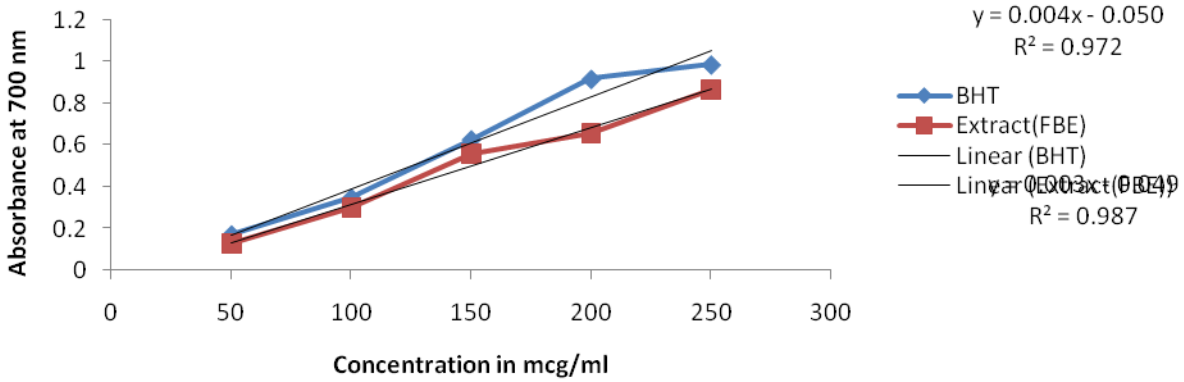
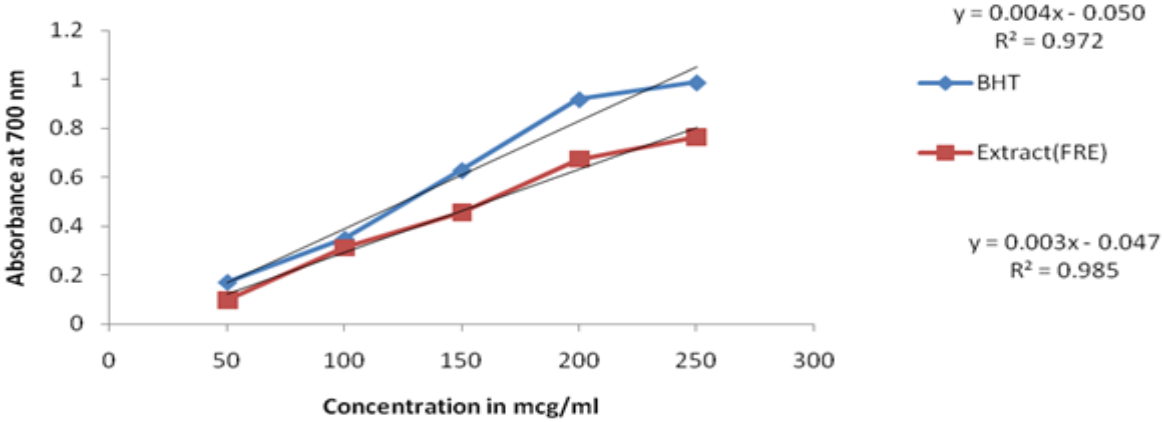
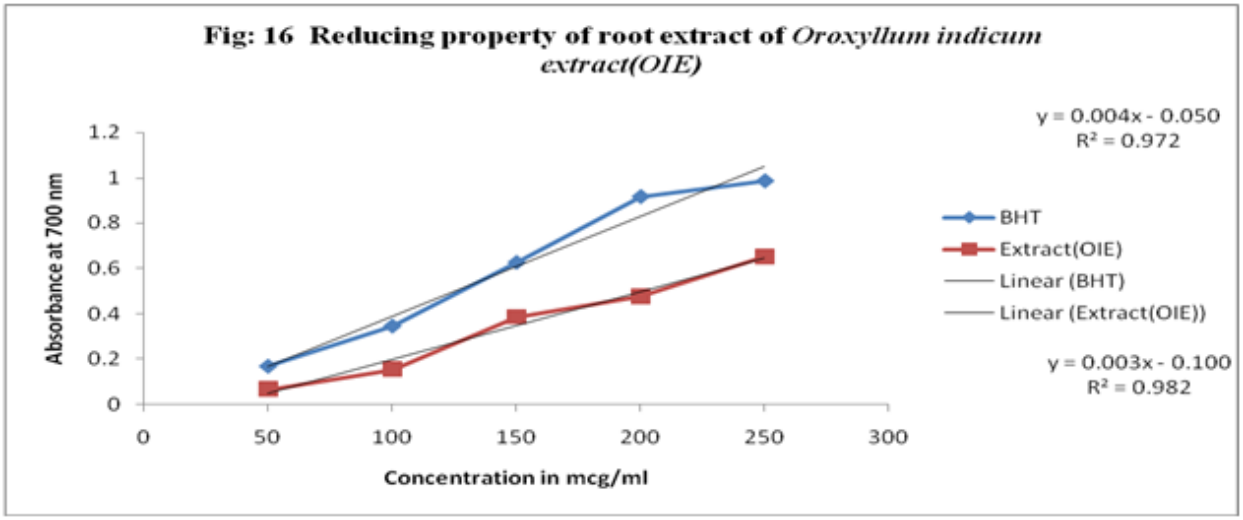


fig: 15 Reducing property of root extract of *Flacourtria romantchi extract(FRE)*





It was observed that all the four *in-vitro* anti-oxidant methods for determining the *in-vitro* anti-oxidant activity showed a dose response relationship in the radicals scavenging activity; the activity was increased as the concentration of plants extracts increased.

DISCUSSION

Various Reactive Oxygen Species (ROS) including superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide, and others. These oxidants can play a variety of roles in both animals and plants *in vivo*. However, the oxidation of lipids, DNA, protein, and carbohydrate by toxic ROS can also often cause DNA mutation, damage target cells or tissues, and result in cellular senescence and death. Recently, knowledge and application of potential antioxidant activities in reducing oxidative stresses *in vivo* have prompted to search for potent natural antioxidants from various plant sources. Thus, in this study, we characterized not only the antioxidant activities but also the protective effect on the DNA scission for the plant extracts of selected herbal medicinal plant sources namely *Saussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root),* and *Oroxyllum indicum (Root).* Based on the results of DPPH-radical assay, the different solvent extracts of selected herbal medicinal plants sources namely *Saussurea lappa* (Root*), Ficus bengallensis* (Root), *Flacourtria romantchi* (stem bark and Root), and *Oroxyllum indicum* (Root) exhibited a significant inhibitory activity against DPPH radical. The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the

unfavorable effects caused by superoxide radicals. Hence, in this report we have also evaluated the effect of *Saussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root),* and *Oroxyllum indicum (Root)* extracts on the inhibition of superoxide radicals. The data in Fig.indicate that among the tested samples, all extracts had the highest inhibitory activity against superoxide radicals induced by hypoxanthine-xanthin oxidase. In other words, all extracts have the most potent effect on antioxidant activity as judged by the free radical and superoxide radical scavenging effects. Hydroxyl radicals are considered to be the most active ROS, which can physically attack DNA to cause strand scission.

It is now recognized that ROS may play major roles in tumor promotion and progression, chronic diseases, and aging. The use of natural antioxidants as a potential preventive for free-radical mediated diseases has become a very important issue for improving the quality of life. This study demonstrates the significant antioxidant activity of plant extracts from selected herbal medicinal plants sources namely *Saussurea lappa* (Root), *Ficus bengallensis* (Root), *Flacourtria romantchi* (stem bark and Root), and *Oroxyllum indicum* (Root). This may indicate that the plant extracts or the derived phytocompounds from all extracts have great potential cancer chemo preventive effects or efficacies on other radical-mediated diseases. MDA may be a new etiology in the pathogenesis of the disease. This may be exploited as a new therapeutic approach. As per current scenario, MDA level can be controlled by increasing the antioxidant level and folate supplementation.[27]

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