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In vitro Antioxidant Evaluation of Adina cordifolia and Cassia angustifolia

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Abstract: The study was aimed to evaluate the antioxidant potential of *Adina cordifolia* and *Cassia angustifolia*. Dry leaf powder of *Adina cordifolia and Cassia angustifolia* was sequentially extracted in organic solvents with increasing polarity by Soxhlet apparatus. The total phenolic content (TPC) was determined by Folin-Ciocalteau method. Antioxidant potential of extracts was determined by DPPH method, Reducing power assay and Nitric oxide scavenging assay. The results revealed that *Cassia angustifolia* has more phenolic content as compared to the *Adina cordifolia*. In *C. angustifolia*, ethanolic extract exhibited the highest phenolic content with 106 µg/ml followed by ethyl acetate, chloroform and petroleum ether extract with total phenolic contents of 69.4 µg/ml, 45.3 µg/ml and 40.2 µg/ml respectively. DPPH assay, Reducing power assay and Nitric oxide scavenging assay revealed that *Cassia angustifolia* has more antioxidant potential than *Adina cordifolia* with ethanolic extract showing the **87.7%**, **90.3%** and **77.86 %** absorbance in the respective assays. The study confirms that the both the medicinal plants have high antioxidant potential.

Key words: Antioxidant activity, TPC, DPPH, FRAP**,** *A .cordifolia* and *C angustifolia*.

Introduction:

Nature has bestowed the mankind with many things, among them, plants occupy the first place. Plants have been used as food, shelter and medicine since the beginning of the civilisation. Plants as a medicine, dates back to the centuries. In the late 1960's, advancement in technology and discovery of novel drugs gave a sigh of relief to the people. But this joy was short lived due to the side effects associated with the synthetic medicine. There has been a surge in the use of plant based medicine since last few decades owing to their effectiveness and no side effects. Scientific evaluation of traditional medicinal plants is the stepping stone for the drug discovery. In the present study, we have evaluated two traditionally used medicinal plants for the antioxidant potential.

Antioxidants are the substances which have the capability to quench free radicals thereby inhibit or delay oxidation process of the substrate, thus plays significant role in protecting biological systems against harmful effects of free radicals (Wilson, 1998). In the contemporary world, the life style, work pressure and environmental pollution cause a heavy oxidative stress on normal human beings which is taking a heavy toll on human health.

The free radicals/oxidants are species with highly reactive, very short half-life and damaging activity towards macromolecules like lipids, proteins and DNA. These free radicals are either Nitrogen derived (RNS) or Oxygen derived (ROS). Peroxyl radicals (ROO), hydrogen peroxide (H_2O_2), superoxide anion (O_2) and reactive hydroxyl radicals (OH) are the most common reactive oxygen species. During the circulation in the body system, the reactive oxygen species tend to react with electrons of other molecules and affects normal enzymatic systems of the body which leads to the conditions like aging, cancer, ischemia, rheumatoid arthritis, adult respiratory distress syndromes, etc. Therefore, there is an urgent need to look for the plant based antioxidants to check the deadly oxidative stress.

Materials and methods

Collection of plant material

The plants *Adina cordifolia* and *Cassia angustifolia* were collected from in and around region of Mysuru. The samples were collected freshly in the air tight clean polythene bags and brought to laboratory, washed under running tap water to remove the dust and debris. The collected samples were shade dried and used for solvent extraction. Dry leaf powder of plants was extracted in organic solvents in order of increasing polarity (petroleum ether, chloroform, ethyl acetate and ethanol) using Soxhlet apparatus. The extracts were evaporated, dried and kept in refrigerator at 5 °C for further use (Harborne, 1998)..

The total phenolic content (TPC) for solvent extractions of *Adina cordifolia* and *Cassia angustifolia* was determined by Folin-Ciocalteau (Tuberoso *et al.*, 2007) method. The Absorbance was measured at 760 nm by spectrophotometer; gallic acid was used as standard phenolic compound. The solvent extracts of 1.0 ml was mixed with 20% of 1.5 ml of Na_2CO_3 and were incubated for 2 min at room temperature. Further 7 ml of deionized water and 50% Folin - Ciocalteau phenol reagent of 500 µl were mixed and the reaction mixture was incubated for further 2 hrs and absorbance was determined at 760 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material.

Antioxidant activity:

DPPH Radical Scavenging Assay: Different concentrations of stock solutions of dried solvent extracts were prepared i.e. 20, 40, 60, 80 and 100 µg in 2 ml of methanol. The DPPH solution of 0.1 m mol was prepared in methanol and one ml from this was added to each of the above different concentrated test solutions. The mixture was vigorously shaken, incubated for about 30 min and the absorbance was determined by spectrophotometrically at 517 nm as described by Miliauskas et al. (2004). Each experiment was carried out in triplicates. Ascorbic acid (AA) was used as standard and methanol served as negative control. Scavenging capacity of the extracts to the DPPH radical was expressed with following formula

`% Inhibition= [(A_B- A_A)/A_B X100]

where A_B, absorption of blank sample

A_A, absorption of tested extract solution

Reducing power assay

The Reducing power ability of the extracts was determined by the Ferric reducing-antioxidant power (FRAP) assay. This method measures the ability of antioxidants to reduce ferric iron. The reducing ability of *Adina cordifolia* and *Cassia angustifolia* was determined by the methods as described by the Yildrim et al., 2000. Various solvent extractions of different concentrations (20, 40, 60, 80 and 100 µg) were mixed in 1 mL of 0.2 mol/L phosphate buffer (pH 6.6) and equal volume of 1% of 1 ml potassium ferric cyanide. Then the mixture was incubated for 20 min at 50 °C. Later 10% of 1 mL trichloro acetic acid (TCA) was added and centrifuged at 3000 rpm for 10 min. After the centrifugation, 2 mL supernatant was mixed with 2 mL of distilled water and 1% of 500 µL ferric chloride. The absorbance was measured at 700 nm. Reducing power ability was expressed as percentage activity using the following formula.

% Reducing Power Activity = 1 - Sample OD / Control OD

Nitric oxide scavenging assay

Radical scavenging capacity of nitric oxide was determined by the method of Nakagawa & Yokozawa., 2002. About 1.5 ml of 10 mM Sodium nitroprusside in phosphate buffer (pH 7.4) was mixed with 1 ml extracts of different concentrations (20, 40, 60, 80 and 100 µg) and the reaction mixture was incubated for 150 min at 25 ºC during which nitric oxide spontaneously is generated from the sodium nitroprusside. The Griess reagent (2% phosphoric acid, 0.1% naphthylethylenediamine dihydrochloride and 1% sulphanilamide) of 1.5 ml was added after incubation. Again the reaction mixture was incubated for 30 min at room temperature. The final volume of test solution was made up to 4 ml with the phosphate buffer (pH 7.4). The absorbance of reaction mixture was determined at 546 nm and IC_{50} values were calculated. The scavenging percent was calculated using the formula:

Nitric oxide scavenging effect (%) = [(Ac – At)/Ac × 100]

Ac= absorbance of control

At= absorbance in presence of the sample of extract

RESULTS

Total phenolic contents

The total phenolic content was determined by Folin-Ciocalteau method. Various solvent extracts of *Adina cordifolia* and *Cassia angustifolia* were evaluated for total phenolic contents. The results revealed that *Cassia angustifolia* has more phenolic contents as compared to the *Adina cordifolia*. In *Cassia angustifolia*, ethanolic extract exhibited the high phenolic content with 106 µg/ml followed by ethyl acetate, chloroform and petroleum ether extract with total phenolic contents of 69.4 µg/ml, 45.3 µg/ml and 40.2 µg/ml respectively Table 1, Fig.1. The solvent extracts of *Adina cordifolia* exhibited the moderate content of phenolic contents, ethanolic extract showed the maximum phenolic contents as compared to others with 49.83 µg/ml. Whereas, petroleum ether exhibited the

least with 25.3 µg/ml. chloroform and ethyl acetate exhibited the 38.1 µg/ml and 45.60 µg/ml respectively Table.1, Fig. 2.

Table 1: Total phenolic contents of solvent extracts

Sl No.	Extract	Total phenolic contents in µg/ml	
		Cassia angustifolia.	*Adina cordifolia*
1	Petroleum ether	40.20	25.30
2	Chloroform	45.30	38.10
3	Ethyl acetate	69.40	45.66
4	Ethanol	106.0	49.83

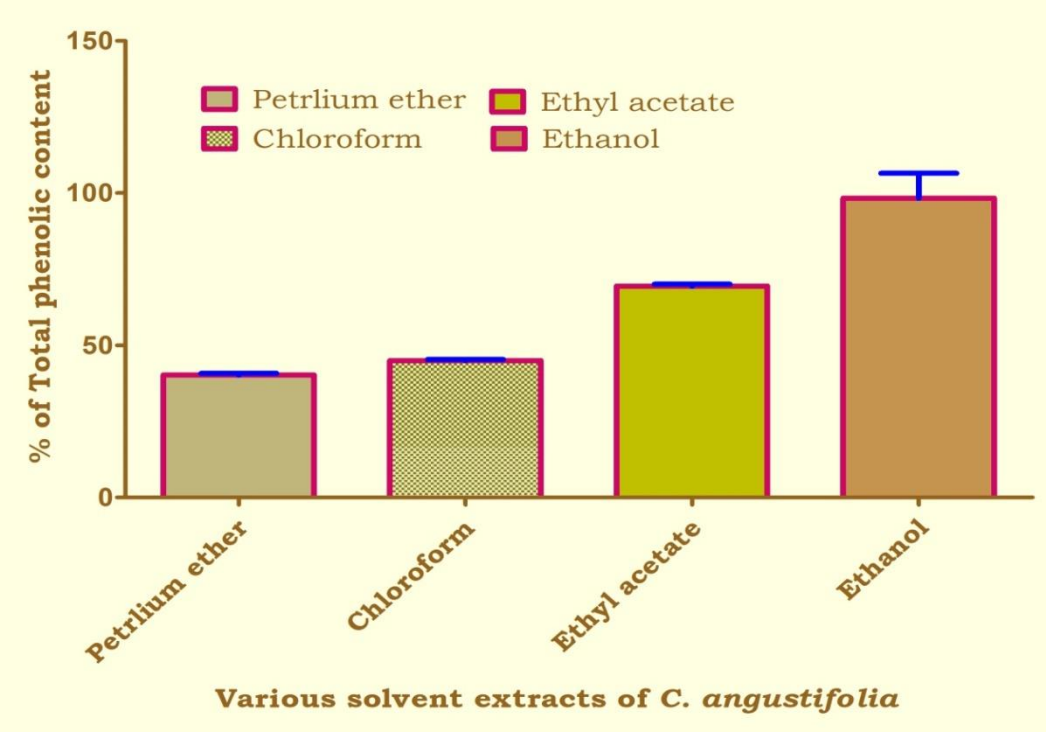

Fig.1. Total phenolic contents of *Cassia angustifolia*

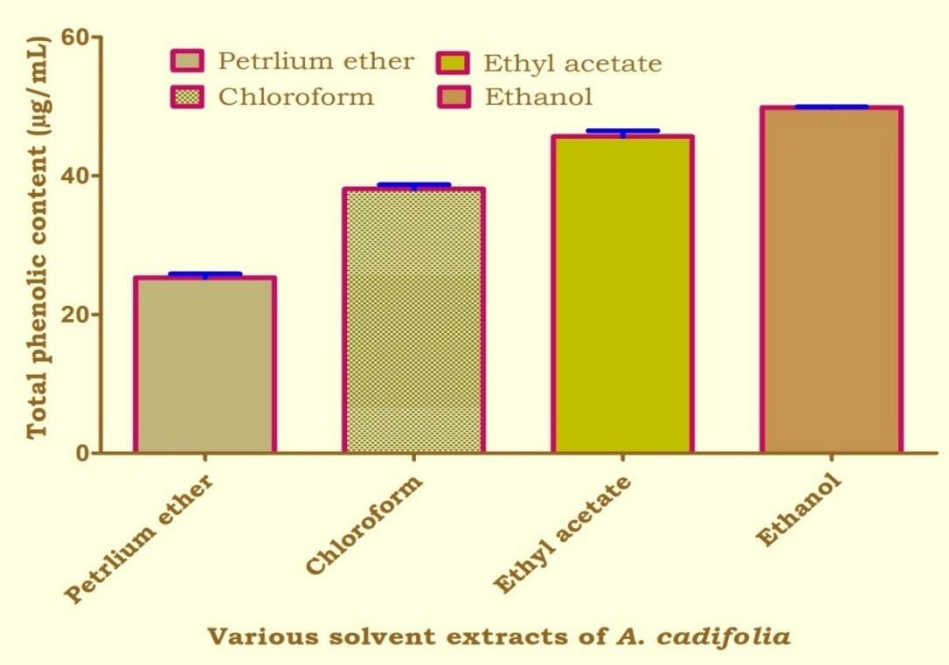

Fig. 2 Total phenolic contents of *Adina cordifolia*

Antioxidant activity

DPPH Radical Scavenging Assay

Free radical scavenging capacity of various solvent extracts of *Adina cordifolia* and *Cassia angustifolia* plants were evaluated by change in the absorbance of reduced DPPH. Maximum scavenging activity was observed in the ethanol extract of *Cassia angustifolia* with 87.7% of scavenging activity and least activity was observed in the petroleum ether extract of *Adina cordifolia* with 20.43 % of scavenging activity. Whereas, ethyl acetate, chloroform and petroleum ether extracts of *Cassia angustifolia* shown the scavenging activity of 58.2%, 40.7% and 48.5% respectively. In *Adina cordifolia* solvent extracts tested for scavenging activity ethanol extract exhibited the maximum scavenging activity with 67.66 % followed by ethyl acetate and chloroform extracts with 45.26% and 28.83% respectively Table 2, Fig. 3, 4.

Reducing power assay

Reducing ability of various solvent extracts of *Adina cordifolia* and *Cassia angustifolia* were carried out by FRAP method. The results revealed that *Cassia angustifolia* very good activity as compared to the *Adina cordifolia*. Among the various solvent extracts of *Cassia angustifolia* tested for their reducing ability ethanolic extract shown maximum percentage (90.3%) followed by ethyl aceteate, chloroform and petroleum ether with reducing ability of 72.3%, 50.63% and 38.5 % respectively Table. 2, fig. 5. Among the solvent extracts of *Adina cordifolia* ethanolic extract shown maximum percentage (59.03%) followed by Chloroform, ethyl aceteate and petroleum ether with reducing ability of 31.33%, 27.8% and 20.9 % respectively Table 2, Fig.6.

Nitric oxide scavenging assay

Various solvent extracts of *Adina cordifolia* and *Cassia angustifolia* plants were tested for nitric oxide scavenging activity. The results revealed that ethanolic extract of *Cassia angustifolia* shown maximum scavenging percentage (77.86 %) followed by ethyl aceteate, chloroform and petroleum ether with reducing ability of 54.3%, 31.16% and

Extracts	*C. angustifolia*			*A. cardifolia*		
	DPPH	FRAP	Nitric oxide	DPPH	FRAP	Nitric oxide
Petroleum ether	48.5	38.5	28.73	20.43	20.9	21.13
Chloroform	40.7	50.63	31.16	28.83	31.33	29.43
Ethyl acetate	58.2	72.23	54.3	45.26	27.8	37.9
Ethanol	87.7	90.3	77.86	67.66	59.03	49.76
Ascorbic acid	93.5	92.1	90.53	93.5	92.1	90.53

25.73% respectively Table 2, Fig 7. Among the solvent extracts of *Adina cordifolia* ethanolic extract shown maximum percentage (49.76%) followed by ethyl aceteate chloroform, and petroleum ether with reducing ability of 37.9%, 29.43 and 21.13 % respectively Table 2, Fig 8.

Table 2: Percentage radical scavenging activity of various solvent extracts of *C. angustifolia* and *A. cardifolia* by DPPH, FRAP and nitric oxide

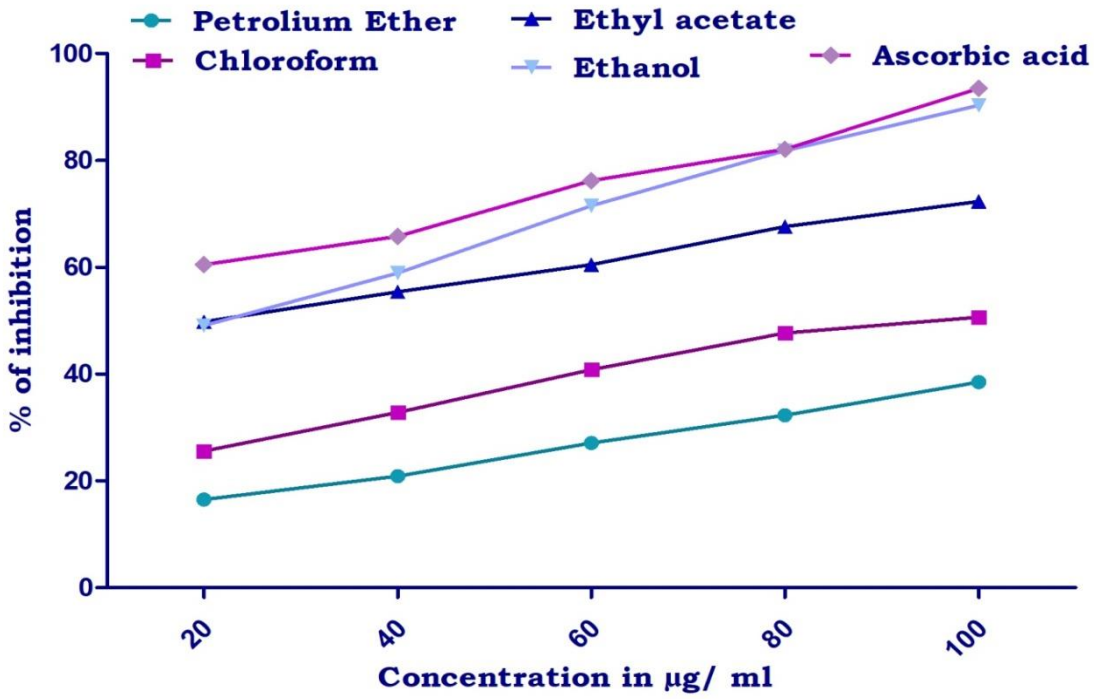

Fig. 3. DPPH free radical scavenging activity of different extracts of *C. angustifolia* and standard Ascorbic Acid at different concentrations.

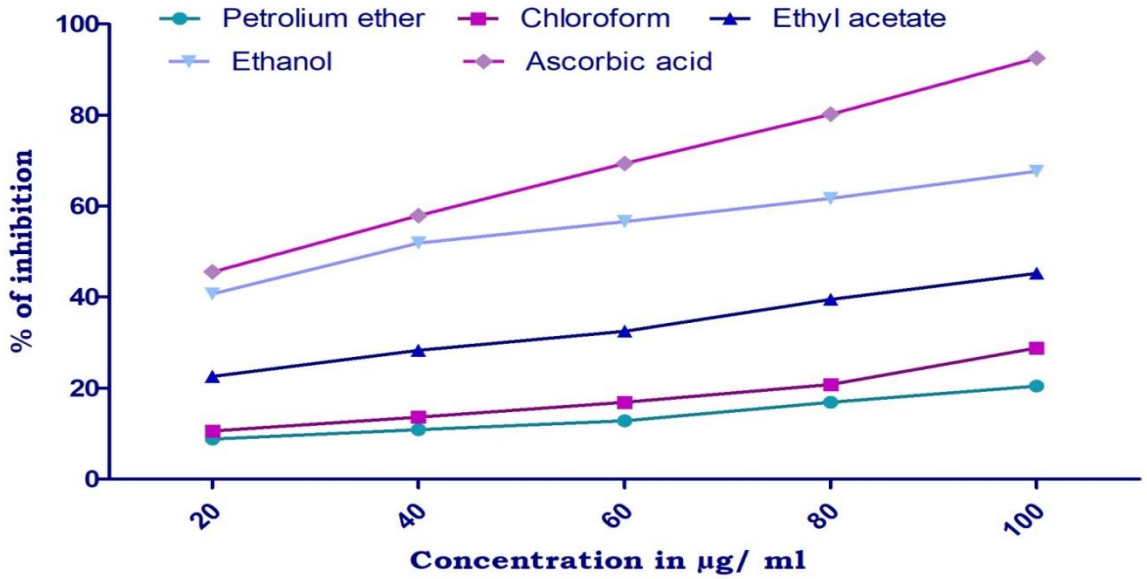

Fig. 4. DPPH free radical scavenging activity of different extracts of *A. cardifolia* and standard Ascorbic Acid at different concentrations.

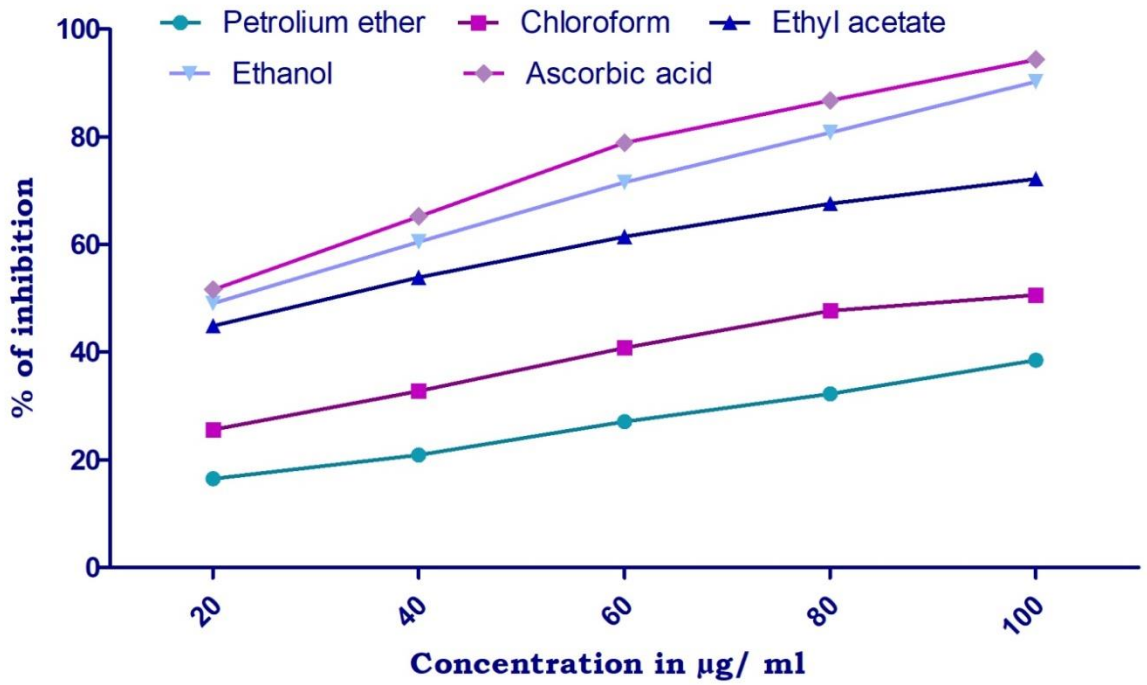

Fig. 5. Reducing scavenging assay by FRAP of different extracts of *C. angustifolia* and standard Ascorbic Acid at different concentrations.

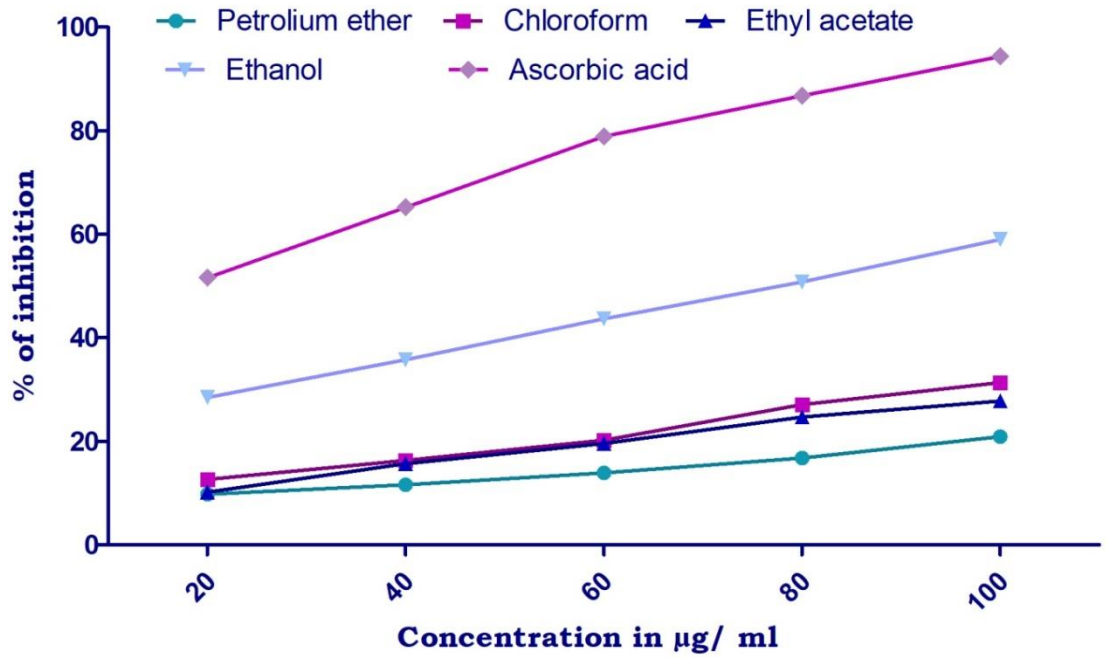

Fig. 6. Reducing scavenging assay by FRAP of different extracts of *A. cardifolia* and standard Ascorbic Acid at different concentrations.

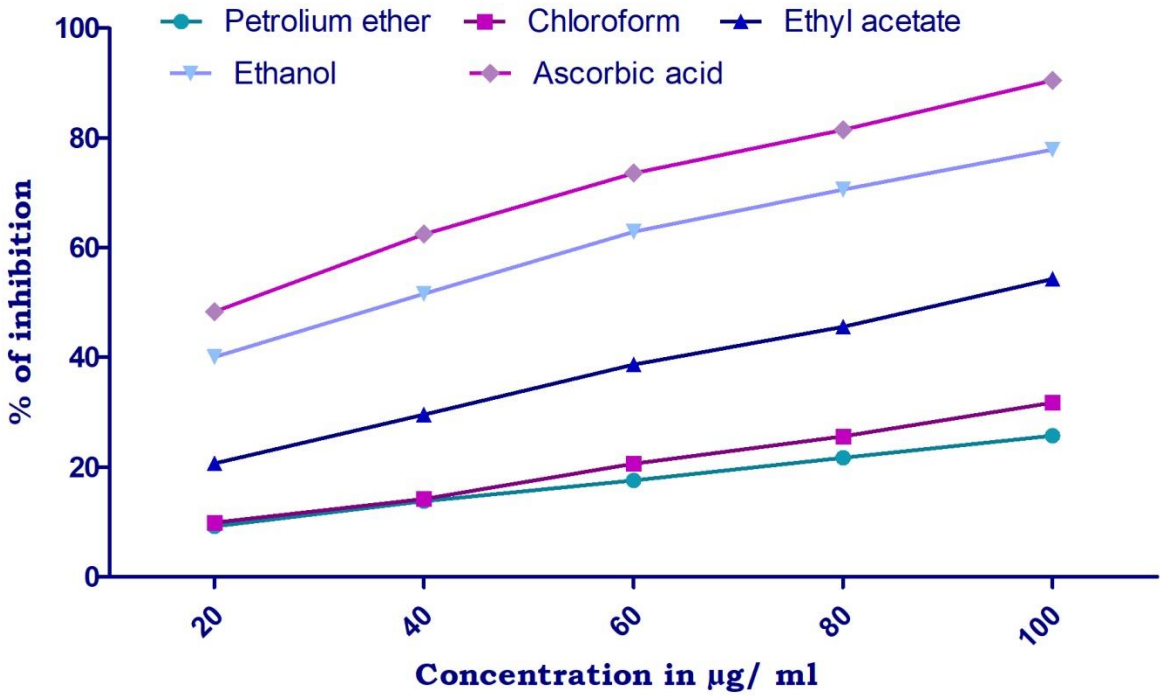

Fig.7. Scavenging effect of nitric oxide radical of different extracts of *C. angustifolia* and standard ascorbic acid at various concentrations.

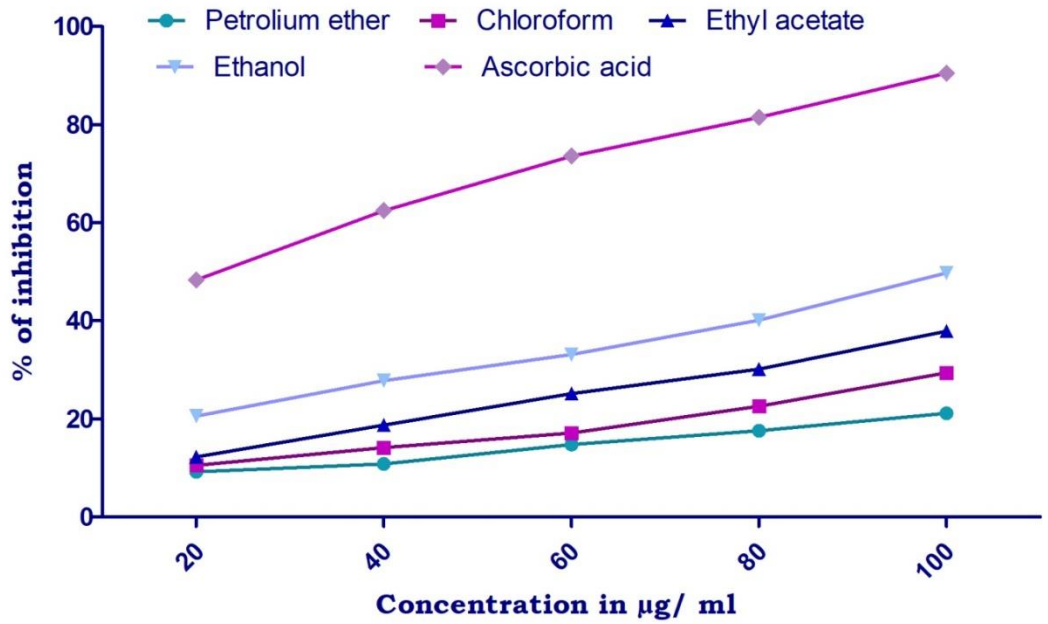

Fig. 8. Scavenging effect of nitric oxide radical of different extracts of *A. cordifolia* and standard ascorbic acid at various concentrations.

Discussion:

In the present investigation various solvent extracts such as Petroleum ether, Chloroform, Ethyl acetate and Ethanol of *C. angustifolia* and *A. cordifolia* were evaluated for antioxidant activities by DPPH, FRAP and Nitric oxide methods. Significant results were observed in the extracts of *C. angustifolia.* Among various solvent extracts ethanol extract shown the significant result 87.7 % in DPPH scavenging assay, 90.3% in FRAP reducing assay and 77.86 in nitric oxide scavenging assay. This result shows the *C. angustifolia* has powerful antioxidant capacity. Numerous reports are available for the antioxidant activities from plant sources such as O*cimum basillicum, Jatropa multifida, Solanum indicum, Alpina calcarata, Hyptis suaveolens, Clitorria ternate Vitis thunbergii, Ludwigia octovalvis, Lindernia anagallis, Rubusn parvifolius, Zanthoxylum nitidum. Desmodium gangeticum, Amaranthus caudatus* (**Shyura** *et al.* **2005; Nik Noor Asma Nik Wil** *et al.* **2014; Ganga** *et al.* **2012; Veeru** *et al.* **2009).** There are some other results which also report the antioxidant activities of the Cassia species. **Ahmed** *et al.* (2016) reported the antioxidant activity of *C. angustifolia* which supports our results. Chakrabarty et al. (1983) also reported the antioxidant activities of *cassia* species. Thus based on the earlier evidences our findings revealed the *C. angustifolia* has strong antioxidant activity.

Phenolic compounds are the secondary metabolite synthesised naturally in the plants and they possess various activities like act against degenerative cells associated with free radicals (Pandey and Mishra, 2009; Kris-Etherton and Mhecker 2002; Johnson et. al. 1994; Murya and Rizavi 2008; Joseph et. al 2005). In the present study total phenolic contents were evaluated from various extracts of *C. angustifolia* and *A. Cordifolia*. It was found that ethanolic extract of *C. angustifolia* possesses maximum total phenolic with 106.0 µg/ml followed by ethyl acetate extract with 69.40 µg/ml. Whereas, others solvent extract of *C. angustifolia* and solvent extracts from *A. cordifolia* did not shown significant results. Earlier reports on cassia species have also reported phenolic components (Hatano et al. 1999; Singh et al. 2012).

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