Antioxidant and antiacetylcholine esterase potential of aerial parts of *Conocarpus erectus, Ficus variegata and Ficus maclellandii*

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Abstract: The current study was designed to check the antioxidant and enzyme inhibition potential of various extracts/ fractions of three selected plants. The aerial parts of *Conocarpus erectus* (Combretaceae), *Ficus variegata* (Moraceae) and *Ficus maclellandii* (Moraceae) were extracted with ethanol (95%) and the resulting crude extracts were partitioned with n-hexane, chloroform and n-butanol successively. Folin-Ciocalteu reagent was used to calculate the total phenolic contents, flavonoids contents were calculated with aluminum chloride while antioxidant and enzyme studies were carried out through standard protocols. All extracts/fractions contained reasonable amount of phenolic compounds ranging from 0.58-58.23mg CE/g of DW and 0.43-30.56mg GAE/g of DW. Total flavonoids were determined using rutin and quercetin standards, ranging from 2.65-18.2 mg rutin equivalent/g of dry weight and 0.92-5.41mg quercetin equivalent/g of dry weight. Antioxidant studies such as DPPH inhibition FRAP and total antioxidant capacity (TAC) was checked. The crude ethanolic extract of *C. erectus* showed maximum antiradical scavenging power (90.43%; IC_{50}=7 µg) and ferric reducing antioxidant power (16.5µM eq.$FeSO_4.7H_2O$), respectively while leave extract of *F. variegata* (chloroform) was the most active (0.6577) in TAC among other extracts of the selected medicinal plants. Butanolic leave extract of *C. erectus* exhibited maximum enzyme inhibition activity (91.62% with IC_{50} 40µg/ml) while other extracts showed significant activity. It was observed from results that all extracts/fractions of under consideration plants, exhibited significant bioactivities especially ethanolic and butanolic fractions, which may be the richest source of such type of activities.

Keywords: Ficus species, antioxidants, enzyme inhibition, medicinal plants

INTRODUCTION

Plant-derived molecules are now become of great attention due to their tremendous applications. Medicinal plants are the richest bio-resource of conventional, folk and recent medicines, food supplements, pharmaceutical intermediates, current medicines, nutraceuticals and chemical entities for synthetic medicine (Ncube *et al.*, 2008; Nirmala *et al.*, 2011). In spite of the recent domination of the methods of synthetic chemistry as to discover and produce drugs, bioactive plants or their extracts are enormously used to provide new and novel products for treatment of diseases (Verpoorte, 2000; Newman *et al.*, 2003; Kviecinski *et al.*, 2008). Many herbal-based medicines are considered to have a wide range of biomedical efficiencies including treatment of inflammation, osteoporosis, bone desorption, hyperlipidemia, arteriosclerosis, nervous system disorders and cancer (Jin *et al.*, 2006; Radad *et al.*, 2006). Several bio-active compounds have been discovered from medicinal plants and used as patented drugs like maprouneacin, artemisinin and taxol (Klayman, 1993; Carney *et al.*, 1999; Goodman and Walsh, 2001). Due to inexpensive, multitargeting effect and safety of plant-based products compared to synthetic agents, there is a need for more detailed search for and discovering the new drugs from plants.

Natural products, including animals, plants and minerals have been the foundation for the treatment of various human diseases. The present widely acclaimed drug (allopathy) has steadily developed by observational and scientific efforts over the years. However, the origin of its growth remains embedded in traditional therapies and medicines. However, ancient knowledge has been the foundation of current medicines and will remain as significant source of future therapeutics and medicine. The future of drugs derived from living cells will be personalized, holistic and engages a wide use of antique and recent therapeutic skills in a complementary manner in order to get maximum benefits for the patients and the community (Bhushan *et al.*, 2004).

Conocarpus erectus L. is one of the two species in Conocarpus genus, in the family Combretaceae distributed on shorelines in tropical and subtropical

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regions of the earth (Nahla, 2010) Jagessar and Cox, 2010). *C. erectus* known in English as button mangrove or button wood; is a 6 m tall an evergreen tree with spreading grey (Bailey, 1976). It is used in some countries as folk remedy for anemia, conjunctivitis, diabetes, catarrh, diarrhoea and fever (Irvin, 1961).

Apart from *Ficus maclellandii* and *Ficus variegate*, the family Moraceae comprises of about 53 genera and 800 species most of which are located in the tropical areas, including mostly wild trees and has a thorn, is about 66% of the world species (Sirisha *et al.*, 2010). The maximum diversity of the genus exhibits in Australian region, Asiatic mainland, New Guinea and Borneo (Chaudhary *et al.*, 2012). The most significant species of genus *Ficus* include; *F. carica, F. bengalensis, Ficus racemosa and F. elastica*. A variety of parts of these plants like bark, tender shoots, leaves, fruits, seeds, and latex are medicinally significant. To our knowledge from the literature, little is known about the biological activities and phytochemical studies of *C. erectus, F. variegata and F. maclellandii*. This study was designed to explore the antioxidant and enzyme inhibition potential of selected plants. Our research group is engaged in evaluating and purification of bio-active compounds from medicinal plants.

MATERIALS AND METHOD

Chemicals and reagents

Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), rutin, gallic acid, quercetin, acetylthiocholine iodide, TPTZ, $FeCl_3$, $FeSO_4.7H_2O$ and 5,5´-dithiobis [2-nitrobenzoic acid] (DTNB) were purchased from Sigma-Aldrich (USA) while erythrocytes (acetylcholine esterase) were obtained from the Biochemistry Lab, Mayo Hospital Lahore. Solvents of analytical grade were purchased from Panerac (Spain). All other chemicals, solvents and reagents of analytical grade were from Merck (Germany).

Plant material

Aerial parts of *C. erectus, F. variegata* and *F. maclellandii* were collected from Hafiz Hayat Campus (University of Gujrat). The plant specimens were identified at Botany Department of UOG, Gujrat.

Extraction

The plant material (leave and branch) was shade dried, powdered and extracted by percolation method for 10 days at room temperature (100 gm each) with 1000 ml ethanol (95%). The crude extracts was filtered through Whatman paper No. 40 and concentrated using rotary evaporator at reduced pressure. The extracts were defatted with hexane and partitioned with chloroform and n-butanol successively.

Determination of total phenolics

The total phenolics in extracts and fractions of *C. erectus, F. variegata* and *F. maclellandii* were determined using Folin-Ciocalteu reagent (Shahwar *et al.,* 2010). The extract (20mg) was dissolved in (10ml) methanol. Forty µl of each sample was mixed with 0.25ml of Folin-Ciocalteu reagent and 0.8ml of 10% sodium carbonate solution. The mixture was allowed to stand for 30 min and absorbance was measured at 765 nm against a blank, which contained 40µl of methanol in place of sample. The total phenolic contents were expressed as mg of gallic acid and catechin equivalent/g of dry weight.

Total flavonoid contents

Total Flavonoid Contents (TFC) of all extracts/fractions of under study plants were determined by colorimetric method using standards (quercetin and rutin equivalent/g of dry weight) (Kamran *et al.,* 2009). The reaction mixture was composed of 100µl of extract (2 mg/ml) and 100µl $AlCl_3$ (1%). The absorbance was noted at 435nm using UV/Visible Spectrophotometer after 30 min by adding 2 ml of water.

In vitro AChE Inhibition Assay

Acetylcholine esterase inhibitory (AChE) activity was measured by Shahwar *et al.,* (2011). Acetylthiocholine iodide was used as substrate in the assay. The reaction mixture contained 2.0ml of 100mM tris buffer (pH 7.8), 100µl of acetylcholine esterase (erythrocytes), A 200µl volume of extracts/fractions, 100µl of DTNB and incubated for 15 min (25°C). The reaction was initiated by the addition of 200µl acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide was monitored at 412nm till 30 min. The percentage inhibition was calculated as follows;

% inhibition = (E – S)/ E x 100

Where E is the activity of the enzyme without sample and S is the activity of enzyme with test sample.

Determination of antioxidant activity

Antiradical activity

Radical scavenging activity of extracts/fractions was assayed according to the method of Shahwar *et al.,* (2011) using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical. A 200µl volume of the sample (2 mg/ml) was mixed with 2.0 ml DPPH solution (methanol), test tubes were kept in dark for 30 min and noted the absorbance at 517 nm. The scavenging of free radical was calculated using following formula;

$$\%\ \text{inhibition of DPPH} = \frac{A - B}{A} \times 100$$

Where A is the absorbance of blank and B is the absorbance of sample.

FRAP assay

FRAP assay of extracts/fractions was carried out according to method of Shahwar *et al.,* (2012). The FRAP reagent consists of 300mM acetate buffer pH 3.6, 10mM TPTZ (2,4,6-tripyridyl-*s*-triazine), and 20mM $FeCl_3.6H_2O$ solution. 150µl plant extracts/fractions were mixed to

Table 1: Total phenolic and total flavonoids contents of selected plants

Plants Name	Part of Plant	Solvents	Extracts	Total phenol		Total flavonoids	
				Eq. to GA	Eq. to Catechin	Eq. to Rutin	Eq. to Quercetin
Conocarpus erectus	Leaves	Ethanol	CELE	30.56±1.03	58.23±1.17	-	-
		n-Hexane	CELH	0.43±0.01	0.68±0.02	-	-
		Chloroform	CELC	0.55±0.02	2.38±0.36	-	-
		n-Butanol	CELB	6.49±0.92	16.65±1.07	14.04±0.87	4.34±0.32
	Branch	Ethanol	CEBE	22.73±1.11	44.7±1.01	-	-
		n-Hexane	CEBH	0.50±0.03	0.61±0.08	-	-
		Chloroform	CEBC	1.50±0.04	2.70±0.32	-	-
		n-Butanol	CEBB	17.37±1.01	35.44±1.91	18.2±1.11	5.41±0.28
Ficus variegata	Leaves	Ethanol	FVLE	4.30±0.24	7.52±0.66	-	-
		n-Hexane	FVLH	0.90±0.02	1.04±0.09	-	-
		Chloroform	FVLC	4.05±0.54	8.27±0.71	-	-
		n-Butanol	FVLB	3.51±0.61	5.90±0.53	-	-
	Branch	Ethanol	FVBE	4.57±0.77	8.36±0.61	-	-
		n-Hexane	FVBH	0.80±0.23	1.13±0.13	-	-
		Chloroform	FVBC	3.20±0.81	0.58±0.09	-	-
		n-Butanol	FVBB	0.92±0.17	1.35±0.14	-	-
Ficus macllendii	Leaves	Ethanol	FMLE	3.04±0.57	6.67±0.63	10.63±0.84	3.34±0.55
		n-Hexane	FMLH	1.67±0.33	2.9±0.12	-	-
		Chloroform	FMLC	1.71±0.17	3.86±0.38	-	-
		n-Butanol	FMLB	2.54±0.36	4.70±0.40	10.46±0.37	4.12±0.37
	Branch	Ethanol	FMBE	1.42±0.28	2.60±0.09	5.68±0.94	2.41±0.41
		n-Hexane	FMBH	3.04±0.53	2.01±0.11	-	-
		Chloroform	FMBC	2.04±0.40	1.05±0.09	-	-
		n-Butanol	FMBB	2.65±0.51	1.89±0.22	2.65±0.08	1.11±0.09

SD (±) was calculated using M.S excel 2007 software (n=3), - = not calculated

FRAP reagent, allowed to stand for six minutes and absorbance was noted at 593nm.

Total antioxidant capacity (TAC)

Total Iron Reducing Power Assay was carried out by taking 100µl of extract (2mg/ml) in individual test tubes added 2.5ml buffer, 1.5ml potassium ferrocyanide (1%) and 100µl $FeCl_3$ then the reaction mixtures were incubated for 30 min at RT. The absorbance was noted at 700 nm by using UV/VIS Spectrophotometer (Shahwar *et al.,* 2012).

RESULTS

Total phenolic contents

Total phenolic contents of extracts/fractions of different parts of the selected plants were calculated using FC method and expressed as mg GAE and CE/g of dry weight of plant using gallic acid and catechen as standards. The amount of TPC ranged from 0.58 (FVBC)-58.23 (CELE) mg CE/g of dry weight. The amount of TPC expressed as mg GAE/g of dry weight ranged from 0.43 (CELH)-30.56 (CELE) mg GAE/g of dry weight.

Total flavonoid contents

TFC (based on colorimetric $AlCl_3$ method) carried out by using standards viz; quercetin and rutin and the amount of TFC was expressed as mg QE/g of dry weight and mg RE/g of dry weight of plant, respectively. Total flavonoids ranged 2.65 (FMBB)-18.2 (CEBB) RE/g of dry weight and 0.92 (CELE)-5.41 (CEBB) QE/g of dry weight while the rest of the extracts gave no response showing the absence of flavonoid skeleton in their extracts/fractions (table 1).

Antioxidant activities

Radical scavenging ability of extracts/fractions was checked using DPPH as free radical and it was noted from results that all extracts exerted remarkable antiradical activity. n-Butanol extract of leave (CEBE) of *C. erectus* showed maximum activity. All other extracts exhibited remarkable scavenging activity except CELH and FVLH showed no response towards DPPH inhibition potential. The n-butanol (CEBE) and ethanol (CELE) extract of leave showed the lowest IC_{50} value of 4 µg with 94.62 % and 7 µg with 90.43 % DPPH inhibition, respectively. The reducing power of the extracts was determined using standard protocols such as, FRAP and total antioxidant activity. All extracts showed significant reducing behavior and it was deduced from the results that ethanolic extracts of leave portion of the three plants exhibited highest activities in the FRAP assay (16.5, 11.4, 11.8 in *C. erectus*, *F. variegata* and *F. maclellandii*, respectively)

Table 2: Antioxidant activity of selected plants

Plants Name	Part of Plant	Solvents	Extracts	DDPH		FRAP	Iron reducing capacity
				% inhibition	IC_{50} (µg)		
Conocarpus erectus	Leave	Ethanol	CELE	90.43±1.27	7±2	16.5±1.0	0.3346±0.0027
		n-Hexane	CELH	-	-	7.9±0.9	0.3790±0.0021
		Chloroform	CELC	48.54±1.00	-	9.6±0.7	0.3678±0.0034
		n-Butanol	CELB	88.45±2.11	26±3	16.5±1.2	0.3548±0.0010
	Branch	Ethanol	CEBE	94.62±1.08	4±1	16.2±0.8	0.2975±0.0037
		n-Hexane	CEBH	59.62±0.28	183±3	0.8±0.2	0.3420±0.0019
		Chloroform	CEBC	73.31±1.77	175±2	11.8±0.8	0.4115±0.0037
		n-Butanol	CEBB	89.95±1.09	22±3	16.6±0.4	0.3369±0.0032
Ficus variegata	Leave	Ethanol	FVLE	41.96±1.11	-	11.4±0.6	0.4101±0.0061
		n-Hexane	FVLH	-	-	9.2±0.9	0.4227±0.0040
		Chloroform	FVLC	58.04±1.54	173±2	10.1±1.1	0.6577±0.0046
		n-Butanol	FVLB	53.81±1.23	191±3	8.37±0.8	0.3655±0.0028
	Branch	Ethanol	FVBE	51.31±1.19	195±2	9.0±1.0	0.5710±0.0010
		n-Hexane	FVBH	0.44±0.08	-	6.3±0.3	0.4527±0.0027
		Chloroform	FVBC	27.90±0.33	-	2.1±0.4	0.2723±0.0036
		n-Butanol	FVBB	20.68±0.28	-	4.3±0.8	0.5329±0.0019
Ficus macllendii	Leave	Ethanol	FMLE	90.07±1.37	15±1	11.8±0.6	0.1821±0.0041
		n-Hexane	FMLH	84.84±1.22	152±3	3.9±0.5	0.5225±0.0057
		Chloroform	FMLC	77.52±1.91	159±3	7.4±1.1	0.4378±0.0035
		n-Butanol	FMLB	92.29±1.06	73±2	10.6±1.0	0.5180±0.0046
	Branch	Ethanol	FMBE	53.08±1.24	183±4	7.4±0.5	0.6051±0.0071
		n-Hexane	FMBH	40.27±1.03	-	3.9±0.2	0.3470±0.0028
		Chloroform	FMBC	25.91±0.89	-	4.7±0.8	0.2942±0.0023
		n-Butanol	FMBB	35.32±0.36	-	4.0±0.2	0.3371±0.0026
Gallic acid				92.09±0.11	5±1	19.6±0.6	0.8039±0.0066

Table 3: Acetylcholine esterase activity of selected plants

Plants Name	Part of Plant	Solvents	Extracts	AChE	
				% inhibition	IC_{50} (µg)
Conocarpus erectus	Leave	Ethanol	CELE	69.21±2.1	351±5
		n-Hexane	CELH	44.30±0.09	-
		Chloroform	CELC	63.08±0.45	310±4
		n-Butanol	CELB	82.51±1.97	48±2
	Branch	Ethanol	CEBE	54.90±1.04	327±5
		n-Hexane	CEBH	46.27±0.99	-
		Chloroform	CEBC	45.03±0.27	-
		n-Butanol	CEBB	91.62±3.01	74±4

SD (±) was calculated using M.S excel 2007 software (n=3), - = not calculated

while total antioxidant activity ranged between 0.1821 (FMLH) to 0.6577 (FVLC) (fig. 4). Acetylcholine esterase (AChE) inhibitory potential of the extracts was determined by colorimetric method at 412nm and the extracts of *C. erectus* inhibited the activity of AChE with IC_{50} values in the range of 40 to 351µg/ml, in the order of % inhibition CEBB>CELB>CELE>CELC>CEBE> CEBH>CEBC>CELH. The correlation studies between total phenols and DPPH, FRAP and total antioxidant were also studied and results revealed that there was a strong correlation between phenolics and antioxidant activities (figs. 1-5).

DISCUSSION

The main compounds exhibited free radical scavenging activity, are molecules having antioxidant activity such as phenolic compounds and flavonoid or plant extracts rich with phenolics (Abdou *et al.,* 2010). The method of DPPH free radical scavenging can be used to assess the antioxidant potential of specific molecules or extracts (Mohammad *et al.*, 2010). Phenolic compounds are the prime antioxidant derived from natural products, comprises of flavonoids and phenolic acids, effective radical terminators by donating hydrogen to radicals

(Amic *et al.*, 2007). The high potential of polyphenols to scavenge free radicals may be because of their many phenolic hydroxyl groups (Sawa *et al.*, 1999). DPPH forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products (Jun *et al.*, 1995).

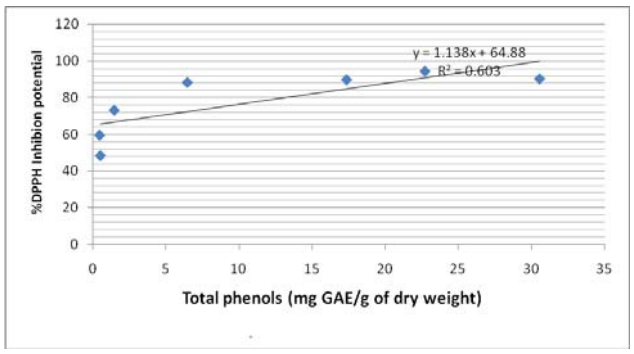

Fig. 1: Correlation between total phenols and DPPH scavenging activity of extracts of *Conocarpus erectus*

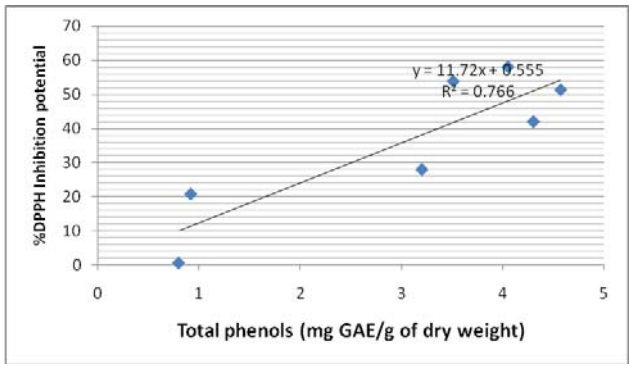

Fig. 2: Correlation between total phenols and DPPH scavenging activity of extracts of *Ficus variegate*

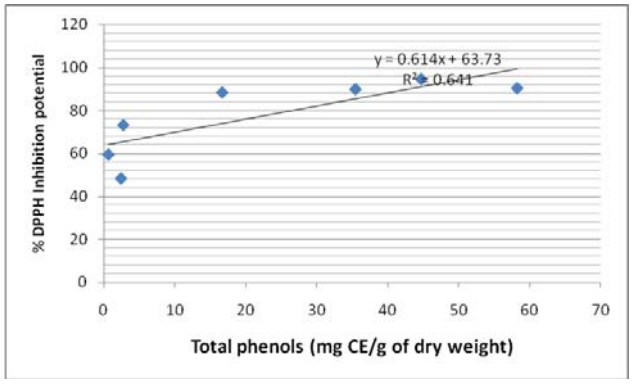

Fig. 3: Correlation between total phenolics and DPPH scavenging activity of extracts of *Conocarpus erectus*

It is reported in a number of research articles that phenolic compounds are good antioxidants and form strong co-relation between phenolic contents and antioxidant activities (Yan and Asmah, 2010). Our results are in agreement with the previous results that non-polar solvent fractions showed absence of flavonoid compounds due to some polar characteristics in their moiety (Yakubu *et al.*, 2014).

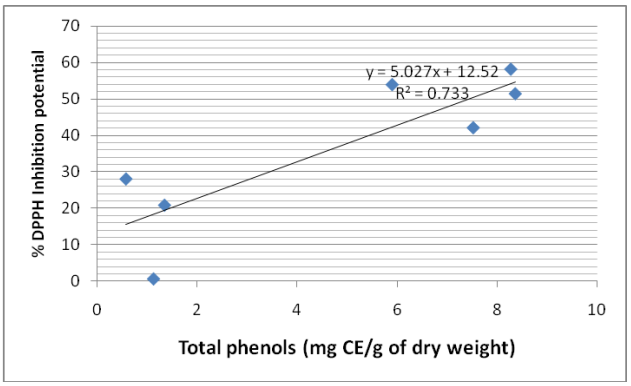

Fig. 4: Correlation between total phenolics and DPPH scavenging activity of extracts of *Ficus variegate*

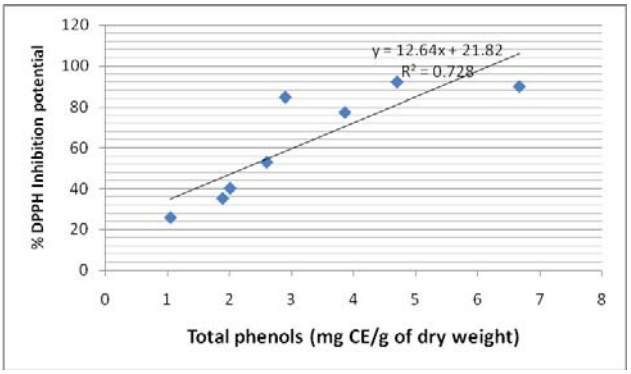

Fig. 5: Correlation between total phenolics and DPPH scavenging activity of extracts of *Ficus maclellendii*

Reactive oxygen species by reacting with biomolecules such as proteins, lipids and nucleic acids, as well as by destruction the body of endogenous enzymatic and non-enzymatic antioxidants cause injury. Problems related to health such as heart disease, diabetics, cancer etc are all co related to oxidative injure. Antioxidants can stop or slow down the oxidative spoil to human body. They perform as "free radical scavengers", hence stop and restore damage done by free radicals (Briviba and Sies, 1994; Georgetti *et al.*, 2003).

Among the *Combretum* species, *C. woodie* has shown significant antioxidant and anti-inflammatory potential (Eloff *et al.*, 2005). Several compounds of interest such as flavonoids, stilbenes, cyclobutanes and triterpenoids have been isolated from this species (Martini *et al.*, 2004). figs are excellent source enriched with bioactive compounds such as flavonoids/ polyphenols, ß- carotines, arabinose, ß-amyrins, ß-setosterols, glycosides and xanthotoxol (Ross and Kasum, 2002; Vaya and Mahmood, 2006; Gilani *et al.*, 2008). The fruits of these plants enclose plentiful amino acids like tyrosine, alanine, threonine, tyrosine and valine in seeds, valine and alanine in proteins. Different parts of *F. religiosa* showed acetyl cholinesterase inhibitory activity, anticonvulsant and anti-

anxiety activity (Damanpreet and Rajesh, 2009). *F. carica* has been documented to contain antiviral, antioxidant, cancer suppressive, antibacterial, hypoglycemic, hypotriglyceridaemic and anthelmintic effects. Different pharmacological trial such as anti-diabetic, anti-ulcer, lipid lowering and antifungal activities have been reported for *F.* exasperate (Wang *et al.,* 2004; Jeong *et al.,* 2005; Solomon *et al.,* 2006. The fruit extracts of *F. benjamina L., F. sycomorous L.,* and *F.bengalensis L.* exhibit anti-tumour activity and anti bacterial activity (Mousa *et al.,* 1994).

CONCLUSION

It is venerable that free radicals are highly reactive, unstable and fundamental/major or primary cause of various diseases such as diabetes, coronary heart diseases, arthritis and several types of cancer etc. The results obtained in the present study suggested that *C. erectus*, *F. variegata* and *F. maclellendii* can be utilized as safer, effective and natural sources of antioxidants with concomitant health benefits. The findings of the current study suggested that these plant fractions could be a potent source of therapeutic agent in preventing or slowing the process of aging and age associated oxidative stresses and related degenerative diseases. Hence, further studies of these plants including isolation, structural elucidation and pharmacological studies of active extracts need to be carried out.

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