

Original article

Medicinal plants of the Western ghats as possible inhibitors of oxidation in various biological lipid substrates

Namratha Pai Kotebagilu, Vanitha Reddy Palvai and Asna Urooj

Department of Studies in Food Science and Nutrition, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India

Received November 1, 2016; Revised November 12, 2016; Accepted November 15, 2016; Published online December 30, 2016

Abstract

Medicinal plants are nature's fighters against several degenerative diseases. The reverse pharmacological approach focuses on traditional knowledge on the usage of medicinal plants for various diseases and revalidating the effect using modern techniques. The Western ghats of Southern India has sheltered a wide range of medicinal plants. The objective of this research work was to evaluate the oxidation inhibiting ability of aqueous extract of four medicinal plants from the Western ghats, namely; *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Costus speciosus* (Koen.) Sm., *Canthium parviflorum* Lam. and *Abrus precatorius* L. in biological lipid substrates, viz., cholesterol, low-density lipoprotein and brain homogenate. The IC₅₀ values of the plant extracts were calculated and the correlation between the phytochemicals and the antioxidant effect exhibited was studied. From the results, it was observed that, the inhibition of oxidation by the aqueous extract of all the four medicinal plants, followed the order cholesterol > LDL > brain homogenate. *C. parviflorum* exhibited highest antioxidant activity in cholesterol (94.8%) and brain (37.35%) than compared to other medicinal plants. In LDL, the highest antioxidant activity (73.75%) was exhibited by *C. speciosus*. The antioxidant activity of CP and CS in the substrates correlated well with the polyphenol and flavonoid content of the extracts. The IC₅₀ values of CS in cholesterol (305 µg, r² = 0.9972) was the least among all the medicinal plants. The overall protective action in the substrates was higher in *C. parviflorum* than other medicinal plants. Thus, the selected medicinal plants can be further explored for its biological activity and utilized as sources of natural antioxidants and phytochemicals against various oxidative stress related diseases.

Keywords: *Andrographis paniculata* (Burm.f.) Wall. ex Nees, *Costus speciosus* (Koen.) Sm., *Canthium parviflorum* Lam., *Abrus precatorius* L., aqueous extract, antioxidant activity, biological substrates, oxidation

1. Introduction

In the current scenario, non-allopathic or complementary and alternative medicine (CAM) has gained momentum in recognition and usage with 70-80% of the population in the world, relying on CAM (Akerele, 1993).

The model of drug discovery and development has observed a paradigm shift. Crisis in newer drug discovery has shifted the focus from normal science towards traditional medicine through reverse pharmacological approach. The abundant wisdom which has run through generations is now being reviewed and revalidated by modern science. The knowledge of traditional medicine has been imparted in modern science for relieving various symptoms associated with a disease in the form of an adjunct or as a therapeutic agent itself (Vaidya, 2014).

Medicinal plants are nature's fighters against several degenerative diseases and have been used in various countries such as China, India, Korea and Africa as part of CAM. In Asia, the knowledge on

the Chinese and the Indian system of traditional medicine have been utilized extensively for treating various diseases. Medicinal plants of China such as *Alphina oxyphylla* Mig., *Rhodiola sacra* Fu, *Astragalus membranaceus* (Fisch.) Bge., *Polygonum multiflorum* Thunb., *Psoralea corylifolia* L., *Astragalus complanatus* R. Br., *Angelica sinensis* (oliv.) Diels, etc. have been evaluated for their antioxidant activity and have been used in Chinese medicine (Wong *et al.*, 2006). Indian medicinal plants such as *Withania somnifera*, *Tinospora cordifolia*, *Mucuna pruriens*, *Phyllanthus amarus*, etc., have been evaluated for various biological activities (Vaidya, 2014). Patwardhan *et al.* (2005) has reported that the PubMed database on Chinese Medicine has received more than 10000 citations and 195 patents whereas the Indian medicine has received more than 1000 citations and 3 patents. The Indian sub-continent has harbored many traditional healthcare systems such as Ayurveda, Unani, Homeopathy and Siddha which emphasize the relationship between nature and mankind (Mukherjee and Wahile, 2006). Seventy percentage of the Indian population makes use of Ayurveda for health care (Vaidya, 2014). Since ancient times, India is known for its usage of different parts of medicinal plants in traditional medical practices of Ayurveda (Govindarajan *et al.*, 2005). The available literature indicates paucity of data on exploration, revalidation and documentation of biological activities of Indian medicinal plants and their use as therapeutic agents.

Author for correspondence: Dr. Asna Urooj

Professor, Department of Studies in Food Science and Nutrition, Manasagangotri, University of Mysore, Mysuru-570006, Karnataka, India

E-mail: asnaurooj@foodsci.uni-mysore.ac.in

Tel.: +91-9448489334

Copyright © 2016 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; **Website:** www.ukaazpublications.com

DOI: 10.21276/ap.2016.5.2.16

It is well known that oxidation by free radicals has been linked to several diseases and antioxidants help in counter acting the action of free radicals. The reverse pharmacological model focuses on evaluating the biological activities of medicinal plants by re-validating the documented effect, using systematic *in vitro*, *ex vivo* and *in vivo* experiments. The thiobarbituric acid reactive substances (TBARS) method has been considered ideal to study lipid peroxidation in biological samples or substrates such as brain, cholesterol, microsomes, RBC, *etc.*, and is one among several *in vitro* assays to determine the protective effect of any food or medicinal plant by inhibition of oxidation (Boligon *et al.*, 2014; Okhawa *et al.*, 1979).

Fatty acid peroxidation has received great attention in causing oxidative stress in the system leading to diseases such as Alzheimer's disease, mild cognitive impairment, atherosclerosis and cardiovascular diseases. A double blind placebo controlled trial of extracts of *Salvia officinalis* (Akhondzadeh *et al.*, 2003a), *Crocus sativus* (Akhondzadeh *et al.*, 2010) and *Melissa officinalis* (Akhondzadeh *et al.*, 2003b) have shown positive effect in the management of mild to moderate Alzheimer's disease. The utilization of herbal medicines prepared from *Echium amoenum*, *Crocus sativus*, and *Rhodiola rosea* as antidepressant agents and *Matricaria recutita*, *Ginkgo biloba*, *Passiflora incanata*, *Echium amoenum*, and *Scutellaria lateriflora* as anxiolytic agents have been reported. These research areas mark the genesis of herbomics and psychopharmacology (Sarris *et al.*, 2011). Medicinal plants have also exhibited protective effects in the management of cardiovascular diseases such as dyslipidemia, hypertension, coronary heart disease, *etc.* (Dahanukar *et al.*, 2000).

The Western ghats of Southern India has sheltered a wide variety of medicinal plants. In our laboratory, plants such as *Morus indica* (Arabshahi-Dlouee and Urooj, 2007), *Ziziphus jujuba* Mill (Esteki and Urooj, 2012) and *Moringa oleifera* (Reddy *et al.*, 2005) and medicinal plants of the Western ghats such as *Andrographis paniculata* (AnP) (Pai Kotabagilu *et al.*, 2014; Pai Kotabagilu *et al.*, 2015), *Costus speciosus* (CS) (Pai Kotabagilu *et al.*, 2014; Pai Kotabagilu *et al.*, 2015), *Canthium parviflorum* (CP) (Pai Kotabagilu *et al.*, 2014; Reddy *et al.*, 2014) and *Abrus precatorius* (AP) (Pai Kotabagilu *et al.*, 2014; Reddy *et al.*, 2014) have been evaluated for various biological activities by *in vitro* and *in vivo* experiments. This study was conducted as part of our attempt to explore the antioxidant activity of medicinal plants of the Western ghats in different food and biological systems.

The antioxidant effect of these medicinal plants in biological substrates such as microsomes, red blood cells (Pai Kotabagilu *et al.*, 2014) and lipid substrates such as cholesterol, low density lipoprotein and brain homogenate was evaluated and positive results were obtained (Pai Kotabagilu *et al.*, 2015). This study was conducted in continuation with our previously published work (Pai Kotabagilu *et al.*, 2015) with the major objective of evaluating the antioxidant activity, using aqueous extracts of the AnP, CS, CP and AP. Though there are several studies reporting various biological activities of these medicinal plants, there are fewer studies on the protective effect of these medicinal plants in biological lipid substrates.

2. Materials and Methods

2.1 Chemicals

The chemicals used in the study were of analytical grade. Protein kit was purchased from Span Diagnostics Ltd, Gujarat, India. LDL diagnostic kit was purchased from Agape Diagnostics Ltd, Kerala, India. Cholesterol was purchased from Himedia.

2.2 Plant materials

The Western ghats of Southern India has a plethora of medicinal plants which have been used in traditional medicine. The leaves of the plant samples selected for the study, *viz.*, (AnP), (CS), (CP) and (AP) were collected from the Western ghats, India. The samples were identified by Botanist, Dr. Janardhan, Department of Studies in Botany, University of Mysore, Mysuru, India. The samples were cleaned, washed and dried in hot air oven at 55°C for 8-10 h. The dried leaves were ground to a fine powder and passed through 60 mesh sieve and stored in air tight containers until use.

2.3 Preparation of extracts

Aqueous extracts of the samples were prepared from the dried powder by adding 50 g of the sample in 250 ml of distilled water. The mixture was shaken in a mechanical shaker for 6 h. The extracts were centrifuged at 4000 rpm for 10 min and filtered using Whatman No. 1 filter paper. The filtrate was frozen at -20°C in a deep freezer overnight. The frozen extract was freeze dried in Modulyo D freeze dryer, Thermo Electron Corporation. The lyophilized aqueous extracts were stored at 0°C until further use.

2.4 Estimation of polyphenol and flavonoid content in the aqueous extract

The polyphenol and flavonoid contents were analyzed in aqueous extracts of all the samples by Folin-Ciocalteu micro and pharmacopoeia methods using Gallic acid and Rutin as standards, respectively (Slinkard and Singleton, 1977; Miliuskas *et al.*, 2004).

2.5 Substrates isolation and preparation

Three biological lipid substrates; LDL, brain and cholesterol were selected to analyze the potency of aqueous extracts of the plant samples in inhibiting oxidation. Cholesterol was obtained from a commercial source and dissolved in ethanol to a known concentration of 10 mg/ml and stored at 0°C until further use (Pai Kotabagilu *et al.*, 2015). 300 µl of the substrate was taken for the experiment.

LDL was isolated by modified method of Schlüssel and Elstner (1996). Permission from Institutional Human Ethics Committee of the University of Mysore was taken for the collection of blood samples from healthy subjects to isolate LDL (IHEC-UOM No. 36 Res/2013-14 date: 16/4/2013). Venous blood was drawn from healthy individuals by Laboratory Technician at University Health Centre, University of Mysore. Samples were added to EDTA vials and centrifuged. The plasma layer was separated followed by the addition KBr. 9 ml saline was added to the tubes and ultracentrifuged (Thermoscientific, Pune) at 43,000 rpm for 3 h at 4°C. Four different layers (L₁, L₂, L₃ and L₄) were obtained and each layer was separated using a Pasteur pipette into separate vials. The layers were dialyzed

and tested for LDL and protein content by standard kit method. The LDL contents of L₁, L₂, L₃ and L₄ were 33.88, 5.32, 3.22 and 17.99 mg/dl, respectively. The top most fraction, L₁ was taken for the experiment. The LDL content was diluted with PBS buffer to a known volume and stored in freezer until further use. Substrate equal to 1mg protein concentration, *i.e.*, 300 µl of the substrate was taken for the experiment.

The preparation of brain homogenate was as per our previously published work (Pai Kotabagilu *et al.*, 2015). Permission from Institutional Animal Ethics Committee of the University of Mysore was obtained for the study (UOM/IAEC/04/2013 dated: 28-09-2013). The protein content of the homogenate was analyzed and substrate equal to 1 mg protein concentration, *i.e.*, 200 µl was taken for the experiment.

2.6 Estimation of thiobarbituric acid reactive substances (TBARS)

Lipid peroxide formation was measured by modified method of Ohkawa *et al.* (1979). Substrates were taken according to their protein content. Aqueous extract of different concentrations (300-500 µl of 1 mg/ml concentration) were added to the substrates. Fenton's reagent was added to induce oxidation in the substrates. The IC₅₀ values were calculated, using the linear regression equation.

$$\% \text{ Inhibition of oxidation} = \frac{(\text{O.D of control} - \text{O.D of sample})}{(\text{O.D of control})}$$

2.7 Statistical analysis

All the experiments were carried out in triplicates (n=3). The correlation was determined by using the Pearson's product-moment correlation coefficient. Data were subjected to One way-ANOVA, Post hoc multiple comparison, bonferroni alpha ($p \leq 0.05$) using SPSS software, 16.0 version. IC₅₀ values were calculated using linear regression equation.

3. Results and Discussion

3.1 Antioxidant activity of medicinal plants in different biological substrates

The TBARS method is a simulation of oxidation process that occurs in the body. Hydrogen peroxide is used in the presence of ferrous sulphate to generate free radicals and to induce oxidation. The formation of malondialdehyde (MDA), caused due to tissue injury combine with proteins to form a protein adduct based on the composition of the substrates. These adducts when heated, form MDA-TBA complex and gives a pink color which can be read spectrophotometrically at 532 nm (Palmieri and Sblendorio, 2007). The antioxidant effect of medicinal plants against oxidation can be analyzed by TBARS method.

3.1.1 Antioxidant activity of *Andrographis paniculata* (Burm.f.) Wall. ex Nees

The antioxidant activity of AnP in three biological lipid substrates is shown in Figure 1. The aqueous extract of *A. paniculata* (AnPAq) at different concentrations (300, 400 and 500 µg) showed the highest

inhibition of oxidation in cholesterol (40.67- 45.63%), followed by LDL (28.43-45.7%) and the least activity in brain homogenate (12-16.09%). There was no significant difference between the inhibition of oxidation in LDL and cholesterol whereas, a significant difference was observed in brain homogenate ($p \leq 0.05$). However, the antioxidant activity of AnPAq was less than 50% in all the substrates. A difference in the protective role between the concentrations of extract was observed. The highest antioxidant activity was shown at 300 µg in cholesterol whereas at 500 µg in LDL. The results indicate the importance of optimizing the concentration of the extracts to exhibit its biological potential. The protective effect of aqueous extract of AnP has been reported in a study, where the extract ameliorated the toxic effects of nicotine in the brain mitochondria of male wistar rats (Das *et al.*, 2009). In our previous work, the methanol and 80% methanol extracts of AnP have shown better antioxidant activity in the substrates than aqueous extract (Pai Kotabagilu *et al.*, 2015).

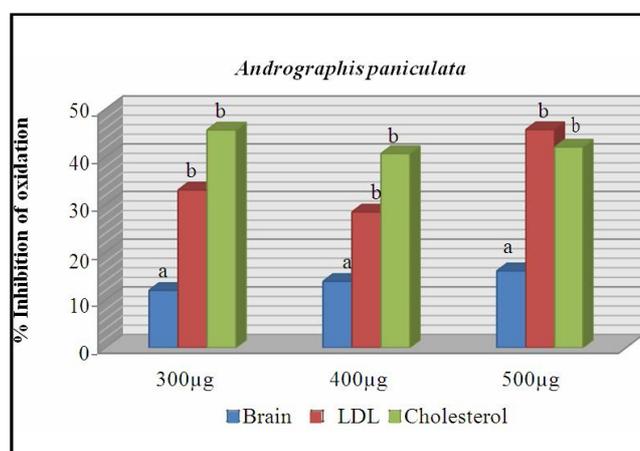


Figure 1: Antioxidant activity of *Andrographis paniculata* a, b, represents significance at 300 µg, 400 µg and 500 µg ($p \leq 0.05$). Values are the mean of triplicates (n=3)

3.1.2 Antioxidant activity of *Costus speciosus* (Koen.) Sm.

The antioxidant activity of CS is shown in Figure 2. At 500 µg, the aqueous extract of *C. speciosus* (CS Aq) exhibited significantly higher antioxidant activity in cholesterol -73.75% ($p \leq 0.05$), followed by LDL -64.46% and brain homogenate -13.15%. It was also observed that the activity of CS Aq was the least in brain homogenate (7.25%) and highest in LDL (64.46%) among all the substrates and all the other medicinal plants. Therefore, the antioxidant activity varies among substrates mainly due to compositional differences. An increasing trend was observed in the antioxidant activity with increasing concentration. In cholesterol, the antioxidant activity of CS Aq at higher concentration was 73.75% which is higher than the methanol extracts (Pai Kotabagilu *et al.*, 2015). This protective action of CS Aq may be contributed to the water soluble phytochemicals such as vitamin C, polyphenols, glutathione and tannins (Pai Kotabagilu *et al.*, 2014). Various studies have reported anti-helminthic and larvicidal activity of aqueous extract of CS leaves (Srivastava *et al.*, 2013; Kanakkanath *et al.*, 2013).

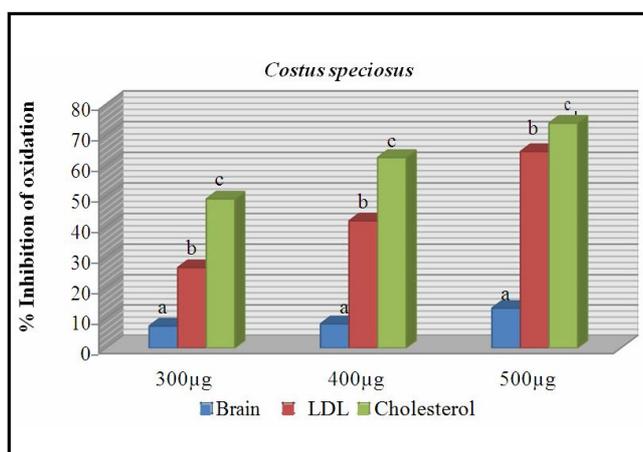


Figure 2: Antioxidant activity of *Costus speciosus*
a, b, c...represents significance at 300 µg, 400 µg and 500 µg ($p \leq 0.05$). Values are the mean of triplicates ($n=3$)

3.1.3 Antioxidant activity of *Canthium parviflorum* Lam.

The antioxidant activity of CP is shown in Figure 3. In comparison with other medicinal plants and substrates, the aqueous extract of *C. parviflorum* (CP Aq) exhibited significantly higher antioxidant activity in cholesterol - 94.8% ($p \leq 0.05$). The inhibition of oxidation at 500 µg followed the order, cholesterol (94.8%), LDL (45.07%) and brain (37.35%). Similar results were observed in methanol extracts of CP in the same substrates, viz., cholesterol, brain and LDL (Pai Kotabagilu *et al.*, 2015). The antioxidant effect could be due to the presence of tannins, alkaloids, flavonoids in aqueous leaf extract of CP (Pasumarthi *et al.*, 2011).

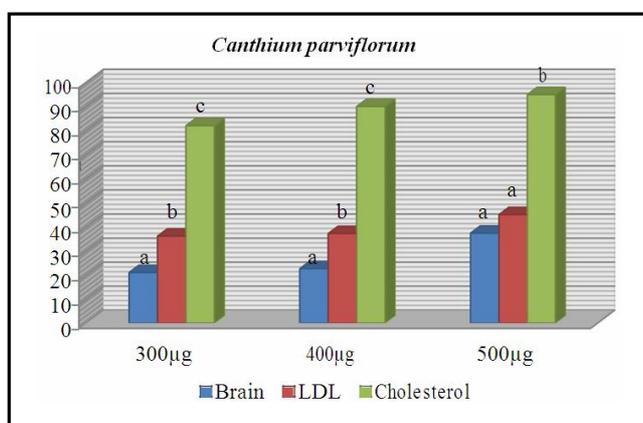


Figure 3: Antioxidant activity of *Canthium parviflorum*
a, b, c...represents significance at 300 µg, 400 µg and 500 µg ($p \leq 0.05$). Values are the mean of triplicates ($n=3$).

3.1.4 Antioxidant activity of *Abrus precatorius* L.

The antioxidant activity of AP is given in Figure 4. The aqueous extract of *Abrus precatorius* (AP Aq) exhibited highest antioxidant activity in cholesterol (60.63% at 300 µg), followed by LDL (39.11% at 500 µg) and brain (22.50% at 500 µg). In case of substrates of brain and LDL, the activity increased with increasing concentration with no significant difference ($p \leq 0.05$) whereas in cholesterol, the activity decreased (38.95%) at 500 µg significantly

($p \leq 0.01$) than at lower concentrations, i.e., 300 µg -60% and 400 µg -56%. Similar trend was observed in our previous findings on the action of methanol extract of AP on cholesterol (Pai Kotabagilu *et al.*, 2015). The aqueous extract of AP has been used in traditional medicine for curing malaria, typhoid, cough, respiratory tract infections and hepatitis (Saganuwan and Onyeyili, 2010).

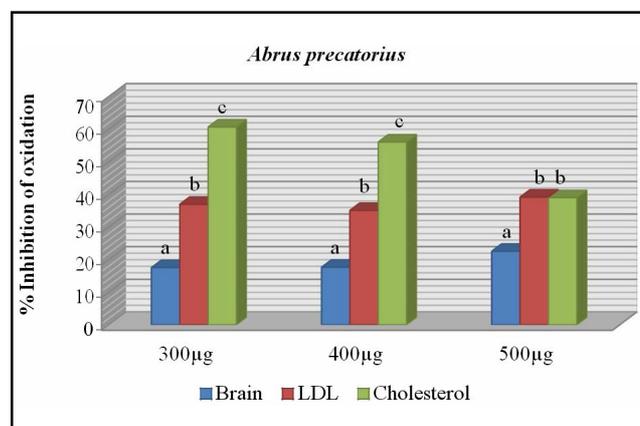


Figure 4: Antioxidant activity of *Abrus precatorius*
a, b, c...represents significance at 300 µg, 400 µg and 500 µg ($p \leq 0.05$). Values are the mean of triplicates ($n=3$)

3.2 The IC_{50} values of aqueous extract of the selected medicinal plants

The IC_{50} values of the aqueous extracts of AnP, CS, CP and AP are given in Table 1. In cholesterol, it was observed that the IC_{50} values of AnP and CP were negative. This observation could probably be due to the presence of IC_{50} values at concentrations lower or greater than 300-500 µg or non-linearity in antioxidant activity with increasing concentration of the extract in AnP or the pro-oxidant effect of phytochemicals at higher concentrations. However, the IC_{50} values of CS was 305 µg ($r^2 = 0.9972$) and AP was 417 µg ($r^2 = 0.9008$). The IC_{50} value of CS in cholesterol was the least among all the medicinal plants.

Table 1: IC_{50} values of aqueous extract of the selected medicinal plants

Substrate	Medicinal plants	IC_{50} values of aqueous extract (µg)	r^2 - value
Cholesterol	AnP	-	0.4867
	CS	305	0.9972
	CP	-	0.9814
Brain	AP	417	0.9008
	AnP	2156	0.9978
	CS	1774	0.8424
LDL	CP	681	0.8235
	AP	1631	0.7650
	AnP	625	0.5017
	CS	430	0.9875
	CP	635	0.8406
	AP	1577	0.2918

In the brain homogenate, concentration of the extract required to exhibit 50% inhibition of oxidation was higher than other substrates.

Brain homogenate is prone to oxidation easily due to high lipid content and, therefore the concentration of extract required to exhibit antioxidant activity will be higher. The least value was observed in CP (681 µg, $r^2 = 0.8235$) and the highest in AnP (2156 µg, $r^2 = 0.9978$). In LDL, CS had the least IC_{50} value (430 µg, $r^2=0.9875$) followed by AnP (625 µg, $r^2=0.5017$), CP (635 µg, $r^2=0.8406$) and AP (1577 µg, $r^2=0.2918$).

3.3 Relationship between polyphenol and flavonoid content of the medicinal plants with the antioxidant activity

The correlation between the phytochemicals and the antioxidant activity was determined, using the Pearson's product-moment correlation coefficient, higher the r-value (close to 1) better is the correlation. The result of correlation of flavonoids and polyphenols with the antioxidant activity is given in Table 2.

In cholesterol, CS Aq and CP Aq had a positive correlation with the polyphenol (CS, $r = 0.988$; CP, $r = 0.931$) and flavonoid (CS, $r = 0.991$; CP, $r = 0.923$) content. The results can be related to the high antioxidant activity exhibited by CS Aq (73.75% at 500 µg) and CP Aq (94.8% at 500 µg) in cholesterol. Though, the polyphenol (25 mg/g) and flavonoid (0.01 mg/g) contents in the extract of CP were lower than other extracts, a positive correlation was observed. Hence, the protective action depends mainly on the nature of the phytochemical present. Similar results were observed in LDL wherein, CS Aq had the highest correlation with the polyphenol (55 mg/g extract, $r = 0.986$) and flavonoid content (0.16 mg/g extract, $r = 0.982$). In brain, CP Aq (polyphenols, $r = 0.816$; flavonoids, $r = 0.867$) and AP Aq ($r = 0.803$) for polyphenols and flavonoids) showed better correlation and the results were comparable to the antioxidant activity, exhibited by both the extracts in brain homogenate.

Table 2: Correlation between the antioxidant activity of plant extracts against oxidation of substrates and the content of polyphenol and flavonoid in the aqueous extract.

Aqueous extracts of medicinal plants	Polyphenol (mg/g) Mean ± SD	r-value cholesterol	r-value brain	r-value LDL
AnP	63.33±4.71	-0.308	0.370	0.689
CS	55.00±0	0.988	0.642	0.986
CP	25.00±0	0.931	0.816	0.748
AP	81.66±8.49	-0.909	0.803	0.260
	Flavonoid (mg/g)	r-value	r-value	r-value
AnP	0.59±0.02	-0.323	0.369	0.661
CS	0.16±0.03	0.991	0.631	0.982
CP	0.01±0	0.923	0.867	0.793
AP	1.02±0.08	-0.909	0.803	0.260

Values are mean of triplicates and expressed as Mean±SD. (n = 3),

The utilization of the selected medicinal plants for their biological activities such as anti-inflammatory, antimicrobial, antitumor, hypoglycemic, antidiabetic, antiviral, hepato and renoprotective effects have been reported (Chao and Lin, 2010; Akbar, 2011; Solanki and Zaveri, 2012; Shirisha *et al.*, 2013; Jayakumar *et al.*, 2013;

Pawar and Pawar, 2014; Kala, 2016;). AnP has been used as a blood purifier in treating blood related diseases such as skin eruptions, boils, scabies, and chronic undetermined fevers (Akbar, 2011). The rhizome of CS is widely used for its health beneficial effects however there are fewer studies reporting the biological activities of the leaves. Though, these medicinal plants have been explored for various biological activities *in vitro* and in animal models, more studies are needed to explore and document the phytochemical constituents and bioactives; and their use as nutraceuticals or a drug in various diseases. The present study has helped in exploring the antioxidant effect of the selected medicinal plants in biological lipid substrates. The inhibition of oxidation by the aqueous extract of all the four medicinal plants, followed the order cholesterol >LDL> brain homogenate. Among these medicinal plants, CP Aq has been the most potent in preventing oxidation of all the lipid substrates.

4. Conclusion

The usage of aqueous extracts of medicinal plants is a safer approach since they are non-carcinogenic and non-genotoxic in nature when compared to most of the organic solvents. From the research findings, it can be inferred that, aqueous extract of all the selected medicinal plants have the potential to inhibit oxidation or free radical generation in biological lipid rich substrates. These plants can be utilized as rich sources of phytochemicals to protect against free radicals at the biological level, thus preventing the progression of degenerative diseases. However, further research is needed on the identification and purification of the bioactive compounds and development of these plants as nutraceuticals and drugs.

Acknowledgements

Authors acknowledge UGC-IOE (Institute of Excellence) for their timely support in providing the financial assistance.

Conflict of interest

We declare that we have no conflict of interest.

References

- Akbar, S. (2011). *Andrographis paniculata*: A review of pharmacological activities and clinical effects. *Altern. Med. Rev.*, **16**(1):66-77.
- Akerele, O. (1993). Nature's medicinal bounty: Don't throw it away. In *World Health Forum*, **14**(4):390-395.
- Akhondzadeh, S.; Noroozian, M.; Mohammadi, M.; Ohadinia, S.; Jamshidi, A. H. and Khani, M. (2003a). *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: A double blind, randomized and placebo controlled trial. *J Clin. Pharm. Ther.*, **28**(1):53-59.
- Akhondzadeh, S.; Noroozian, M.; Mohammadi, M.; Ohadinia, S.; Jamshidi, A. H. and Khani, M. (2003b). *Melissa officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: A double blind, randomized, placebo controlled trial. *J. Neurol. Neurosurg. Psychiatry*, **74**(7):863-866.
- Akhondzadeh, S.; Sabet, M. S.; Harirchian, M. H.; Togha, M.; Cheraghmakani, H.; Razeghi, S. and Rezazadeh, S. A. (2010). A 22-week, multicenter, randomized, double-blind controlled trial of *Crocus sativus* in the treatment of mild-to-moderate Alzheimer's disease. *Psychopharmacol.*, **207**(4):637-643.
- Arabshahi-Delouee, S. and Urooj, A. (2007). Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food. Chem.*, **102**(4):1233-1240.

- Boligon, A. A., Machado, M. M. and Athayde, M. L. (2014).** Technical evaluation of antioxidant activity. *Med. Chem*, **4**(7):517-522.
- Chao, W. W. and Lin, B. F. (2010).** Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chin. Med.*, **5**(17):1-15.
- Dahanukar, S. A.; Kulkarni, R. A. and Rege, N. N. (2000).** Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.*, **32**(4): S81-S118.
- Das, S.; Gautam, N.; Dey, S. K.; Maiti, T. and Roy, S. (2009).** Oxidative stress in the brain of nicotine-induced toxicity: Protective role of *Andrographis paniculata* Nees and vitamin E. *Appl. Physiol. Nutr. Metab.*, **34**(2):124-135.
- Esteki, T., and Urooj, A. (2012).** Antioxidant components and activity in the peel of *Ziziphus jujuba* Mill. *J. Pharm. Res.*, **5**(5):2705-2709.
- Govindarajan, R.; Vijayakumar, M. and Pushpangadan, P. (2005).** Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *J. Ethnopharmacol.*, **99**(2):165-178.
- Jayakumar, T.; Hsieh, C. Y.; Lee, J. J. and Sheu, J. R. (2013).** Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide. *Evid. Based. Complement. Alternat. Med.*, pp:1-16.
- Kala, S. C. (2016).** A review on phytochemical and pharmacological significance of *Canthium parviflorum* LAM. *Int J. Curr. Pharm. Res.*, **8**(1):1-3.
- Kanakkanath, S.; Narayanan, A. and Ganesan, B. (2013).** Evaluation of *Costus speciosus* Koen aqueous extract for larvicidal activity. *Pharm. Lett.*, **5**(4):283-285.
- Miliauskas, G.; Venskutonis, P. R. and Van Beek, T. A. (2004).** Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food. Chem.*, **85**(2):231-237.
- Mukherjee, P. K. and Wahile, A. (2006).** Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J. Ethnopharmacol.*, **103**(1):25-35.
- Ohkawa, H.; Ohishi, N. and Yagi, K. (1979).** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, **95**(2): 351-358.
- Pai Kotebagilu, N.; Reddy Palvai, V. and Urooj, A. (2014).** Protective effect of selected medicinal plants against hydrogen peroxide induced oxidative damage on biological substrates. *Int. J. Med. Chem.*, pp: 1-7.
- Pai Kotebagilu, N.; Reddy Palvai, V. and Urooj, A. (2015).** *Ex vivo* Antioxidant activity of selected medicinal plants against fenton reaction-mediated oxidation of biological lipid substrates. *Biochem. Res. Int.*, pp:1-7.
- Palmieri, B. and Sblendorio, V. (2007).** Oxidative stress tests: overview on reliability and use. *Eur. Rev. Med. Pharmacol. Sci.*, **11**(6):383-399.
- Pasumarthi, S.; Chimata, M. K.; Chetty, C. S. and Challa, S. (2011).** Screening of phytochemical compounds in selected medicinal plants of Deccan Plateau and their viability effects on Caco-2 cells. *J. Med. Plant. Res.*, **5**(32):6955-6962.
- Patwardhan, B.; Warude, D.; Pushpangadan, P. and Bhatt, N. (2005).** Ayurveda and traditional Chinese medicine: a comparative overview. *Evid. Based. Complement. Alternat. Med.*, **2**(4):465-473.
- Pawar, V. A. and Pawar, P. R. (2014).** *Costus speciosus*: An important medicinal plant. *Int. J. Sci. Res.*, **3**(7):28-33.
- Reddy Palvai, V.; Mahalingu, S. and Urooj, A. (2014).** *Abrus precatorius* leaves: antioxidant activity in food and biological systems, pH, and temperature stability. *Int. J. Med. Chem.*, pp:1-7.
- Reddy Palvai, V.; Mahalingu, S. and Urooj, A. (2014).** *Canthium parviflorum* leaves: antioxidant activity in food and biological systems, pH, and temperature stability. *Chin J. Biol.*, pp:1-7
- Reddy, V.; Urooj, A. and Kumar, A. (2005).** Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food. Chem.*, **90**(1):317-321.
- Saganuwan, S. A. and Onyeyili, P. A. (2010).** Biochemical effects of aqueous leaf extract of *Abrus precatorius* (Jecquirity bean) in Swiss albino mice. *Herba. Pol.*, **56**(3):63-80.
- Sarris, J.; Panossian, A.; Schweitzer, I.; Stough, C. and Scholey, A. (2011).** Herbal medicine for depression, anxiety and insomnia: A review of psychopharmacology and clinical evidence. *Eur. Neuropsychopharmacol.*, **21**(12):841-860.
- Schlüssel, E. H. and Elstner, E. F. (1996).** Desialylation of low density lipoprotein-metabolic function versus oxidative damage? *Z. Naturforsch. C. Bio. Sci.*, **51**(5-6):395-400.
- Shirisha, K.; Begum, B. S. and Mastan, M. (2013).** Protective effect of *Andrographis paniculata* against Atrazine induced renotoxicity in albino rats. *IOSR J. Pharm.*, **10**:17-21.
- Slinkard, K. and Singleton, V. L. (1977).** Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic.*, **28**(1): 49-55.
- Solanki, A. and Zaveri, M. (2012).** Pharmacognosy, phytochemistry and pharmacology of *Abrus precatorius* leaf: A review. *Int J. Pharm. Sci. Rev. Res.*, **13**(2):71-76.
- Srivastava, S.; Singh, P.; Jha, K. K.; Mishra, G.; Srivastava, S. and Khosa, R. L. (2013).** Anti-inflammatory, analgesic and antipyretic activities of aerial parts of *Costus speciosus* koen. *Indian J. Pharm. Sci.*, **75**(1): 83-88.
- Vaidya, A. D. (2014).** Reverse pharmacology-A paradigm shift for drug discovery and development. *Curr. Res. Drug. Discovery*, **1**(2):39-44.
- Wong, C. C.; Li, H. B.; Cheng, K. W. and Chen, F. (2006).** A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food. Chem.*, **97**(4):705-711.