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PLANT SCIENCE TODAY, 2020
Vol 7(3): 383–390
https://doi.org/10.14719/pst.2020.7.3.765
ISSN 2348-1900 (online)

RESEARCH ARTICLE

Assessment of total phenolic content and antioxidant potentiality of selected Indian folk medicinal plants by spectrophotometric method

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ARTICLE HISTORY

Received: 04 March 2020
Accepted: 30 May 2020
Published: 10 July 2020

KEYWORDS

Reactive oxygen species
Antioxidants
Polyphenols
Leaf extract
Folk Medicines

ABSTRACT

Natural antioxidant capacity of five important folk medicinal plants measured *in vitro*. Total phenolic content (TPC), flavonoid content (FC) and free radical scavenging capacity of ethanolic, methanolic and aqueous leaf extracts of *Lippia alba* (LA), *Annona squamosa* (AS), *Hyptis suaveolens* (HS), *Commiphora wightii* (CW) and *Milletia pinnata* (MP) was assessed using spectrophotometric method. Folin ciocalteu and aluminium chloride method employed to optimise TPC and FC. Free radical scavenging potentiality of leaf extracts was assessed using Ferrous ion chelation (FIC), 2, 2'-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) scavenging, Hydroxyl (OH$^\bullet$) radical scavenging (HRS) and Superoxide (O^-_2) radical scavenging (SRS) methods. Results revealed that the TPC (96.22±5.85 to 519.23±34.90 µg GAE/gm dry weight) were found significant in aqueous extracts from all the plants except AS ($p<0.05$). For FC (µg QCE/gm dry weight), ethanol was found optimum for LA (463.94±6.49), CW (289.99±2.70) and MP (347.47±4.50) whereas, aqueous was found more appropriate for rest two plants were found significant instead of ethanol and methanol (all $p<0.05$). The lowest IC_{50} (µg/ml) were recorded from *A. squamosa* (27.72±8.95), *H. suaveolens* (27.78±0.88), *C. wightii* (27.18±0.16) and *M. pinnata* (27.30±0.03). All plants have reflected a high antioxidant capacity; however, the highest antioxidant activity was reported from ethanolic extract of *H. suaveolens* followed by *L. alba*, *A. squamosa*, *C. wightii and M. pinnata*. Hence, these studies show that all folk medicinal plants contain potential antioxidant bioactive compounds.

Introduction

Reactive oxygen species or free radicals like hydroxyl radical, hydrogen peroxide and superoxide anions generated during the cell metabolism. Survival of the cell largely depends on the oxidative metabolism. Production of free radical and other oxidative oxygen species are result of this dependency, which causes oxidative changes. A wide range of scavenging enzymes such as glutathione, NADH, NADPH, superoxide dismutase and catalase reacts with free radicals and protect the body against noxious oxidative stress, cellular and molecular damage (1). Disintegration of free radicals and scavengers balance may trigger various diseases such as neurodegenerative disease, Parkinson's, mongolism, cancer and the aging process (2, 3). Antioxidant inhibits the initiation of oxidative chain reactions, which prevents the oxidation of lipids or other molecules by different mechanisms like free radical-scavenging, potential complexity of pro-oxidant metals and by single oxygen quenching (4). Synthetic antioxidants like butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) along with other food preservatives have toxic and carcinogenic effects by high doses which causes tumour in stomach and liver (5). Plants possess phytochemicals like flavonoids, carotenoids, benzoic acids, polyphenols, cinnamic acids, ascorbic acid, tocotrienols and tocopherols, which act as natural antioxidants at low concentration without any risk (6). Naturally, polyphenols derived from various parts of plants in active form with anti-radical properties such as leaf, flower, seed, root, fruit and barks (7). Antioxidant activity and yield of plant

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To cite this article: Dubey S, Ojha K, Chandrakar J, Dehariya R, Vinodia S, Singh A, Dixit A K. Assessment of total phenolic content and antioxidant potentiality of selected Indian folk medicinal plants by spectrophotometric method. Plant Science Today. 2020;7(3):383–390. https://doi.org/10.14719/pst.2020.7.3.765

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compounds totally depends on different extraction techniques (such as maceration, Soxhlet extraction, ultrasound-assisted extraction, subcritical water extraction) as well as polarity of solvents. Antioxidant compounds, characterized by different chemical nature like their structure, polarity and solubility (8). Present study-employed ethanol, methanol and deionized water for the extraction of phytochemicals obtained from medicinal plants. Ethanol is a good solvent for extraction and safe for human consumption. Methanol generally used for the extraction of the polar and lower molecular weight polyphenols. While, deionised water is exploit for extraction of highly polar polyphenols from plant parts (9). Plants were used as crude drugs for treatment of different ailments in different cultures (10). However, the medicinal actions are unique for each plant species. This consistency with the concept of combination of secondary products in particular plant is taxonomically different (11).

Materials and Methods

Plant Materials

Five locally available plants used as folk medicine against many diseases by tribal and rural communities of Chhattisgarh. These plants viz. *Lippia alba* (LA), *Annona squamosa* (AS), *Hyptis suaveolens* (HS), *Commiphora wightii* (CW) and *Millettia pinnata* (MP) are described in Ayurveda for curing various ailments (Table 1).

Chemicals and Reagents

Ascorbic acid (ACa), Gallic acid (GA), Folin reagent, Aluminium chloride ($AlCl_3$), Quercetin, Hydrogen peroxide (H_2O_2), Phosphate buffer (pH 7.4, 7.8), 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^•$), Ethylenediamine tetra acetic acid (EDTA), Phenanthroline, Ferric chloride ($FeCl_3$), Ferrous sulphate ($FeSO_4$), Ferrozine, Nitro blue tetrazolium (NBT), Nicotinamide adenine dinucleotide reduced (NADH$^•$), Phenazinemethosulphate (PMS). All chemicals purchased from HiMedia (India) were of analytical grade.

Collection of plants

Guru Ghasidas University campus with around 700 acres of land, lies between 22° 7' 54" North to 82° 8' 32" East in Koni, Bilaspur, Chhattisgarh, India. It is an adobe of many naturally growing medicinal plants with rich diversity without pollution and any human disturbance. Selected plant species are already found as wild plants in the area of university campus. The collected plants were identified using APG IV criteria and deposited in the Department of Botany as herbarium with voucher number (Table 1).

Preparation of plant extract

Healthy plant leaves were surface sterilized by using 1% H_2O_2 and dried at 30 °C and finally ground to coarse powder. Obtained powder was dissolved separately into ethanol, methanol and aqueous in 1:10 ratio (gm/ml) than kept on the rotary shaker for 24 hrs. All extracts filtered through the Whattman No. 1 filter paper and stored in refrigerator (GL-F282RSOL/2016) at 4 °C until analysis (12).

Determination of total amount of antioxidant compounds

Determination of the total phenolic content (TPC)

The total phenolic content (TPC) was determined by folin ciocalteu assay with slight modifications (13). The sample (1 ml) was placed into the test tube and 0.5 ml of Folin-Ciocalteu reagent was added. After five min, 1.5 ml of sodium carbonate (20%) added in the mixture, volume make up to 10 ml with deionized water and left for 30 min at room temperature. The deep blue colour indicated that presence of high phenolic compound in the samples. Absorbance measured at 750 nm wavelength using UV-visible, spectrophotometer (Elico Double beam SL-210, India). TPC, expressed as Gallic acid equivalents (GAE) in µg GAE/gm dry weight which calculated by the following equation:

$$C = \frac{c \times V}{m}$$

Where, C = total content of phenolic compounds, c = the concentration of GA established from the calibration curve, V = the volume of extract and m = the weight of crude plant extract.

Determination of the flavonoid content (FC)

For the determination of flavonoid content (FC) aluminium chloride colorimetric method was used as standard protocol (14). Each plant extract (0.5 ml) was diluted with 4 ml of deionized water and added 0.3 ml of sodium nitrate (5%). After 5 min later 0.3 ml of aluminium chloride (10%) was added and incubated for six min. After incubation 2 ml sodium hydroxide (1M) added in the mixture and final volume make with 10 ml deionized water and shaken well. Reaction mixture was incubated at room temperature for 30 min. Appearance of Orange yellowish colour was the positive indicator for the presence of flavonoid content. Absorbance measured with the help of the UV-visible, spectrophotometer (Elico Double beam SL-210, India) at 510 nm wavelengths. FC expressed as Quercetin equivalents (QUE) in µg QCE/gm dry weight and calculated by the following formula:

$$C = \frac{c \times V}{m}$$

Where, C = flavonoid content (FC), c = the concentration of quercetin established from the calibration curve, V = the volume of extract and m = the weight of crude plant extract.

In-vitro determination of free radical scavenging activity

Below described four methods were used with minor modifications for evaluation of free radical scavenging activity of selected plants. Inhibition percent of leaf extract calculated by the following formula:

$$Inhibition\,\% = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 = Absorbance of the blank, A_1= absorbance of extracts. However, the extract concentration provided 50% inhibition (IC_{50}) which calculated through interpretation of data as a linear regression analysis.

Determination of antioxidant activity by $DPPH^{\bullet}$ method

$DPPH^{\bullet}$ scavenging activity was measured by standard method (15). Plant extract (1 ml) mixed with 3 ml of $DPPH^{\bullet}$ (0.1 mM) and allowed reacting at room

Table 1. General description, utilization and specimen voucher number of selected folk medicinal plants.

Name of plant species		Family	Plant Habit	Specimen Voucher Number	Utilization
Botanical name	Local name				
Lippia alba (Mill.) N. E. Brown	Hanuman sund	Verbenaceae	Aromatic shrub	GGV/BOT/S/VER/DKS/420	Diabetes, hepatic disease, bronchitis, cardiovascular diseases. (32)
Annona squamosa L.	Sitafal	Annonaceae	Tree	GGV/BOT/T/ANA/DKS/050	In Epilepsy, Dysentery, worm infestation, Fever, malignant tumours, ulcers, as an abortifacients. (33)
Hyptis suaveolens (Poit.)	Ganga tulsi	Lamiaceae	Aromatic herb	GGV/BOT/H/LAM/DKS/250	Stimulant, carminative, parasitic skin disease, antibacterial, anti-malarial anti-inflammatory, anti-plasmodial, activities. (34)
Commiphora wightii (Arnott) Bhandari	Guggul	Burseraceae	Bushy tree	GGV/BOT/BT/BUR/DKS/130	Arthritis, inflammation, rheumatism, fatness and disorders of lipids metabolism. (35)
Millettia pinnata (L.) Panigrahi	Karanj	Fabaceae	Evergreen medium-sized tree	GGV/BOT/T/FAB/DKS/234	Anti-diarrheal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory and analgesic activities. (36)

Table 2. Effect of extracting solvents on the total phenolic content (TPC) and flavonoid contents (FC) of leaf extract of medicinal plant species

Plants name	Total Phenolic content (µg GAE/gm dry weight)			Flavonoid content (µg QCE/gm dry weight)		
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous
Lippia alba	$117.78{\pm}2.69_a^a$	$367.49{\pm}38.90_c^b$	$505.11{\pm}2.55_d^c$	$463.94{\pm}6.49_d^b$	$435.01{\pm}25.03_c^b$	$371.33{\pm}4.50_c^a$
Annona squamosa	$206.86{\pm}2.52_d^b$	$205.38{\pm}4.57_{ab}^b$	$96.22{\pm}5.85_a^a$	$256.08{\pm}1.66_a^b$	$230.59{\pm}1.19_a^a$	$265.73{\pm}7.98_b^b$
Hyptis suaveolens	$121.16{\pm}4.09_a^a$	$237.04{\pm}2.69_b^b$	$519.23{\pm}34.90_d^c$	$345.00{\pm}4.00_c^a$	$243.00{\pm}6.00_a^b$	$560.17{\pm}25.91_d^c$
Commiphora wightii	$153.17{\pm}0.30_b^a$	$167.46{\pm}3.52_a^b$	$186.74{\pm}3.41_b^c$	$289.99{\pm}2.70_b^a$	$256.37{\pm}1.17_a^b$	$146.33{\pm}3.06_a^b$
Millettia pinnata	$176.53{\pm}2.76_c^a$	$197.40{\pm}1.53_{ab}^b$	$280.61{\pm}1.50_c^c$	$347.47{\pm}4.50_c^a$	$288.81{\pm}3.71_b^b$	$289.67{\pm}5.03_b^c$

GAE= Gallic Acid Equivalent; QCE= Quercetin Equivalent, Data is represented in Mean± SEM;
Different Superscript alphabets in left to right show variation between solvents.
Different subscript alphabets in top to bottom in separate column show significant variation within plants.
Significance was calculated by post hoc ANOVA Tukey test at $p<0.05$.

temperature for 30 min in the dark. Absorbance measured at 517 nm after 30 min and converted into the percentage of antioxidant activity using the above-mentioned formula.

Determination of ferrous ion chelating activity

Estimation of Chelation of ferrous ions was carried out with the help of described standard method (15). Each plant extracts (1.5 ml) taken in separate test tubes and added 100 and 300 µl ferrous sulphate solution (2 mM) and Ferrozine (5 mM). This mixture were incubated for 10 min at room temperature and absorbance were measured at 562 nm using EDTA as a standard.

Determination of hydroxyl ($OH^{\bullet}$) radical scavenging activity

The Hydroxyl radical scavenging activity (HRS) of the leaf extracts was measured (16). In each test tube 1.5 ml of each diluted extract was mixed with 60 µl, 90 µl, 2.4 ml, 150 µl of $FeCl_3$ (1 mM L^{-1}), 1, 10-phenanthroline (1 mM L^{-1}), phosphate buffer (0.2 M, pH 7.8) and H_2O_2 (0.17 mM) respectively. All homogenized mixtures were incubated at room temperature for 5 min and then absorbance was measured at 560 nm.

Superoxide (O^{-}_2) radical scavenging activity

Superoxide radical scavenging activity was determined by the method of (17). First superoxide anion radicals generated in the 3.0 mL of Tris–HCl buffer (16 mM, pH 8.0) which contain 0.5 ml of nitro blue tetrazolium (0.3 mM) (NBT), 0.5 ml of NAD H (0.936 mM) solution. Plant extract (1.0 ml), 0.5 ml Tris–HCl buffer (16 mM, pH 8.0) were added in the above mixture. Reaction started after adding the 0.5 ml of PMS solution (0.12 mM) in the mixture. Finally mixture incubated for 5 min under florescent lamp light at 25 ^{0}C. Absorbance was measured at 560 nm against a blank (Tris–HCl buffer).

Statistical analysis

Results were recorded from triplicate observations and expressed as mean with standard deviation (± SD). Post hoc ANOVA Tukey test at $p<0.05$ was performed with the help of SPSS version 16.0 software. While graphical analysis carried out by the Sigma Plot 12.0 software.

Results

Data obtained from ethanolic, methanolic and aqueous leaf extracts showed significant results. Results from all parameters are described in below (Table 2 and 3).

Total phenolic content and flavonoid content

TPC and FC was predicted by Gallic acid (Y = 0.006x + 0.036, R^2 = 0.998) and Quercetin (Y = 0.005x + 0.115, R^2 = 0.981) calibration curves. TPC (96.22±5.85 to 519.23±34.90) were found significant in aqueous extracts from all plant except AS (all $p<0.05$). For FC ethanol was found optimum for LA (463.94±6.49), CW (289.99±2.70) and *MP* (347.47±4.50) while aqueous extract of both AS (265.73±7.98) and HS (560.17±25.91) was found significant for flavonoid content instead of ethanolic and methanolic extracts (all $p<0.05$, Table 2).

Antioxidant Assay

Free radical scavenging activity of selected plant leaf extracts revealed the concentration (10-100 µg/ml) dependent. Data of different antioxidant methods was plotted in the Fig. 1 and 2. Concentration (µg/ml) of Ascorbic acid (28.76±2.16) and EDTA (48.16±0.25) for 50% inhibition (IC_{50}) was recorded (Table 3).

DPPH$^{\bullet}$ radical scavenging activity

The scavenging effect of ethanolic (93.02±0.08µg/ml) and aqueous extract (90.64±0.35 µg/ml) of LA, AS (94.52±0.74 µg/ml, 93.78±0.56 µg/ml), CW (84.92±1.34 µg/ml, 84.92±1.34 µg/ml), MP (94.73±0.83 µg/ml, 85.11±0.57 µg/ml) and HS (97.02±0.17 µg/ml, 98.21±0.56 µg/ml) shows significant scavenging activity as compared to methanolic extract. It is reported as the best scavenger over others (Fig. 1 (IV), and Fig. 2 (II). Significant (P <0.05) differences between IC_{50} mean values of the extracts were observed (Table 3). Methanolic extract of CW for 50% inhibition was found to be 27.18±0.16 µg/ml. whereas, ethanolic and aqueous extract of CW was reported to be 38.08±0.61 µg/ml and 33.29±0.33 µg/ml respectively. Methanolic and aqueous extract of AS needed 28.81±0.04 µg/ml for 50% inhibition in comparison with ethanolic extract (42.02±0.58 µg/ml).

Ferrous ion chelating activity

In all the extract, significant result was found in case of ion chelation i.e. $p<0.05$. Both methanolic and ethanolic extract were found to be optimal as compared to aqueous extract. The highest chelation percentage reported from aqueous (88.798±0.32) followed by ethanolic (92.57±2.06) and methanolic (94.39±0.02,) extracts of LA. IC_{50} with minimum trapping activity was reported from the aqueous (47.06±0.48) and methanolic (42.88±0.18) extracts of LA as compare to EDTA (48.16±0.25). But IC_{50} for aqueous extracts of AS (27.72±8.95) and CW (27.4 ±0.27) which was found low from standard value (Table 3, Fig. 1I).

Hydroxyl (OH^{-}) radical scavenging activity

All extract showed significant ($p<0.05$) difference from each other. Results were reported from ethanolic extract of AS (95.28±0.31), methanolic extracts of LA (94.39±0.02) and aqueous extracts of AS and MP (89.00) which showed similar result. But IC_{50} of AS, LA and MP were reported higher than standard values (28.76±2.16) while, methanolic extract of HS was reported with lower IC_{50} (27.78±0.88 33) than standard values (Fig.1 (III).

Superoxide radical (O^{-}_2) scavenging activity

Result indicated an outstanding effect on superoxide scavenging. All extracts shows the significant ($p<0.05$) result for superoxide radical scavenging percentage. Ethanolic extract of AS (96.28±1.02) shows the highest percentage of inhibition followed by methanolic and aqueous extract of CW (95.36± 1.31 and 92.98±1.27) respectively. IC_{50} of ethanolic extract of AS (50.06±0.19) reported higher than the standard value that was ascorbic acid (28.76±2.16). But IC_{50} of ethanolic extract of MP was observed at 29.55±4.81 from other plant extracts but statistically all other three extracts of MP has not shown significant differences ($p>0.05$, Table 3).

Table 3. Result of IC_{50} values (in µg/ml) of the Methanolic, Ethanolic and Aqueous leaf extracts of five medicinal plant species

Methods	**Extract**	***Lippia alba***	***Annona squamosa***	***Hyptis suaveolens***	***Commiphora wightii***	***Millettia pinnata***
DPPH$^{\bullet}$ method (µg/ml)	Ethanolic	$36.21\pm1.44^{a}_{b}$	$42.02\pm0.58^{a}_{d}$	$38.34\pm1.24^{b}_{b}$	$38.08\pm0.61^{a}_{b}$	$30.02\pm0.68^{a}_{a}$
	Aqueous	$37.85\pm0.24^{b}_{a}$	$28.81\pm0.04^{a}_{a}$	$36.93\pm0.34^{b}_{a}$	$33.29\pm0.33^{b}_{a}$	$31.54\pm0.74^{b}_{a}$
	Methanolic	$35.41\pm0.11^{a}_{d}$	$28.81\pm0.04^{a}_{b}$	$31.48\pm0.04^{a}_{c}$	$27.18\pm0.16^{c}_{a}$	$27.30\pm0.03^{c}_{a}$
Ion chelating method (µg/ml)	Ethanolic	$56.38\pm0.54^{c}_{d}$	$50.64\pm0.23^{b}_{c}$	$36.98\pm0.10^{b}_{a}$	$42.61\pm0.43^{c}_{b}$	$41.40\pm0.74^{a}_{b}$
	Aqueous	$47.06\pm0.48^{a}_{c}$	$27.72\pm8.95^{a}_{a}$	$35.83\pm0.59^{a}_{ab}$	$27.41\pm0.27^{a}_{a}$	$41.35\pm0.41^{a}_{bc}$
	Methanolic	$42.88\pm0.18^{b}_{b}$	$59.00\pm2.88^{b}_{c}$	$46.92\pm0.35^{c}_{b}$	$31.38\pm0.27^{b}_{a}$	$46.85\pm0.13^{b}_{b}$
Hydroxyl radical scavenging activity (µg/ml)	Ethanolic	$33.36\pm0.47^{a}_{a}$	$63.17\pm14.85^{a}_{b}$	$41.75\pm0.49^{c}_{a}$	$31.62\pm0.53^{a}_{a}$	$38.39\pm0.69^{a}_{a}$
	Aqueous	$39.31\pm0.28^{b}_{a}$	$59.06\pm7.75^{a}_{b}$	$36.01\pm0.27^{b}_{a}$	$31.25\pm0.17^{a}_{a}$	$38.95\pm1.08^{a}_{a}$
	Methanolic	$42.92\pm0.14^{c}_{ab}$	$49.02\pm14.64^{a}_{b}$	$27.78\pm0.88^{a}_{a}$	$38.94\pm0.06^{b}_{ab}$	$41.23\pm0.25^{b}_{ab}$
Superoxide radical scavenging activity (µg/ml)	Ethanolic	$39.54\pm2.77^{ab}_{b}$	$50.06\pm0.19^{a}_{c}$	$47.76\pm1.02^{b}_{c}$	$54.49\pm1.11^{b}_{c}$	$29.55\pm4.81^{a}_{a}$
	Aqueous	$37.07\pm0.78^{a}_{a}$	$37.99\pm0.66^{b}_{a}$	$47.90\pm0.33^{b}_{c}$	$45.11\pm0.82^{a}_{b}$	$38.03\pm0.53^{a}_{a}$
	Methanolic	$42.74\pm2.41^{b}_{ab}$	$41.61\pm0.13^{c}_{ab}$	$33.23\pm0.72^{a}_{a}$	$52.60\pm0.12^{b}_{b}$	$42.33\pm10.37^{a}_{ab}$
Standard (µg/ml)	Ascorbic acid	28.76±2.16				
	EDTA	48.16±0.25				

Data is represented in Mean± SEM; same superscript alphabets in left to right show homogeneity between medicinal plants. Different subscript alphabets in top to bottom in separate column show significant variation within plants and different solvents methods. Significance was calculated by post hoc ANOVA Tukey test at $p<0.05$.

Discussion

There are varieties of phytocompounds found in medicinal herbs with antioxidant properties. Phenolics and flavonoids are mostly used in polymer

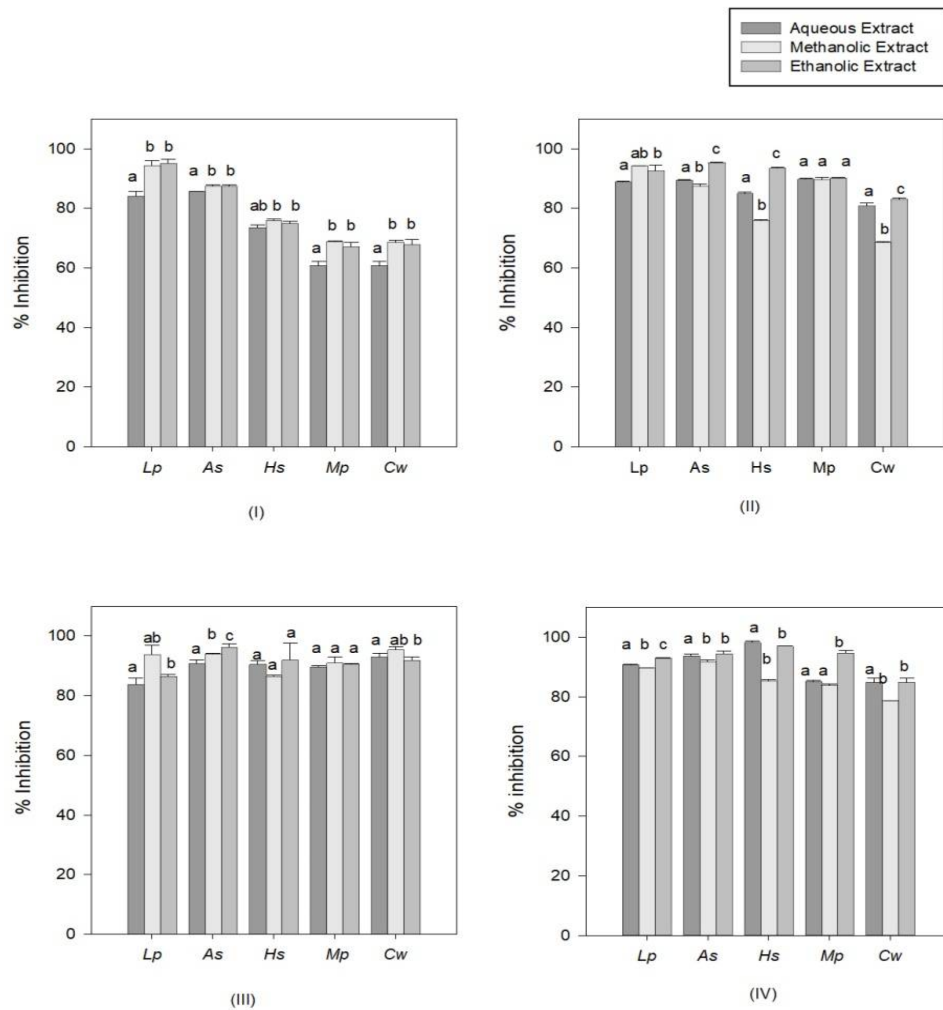

Fig. 1. Comparison of inhibition percentage between ethanolic, aqueous and methanolic leaf extracts of five plants; (I) Ion chelating activity; (II) Hydroxyl radical scavenging activity; (III) Superoxide radical scavenging activity; (IV) DPPH$^{\bullet}$ method; Same alphabets in separate plant show homogeneity, significance using post hoc ANOVA Tukey test at p<0.05.

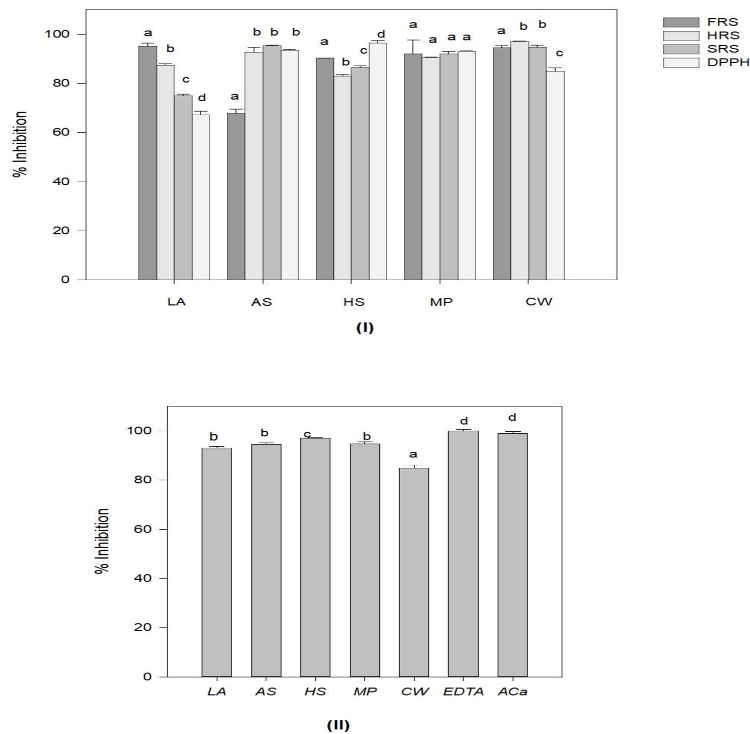

Fig. 2. Comparison between inhibition percentages; (I) between methods; (II) between plants. Same alphabets in separate plant show homogeneity, significance using ANOVA, Tukey test (Post hoc) set P value is *p<0.05*.

chemistry, biochemistry, and food chemistry, but tannins, Coumarins, xanthones, and procyanidins that depend on doses, have been promising as therapeutic drugs in free radical pathologies. Due to the presence of hydroxyl groups and phenolic rings, they have many other beneficial effects on human health (18- 20). The present study revealed that, the highest poly phenols (TPC, 519.23±34.90 & FC, 560.17±25.91) found in the aqueous extract of *H. sauveolans* which is more polar solvent from others two. During the study of selected Indian folk medicinal plants similar results were reported (21). Whereas the lowest TPC and FC from the aqueous extract of *A. squamosa, L. alba*, *C. wightii* and *M. pinnata* could be correlated to the responsiveness of folin reagent as well as chemical compositions of polyphenols in the solvents (22).

The FIC, HRS, SRS and DPPH$^{\bullet}$ quenching assays are most common methods for the evaluation of antioxidant activity of extracts. A comparable result was obtained for the antioxidant activity of ethanolic, methanolic, and aqueous leaf extracts of these five folk medicinal plant species. The data were presented in Fig. 2 (I). Results obtained in the present investigation shows similarity with previous studies (23) for DPPH$^{\bullet}$, ABTS and FRAP assays. These extracts disrupted the ferrozine Fe^{2+} complex as chelating agents and decreased the developed red colour of the complex with the increase in their concentration in the mixture which competes with Ferrozine for the ferrous ions (15). Our findings displayed a noticeable efficiency about ion binding capacity of *L. alba* which support the study from essential oil of two morph types of *L. alba* (24). The aim of ferrous ion chelating assay is to find out the oxidation catalytic ferrous ion potentiality of selected materials. It is reported that chelating agents work as secondary antioxidants because they oxidized the metal ion by reducing the redox potential. *L. alba* was reported most effective metal ion chelator from others. H_2O_2 is not a reactive species, but it can be made toxic by pre-existing hydroxyl radicals in the cells. It can disturb the pro-oxidant–antioxidant balance hence, elimination of H_2O_2 is important (25).

The leaf extract of five medicinal plant samples exhibited significant (p<0.05) hydroxyl radical scavenging percentage. Hydroxyl radical substitution by phenolic substances is known that's scavenging activity. And this stronger scavenging percentage was shown its maximum in *L. alba*, *A. squamosa* and *M. pinnata.* Out of which, *L. alba* was reported as best ferrous ion chelator and best hydroxyl radical scavenger. On the other hand, Polyphenols inhibits the hydroxyl radical formation and lipid per-oxidation that correlates to their iron chelating properties (26).

Statistically, ethanolic extract of *L. alba* was found as a powerful hydroxyl radical scavenger that's play a role of shield for hydroxyl radicals. Superoxide radicals are very harmful to cellular components as a precursor of the highly reactive oxygen species, which is responsible for the tissue damage and numerous pathological conditions (27). Findings reveals that all plant extracts have significant (p<0.05) and potential superoxide radicals scavenging percentage. High FC in extracts may contribute to the high superoxide radical scavenging potential as compared to TPC. Ethanolic and methanolic extracts could be dissolved most FC from *A. squamosa* in comparison with aqueous extract, which mainly dissolve in phenolic compounds. Similar results were reported from comparative study of Indian leafy vegetables and six medicinal plants respectively (28, 29). In the DPPH radical scavenging assay 98.21±0.56 % and 97.02±0.17 scavenging percentage reported from the aqueous and ethanolic extract of *H. sauveolens* that is indication of presence of highest amount of total phenolic content in this extracts. Lowest (83.86±0.55) antioxidant activity and total phenolic content was obtained from methanolic extract of *M. pinnata*. IC_{50} value of aqueous (36.93±0.34) and ethanolic (38.34±1.24) extracts was reported lower than the standard (28.76±2.16) which was Ascorbic acid. Comparable correlation was found between the total phenolic content and antioxidant activities from six medicinal plants and *C. fistula* during the study of antioxidants potential of their different parts (29, 30). Likewise flavonoids such as kaempferol, rutin, myricetin and quercetin also contributed as free radical scavenger (31-33). The data obtained from the present study revealed that, all selected folk medicinal plants significantly (p<0.05) followed the following trends, *i.e.* EDTA>ACa> HS>MP>AS>LA>CW for free radical scavenging activity (Fig. 2 (II).

Conclusion

The DPPH$^{\bullet}$, Ion chelating method, Hydroxyl radical scavenging and superoxide radical scavenging methods of ethanolic, aqueous and methanolic leaf extracts of five medicinal plants gave comparative results of antioxidant activities, confirms that all the five plants have significant capacity to reduce free radicals. As more polyphenols were found in aqueous extracts in comparison with ethanolic and methanolic leaf extracts of these plants, the values of different antioxidant assays and total phenolic content is more for aqueous extracts. Furthermore, this research shall be useful for future researchers working in the field of biologically active drug formulation of novel drugs from these folklore medicinal plants.

Acknowledgement

The authors are thankful to Department of Biotechnology (DBT- BUILDER), Government of India (BT/PR7020/INF/22/172/2012) for the financial support.

Authors' contributions

Concept and work plan was supervised by AKD and SD. All the analytical experiment was performed by JC and KO. The manuscript writing and statistical calculation were done by SD and RD. Necessary and final correction done by SV and AS.

Conflict of interest

Authors do not have any conflict of interest to declare.

References

1. Aslani BA, Ghobadi S. Studies on oxidants and antioxidants with a brief glance at their relevance to the immune system. Life Sciences. 2016 Feb 1;146:163-73. https://doi.org/10.1016/j.lfs.2016.01.014

2. Makari HK, Haraprasad N, Patil HS, Ravikumar S. *In vitro* antioxidant activity of the hexane and methanolic extracts of *Cordia wallichii* and *Celastrus paniculatus*. Internet J Aesthetic Anti-aging Med. 2008; 1:1-0. https://doi.org/10.5580/618

3. Dehariya R, Chandrakar J, Dubey S, Ojha K, Dixit AK. Scavenging and metal chelating potential of *Carthamus tinctorius* L. extracts. Current Botany. 2020 May 19:43-50. https://doi.org/10.25081/cb.2020.v11.6009

4. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford University Press, USA; 2015. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

5. Kahl R, Kappus H. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Zeitschrift fur Lebensmittel-untersuchung Und-forschung. 1993 Apr;196(4):329-38. https://doi.org/10.1007/BF01197931

6. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition. 2005 Jun 1;45(4):287-306. https://doi.org/10.1080/1040869059096

7. Walton NJ, Brown DE. Chemicals from plants: perspectives on plant secondary products, 1999. https://doi.org/10.1142/3203

8. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry. 2006 Jan 1;99(4):835-41. https://doi.org/10.1016/j.foodchem.2005.08.034

9. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010 Oct;15(10):7313-52. https://doi.org/10.3390/molecules15107313

10. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacologica Sinica. 2002 Dec 1;23(12):1127-41

11. Wink M, editor. Biochemistry of plant secondary metabolism. CRC Press; 1999 Sep 21

12. Jones Jr JB, Case VW. Sampling, handling and analyzing plant tissue samples. Soil Testing and Plant Analysis. 1990 Jan 1;3:389-427. https://doi.org/10.2136/sssabookser3.3ed.c15

13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965 Jan 1;16(3):144-58

14. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002 Jul 1;10(3)

15. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid per-oxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics. 1994;315(1):161-69. https://doi.org/10.1006/abbi.1994.1485

16. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry. 1981 Oct 1;20(21):6006-12. https://doi.org/10.1021/bi00524a013

17. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology. 1988 Mar 1;37(5):837-41. https://doi.org/10.1016/0006-2952(88)90169-4

18. Harborne JB, Herbert H. Phytochemical dictionary: a Handbook of Bioactive Compounds from Plants. London: Taylor and Francis;1993

19. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Tech Metall. 2005;40:255-60

20. Okpuzar J, Ogbunugafor H, Kareem GK, Igwo-Ezikpe MN. *In vitro* investigation of antioxidant phenolics compounds in extract of *Senna alata*. Res J Phytochem. 2009;3:68-76. https://doi.org/10.3923/rjphyto.2009.68.76

21. Sharma RK, Chatterji S, Rai DK, Mehta S, Rai PK, Singh RK, Watal G, Sharma B. Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants. J Med Plants Res. 2009 Nov 1;3(11):944-48

22. Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food and Chemical Toxicology. 2006;44(2):198-206. https://doi.org/10.1016/j.fct.2005.06.013

23. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 2006 Sep 1;19(6-7):669-75. https://doi.org/10.1016/j.jfca.2006.01.003

24. Joshi A, Prakash O, Pant AK, Kumar R, Negi MS. Chemical analysis and antioxidant activity of essential oils of two morpho types *of Lippia alba* (Mill.) N. E Br. ex Britton & P. Wilson (Verbenaceae). Journal of Essential Oil Bearing Plants. 2018 May 4;21(3):687-700. https://doi.org/10.1080/0972060X.2018.1486232

25. Shahat AA, Ibrahim AY, Alsaid MS. Antioxidant capacity and polyphenolic content of seven Saudi Arabian medicinal herbs traditionally used in Saudi Arabia. Indian Journal of Traditional Knowledge. 2015;14(1):28-35

26. Satoh K, Sakagami H. Effect of metal ions on radical intensity and cytotoxic activity of ascorbate. Anticancer Research. 1997;17(2A):1125-29

27. Yen GC, Duh PD, Tsai HL. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chemistry. 2002 Nov 1;79(3):307-13. https://doi.org/10.1016/S0308-8146(02)00145-0

28. Dasgupta N, De B. Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chemistry. 2007 Jan 1;101(2):471-74. https://doi.org/10.1016/j.foodchem.2006.02.003

29. Choudhary RK, Swarnkar PL. Antioxidant activity of phenolic and flavonoid compounds in some medicinal plants of India. Natural Product Research. 2011 Jul 1;25(11):1101-19. https://doi.org/10.1080/14786419.2010.498372

30. Luximon-Ramma A, Bahorun T, Soobrattee MA, Aruoma OI. Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in extracts of *Cassia fistula*. Journal of Agricultural and Food Chemistry. 2002 Aug 28;50(18):5042-47. https://doi.org/10.1021/jf0201172

31. Silva MM, Santos MR, Caroço G, Rocha R, Justino G, Mira L. Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radical Research. 2002 Jan 1;36(11):1219-27. https://doi.org/10.1080/198-1071576021000016472

32. De Albuquerque UP, De Medeiros PM, De Almeida AL, Monteiro JM, Neto EM, de Melo JG, Dos Santos JP. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: a quantitative approach. Journal of Ethnopharmacology. 2007 Dec 3;114(3):325-54. https://doi.org/10.1016/j.jep.2007.08.017

33. Chandrakar J, Dubey S, Ojha K, Dehariya R, Dixit, AK. *In vitro* antioxidant activity, total phenolic contents and phytochemical evaluation from crude extracts of an endangered plant *Cordia macleodii* Hook. f. & Thomson. Journal of Biomedical and Pharmaceutical Research. 2019 June; 8(3):111-22. https://doi.org/10.32553/jbpr.v8i3.620

34. Di Stasi LC, Oliveira GP, Carvalhaes MA, Queiroz-Junior M, Tien OS, Kakinami SH, Reis MS. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. Fitoterapia. 2002 Feb 1;73(1):69-91. https://doi.org/10.1016/S0367-326X(01)00362-8

35. Grassi P, Reyes TS, Sosa S, Tubaro A, Hofer O, Zitterl-Eglseer K. Anti-inflammatory activity of two diterpenes of *Hyptis suaveolens* from El Salvador. Zeitschriftfür Natur for schung C. 2006 Apr 1; 61(3-4):165-70. https://doi.org/10.1515/znc-2006-3-402

36. Yoganarasimhan SN. Medicinal Plants of India (Tamil Nadu), Vol II. Bangalore: Cyber Media. 2000;276

37. Sangwan S, Rao DV, Sharma RA. A review on *Pongamia pinnata* (L.) Pierre: A great versatile leguminous plant. Nature and Science. 2010; 8(11):130-39