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Antioxidants and free radicals scavenging activity of Medicinal Plants

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Abstract
Background: The medicinal plants have bioactive compounds which provide them unique properties against different diseases.
Objective: The aim of this study was to determine the antioxidant and free radicals scavenging activities of medicinal plants such as *Withania coagulans, Cestrum nocturnum, Cymbopogon citratus* and *Catharanthus roseus*.
Materials and methods: The antioxidant and free radicals scavenging property was evaluated using the 2, 2 Diphenyl-1-Picrylhydrazyl, Hydrogen peroxide scavenging, hydroxyl radicals scavenging, superoxide scavenging and reducing power assay methods. Moreover, the phytochemical analysis of each plants extract was determined using standard methods.
Results: The results of phytochemical analysis confirmed the proteins, phenols/tannins and flavonoids were presents in each plants extract. Moreover, saponins present in the *Withania coagulans and Cymbopogon citratus* extract while alkaloids present in the *Cymbopogon citratus and Catharanthus roseus* extract. The 2, 2 Diphenyl-1-Picrylhydrazyl results confirmed the each extract have the antioxidant property. The hydrogen peroxide scavenging activity confirmed the each extract has strong hydrogen peroxides scavenging activity. The Hydroxyl radicals scavenging activity confirmed the each extracts have strong hydroxyl radicals scavenging activity. The superoxide scavenging methods confirmed the each plant extract have strong superoxide scavenging activity. The reducing power assay results confirmed the each plant extracts has strong reducing activity.
Conclusions: The selected plant extract have strong antioxidant and free radicals scavenging properties. These property of plants extract due to the presence of bioactive compounds

Keywords: antioxidant, free radicals, *Withania coagulans*, *Cestrum nocturnum*, *Cymbopogon citratus* and *Catharanthus roseus*

Introduction
The free radicals are generated during immune system function and normal metabolic process [1]. The oxidation of cell forms the reactive oxygen species (ROS) and reactive nitrogen species (RNS) in many living organisms. ROS such as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are formed as the byproduct of biological reaction [2]. The free radicals have dual behavior such as deleterious and beneficial depends upon the concentration of free radicals present in the body [3]. *In vivo* generation of ROS play important role in cell metabolism (energy production, intercellular signaling, and phagocytosis) and *In vitro* synthesize ROS (sunlight, UV light, and chemical reaction) damage the DNA, proteins, and lipids [4]. The excess free radicals are responsible for the generation of various diseases such as atherosclerosis, cancer, diabetes, Parkinson, Alzheimer, cirrhosis, hypertension, and aging. The imbalance between reactive oxygen species and antioxidants leads to the oxidative stress. Oxidative stress damage the proteins, nucleic acid and lipids which responsible for the generation of various diseases [5]. The antioxidants neutralize the effect and action of free radicals and prevent the development of various diseases [6]. The antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage. The endogenous antioxidant (superoxide dismutase, thioredoxin, vitamin E, vitamin C, flavonoids and glutathione) present in the humans and protect the biomolecules from free radicals injury [7]. The natural antioxidants present in different parts of plants (leaves, fruits, roots, stem, and seeds) and this property occurs due to the presence of phytocompounds such as phenolic, flavonoids, alkaloids and tannins [8-9]. Recent investigations suggest the plant origin antioxidants with free radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process [10]. The medicinal plants (*Withania coagulans* fruits (WCF), *Cestrum nocturnum* leaves (CNL),

Cymbopogon citratus leaves (CCL) and *Catharanthus roseus* leaves (CRL)) have bioactive compounds which provide them unique properties against the diseases [11]. The WCF (Family, *Solanaceae)* is rich in steroidal lactones such as polyhydroxy C28, phenols tannins, carbohydrates etc. It has been reported that the WCF used as Anti-inflammatory, wound healing, Antifungal, Antibacterial, anthelmintic, Antimutagenic, anticarcinogenic, Antihyperglycaemic, Hypolipidaemic, free radical scavenging and cardiovascular effects [12]. The CNL (family, Solanaceae) have bioactive compounds such as sapogenin steroids tigogenine, smilagenin, and yuccagenine. The CNL have been used as anti-inflammatory, antioxidant, antinociceptive, antipyretic, larvicidal, antileishmanial, antihistaminic, antidiabetic, anti-proliferative, ulcerogenic and cytotoxic [13]. The CCL (family of Poaceae) flavonoids, lemonal or citral and phenolic compounds (luteolin, glycosides, quercetin, kaempferol, elimicin, catechol, chlorogenic acid and caffeic acid) which help in providing medicinal aids. The CCL have been used as antioxidants, antifungal and antimicrobial activities [14]. The CRL (family, Apocynaceae) have different Phyto compounds such as vincristine and vinblastine, ajmalicine. The CRL have been used as anti-plasmodium antibacterial, antifungal, antibiotics, antioxidant, wound healing and antiviral activities [15]. In our best of knowledge, there is no literature regarding the phytochemical analysis of medicinal plants and its antioxidant and free radicals scavenging activity. The present study was tried to check the presence of bioactive compounds of WCF, CNL, CCL and CRL which might provide the antioxidant and free radicals scavenging property to the plants.

Materials and methods

Materials

$AgNO_3$ (Sigma Aldrich, 99%), Plants sample [*Withania coagulans* fruits (WCF), *Cestrum nocturnum* leaves (CNL), *Cymbopogon citratus* leaves (CCL) and *Catharanthus roseus* leaves (CRL)], EDTA (Fisher, 98 %), Ascorbic acid (Himedia, 99 %), hydrogen peroxide (Fisher, 30 %), 2-Deoxyribose (SRL, 85%), Nitroblue tetrazolium (SRL, 99 %), Thiobarbituric acid (Himedia, 99 %), Nicotinamide Adenine Dinucleotide (SRL, 98%) and Phenazinemethosulphate (SRL, 99 %).

Methods

Preparation of plant extract

Plants sample (WCF, CNL, CCL and CRL) were collected from the Botanical Garden of Banaras Hindu University, Varanasi. The sample was dried in an oven at 40 0C for 7 days and powdered with the help of mortar and pestle. The each crude plants extract (5 % w/v) was prepared using 5 gram each sample in Erlenmeyer flask containing 100 ml deionized water and boiling for 5 minutes. Then each extract was filtered using Whatman no 1 filter. The each crude plants extract were collected and stored in a refrigerator for further study [16].

Phytochemical Analysis (Qualitative)

The phytochemical analysis was performed according to the Yadav *et al*; 2011 method [17].

Analysis of proteins

The each crude plant extracts (WCF, CNL, CCL and CRL) was mixed with 2 ml ninhydrin solution (0.2%) and the mixture boiled for a few minutes. Then the appearance of violet color confirmed the presence of proteins in the extract [17].

Analysis of carbohydrates

Molish reagent (2 ml) mixed with each crude plants extract (WCF, CNL, CCL and CRL) and shaken vigorously and added 2 ml H_2SO_4. The appearance of a violet ring at the interface confirmed the presence of carbohydrates [17].

Analysis of starch

Each crude plant extract (WCF, CNL, CCL and CRL) was mixed with 2 ml of iodine solution. The presence of blue/violet color indicated the presence of starch in the extract [17].

Analysis of phenols/tannins

Each crude plant extract (WCF, CNL, CCL and CRL) was mixed with 2 ml of FeCl3 (2%) solution. Then the appearance of blue-green or black color indicated the presence of phenols and tannins in the extract [17].

Analysis of flavonoids

Each crude plant extract (WCF, CNL, CCL and CRL) was mixed with 2 ml NaOH (2%) and the intense yellow color was observed. Then few drops of dilute HCl were added and the solution turned to colorless indicated the presence of flavonoids [17].

Analysis of saponins

Deionized water (5 ml) was mixed with each crude plants extract (WCF, CNL, CCL and CRL) and vigorously shaken. Then the formation of stable foam indicated the presence of saponins [17].

Analysis of glycosides

Each crude plant extract (WCF, CNL, CCL and CRL) was mixed with glacial acetic acid (5 ml). Then few drops of FeCl3 (2%) and conc. H2SO4 (2 ml) was mixed. The formation of a brown ring at the interphase indicated the presence of cardiac glycosides [17].

Analysis of Steroids

The each crude plant extract (WCF, CNL, CCL and CRL) was added with chloroform (2 ml). Then conc. H2SO4 (2 ml) and glacial acetic acid (2 ml) was mixed. The appearance of greenish color indicated the presence of steroids [17].

Analysis of terpenoids

The each 2 ml crude plants extract (WCF, CNL, CCL and CRL), chloroform (2 ml) and conc. H_2SO_4 (1.5 ml) was mixed. Then the appearance of reddish-brown color indicated the presence of terpenoids [17].

Analysis of quinones

The each crude plants extract (WCF, CNL, CCL and CRL) was mixed with 2 ml of NaOH. Then the appearance of blue-green or red color indicated the presence quinones [17].

Analysis of phlobatannins

The each crude plants extract (WCF, CNL, CCL and CRL) was mixed with HCl (1%) and boiled for few minutes. Then the appearance of red precipitate indicated the presence of phlobatannins [17].

Analysis of alkaloids

The each 0.2 gram plants sample (WCF, CNL, CCL and CRL) was mixed with 3 ml hexane, shaked and filtered. Then 5 ml HCl (2%) was added and heated for few minutes. Further, few drops of picric acid was mixed. The formation of yellow color precipitate indicated the presence of alkaloids [18].

Antioxidant property

The antioxidant property of plants sample (WCF, CNL, CCL and CRL) was analyzed using following methods.

2, 2-Diphenyl-1-Picrylhydrazyl method (DPPH)

DPPH method was performed according Keshari *et al* 2017 [16]. The free radicals scavenging activity of each plant extract (WCF, CNL, CCL and CRL) and standard vitamin C was analyzed using stable free radical DPPH. 1 ml each plant extract (WCF, CNL, CCL and CRL) (25-500 µg/ml in ethanol) was mixed with 4 ml DPPH (0.004 % in methanol) solution. Then solution was incubated at room temperature in the dark and absorbance was recorded at 517 nm. The antioxidant percentage was calculated using the formula.

$$\text{Antioxidant activity (\%)} = \frac{OD\ (c) - OD\ (s)}{OD\ (c)} \times 100$$

(Formula no. 1) [16]

Hydroxyl radical scavenging activity

The effect of plants sample on hydroxyl radicals was determined according to the Keshari *et al* 2017 [16]. 75 µl each plant sample (WCF, CNL, CCL and CRL) solution (50-250 µg/ml in methanol), 150 µl 2- deoxyribose (10 mM), 450 µl sodium phosphate buffer (200 mM, pH, 7.0), 150 µl H_2O_2 (10 mM), 150 µl FeSO4-EDTA (10 mM), and 525 µl distilled water were mixed in the test tubes. Then mixture was kept at 37°C for 4 hours. 750 µl TBA (1% in 50 mM NaOH solution) and 750 µl trichloroacetic acid (2.8%) was mixed to stopped the reaction. Then mixture was kept in the boiling water bath for 10 minutes and cooled by the tap water. Then the absorbance was recorded at 520 nm. The vitamin C was used as standard while methanol was used as blank. The hydroxyl radical scavenging activity was determined using the formula no.1.

Superoxide radical scavenging activity

This method used according to the Keshari *et al*, 2017 [16]. 200 µl plants sample (WCF, CNL, CCL and CRL) [100-500 µg/ml in methanol], 1 ml Tris-HCl buffer (16 mM, pH, 8), 1 ml NBT (50 µM), 1 ml NADH (78 µM), and 1 ml PMS (10 µM) solution were mixed. The solution was kept at 25°C in the incubator for 5 minutes and absorbance was recorded at 560 nm using UV-Vis spectrophotometer (Systronics, AU-2701). The vitamin C was used as standard antioxidant. The percentage of superoxide scavenging activity was determined using the formula no. 1.

Hydrogen peroxide radical scavenging activity

This method was performed according to Keshari *et al*, 2017 [16]. 100 µl each plants sample (WCF, CNL, CCL and CRL) [25- 250 µg/ml in 50 mM phosphate buffer, pH, 7.4] was mixed with 300 µl phosphate buffer (50 mM, pH,7.4) and 600 µl H_2O_2 solution (2 mM in 50 mM phosphate buffer) and vortexed. Then after 10 minutes the absorbance was recorded at 230 nm using UV- Vis spectrophotometer (Systronics, AU-2701). The phosphate buffer (50 mM, pH, 7.4) and vitamin C was used as blank and standard respectively. The hydrogen peroxide scavenging activity was determined using the formula no1.

Reducing power Assay

This method was performed using Anjali Soni *et al*, 2013 with slight modification [19]. In this method absorbance of the solution is increased as sample concentration increases. The increase in absorbance confirms the increase in the antioxidant activity. 1 ml each plant extract (WCF, CNL, CCL and CRL) [100-500 µg/ml] was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml $K_3Fe\ (CN)_6$ (1 %) and incubated at 50 °C for 20 minutes. Then 2.5 ml trichloroacetic acid (10 %) was added to the solution and centrifuged at 3000 rpm for 10 minutes. The 2.5 ml of this solutions was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$. Then absorbance was measured at 700 nm using UV-Vis spectrophotometer (Systronics, AU-2701). The vitamin C was used standard and reducing power was determined as vitamin C equivalent per 100 gm of dry sample.

Results & Discussion

Phytochemical analysis

The results indicated that the proteins, phenols/tannins, and flavonoids are presents and carbohydrates, glycosides, quinones, terpenes, steroids, phlobatannins and iodine absent in the WCF, CNL, CCL and CRL extract. Moreover, the saponins present in the WCF and CCL extract and alkaloids presents in the CCL and CRL extract (Table 1 & Table 2).

Table 1: Table shows the phytochemical analysis (Proteins, carbohydrates, phenols/ tannins, glycosides, saponins and flavonoids) of plants sample.

S.N.	Plant sample	Phytochemical analysis					
		Proteins	Carbohydrates	Phenols/tannins	Glycosides	Saponins	Flavonoids
1	*Withania coagulans*	+ve	-ve	+ve	-ve	+ve	+ve
2	*Cestrum nocturnum*	+ve	-ve	+ve	-ve	-ve	+ve
3	*Cymbopogon citratus*	+ve	-ve	+ve	-ve	+ve	+ve
4	*Catharanthus roseus*	+ve	-ve	+ve	-ve	-ve	+ve

Table 2: Table shows the phytochemical analysis (Quinones, terpenes, steroids, phlobatannins, iodine and alkaloids) of plants sample.

S.N.	Plant sample	Phytochemical analysis					
		Quinones	Terpenes	Steroids	Phlobatannins	Iodine	Alkaloids
1	*Withania coagulans*	-ve	-ve	-ve	-ve	-ve	-ve
2	*Cestrum nocturnum*	-ve	-ve	-ve	-ve	-ve	-ve
3	*Cymbopogon citratus*	-ve	-ve	-ve	-ve	-ve	+ve
4	*Catharanthus roseus*	-ve	-ve	-ve	-ve	-ve	+ve

DPPH
The results indicated the WCF, CNL, CCL and CRL extract have antioxidant property compared with vitamin C. The antioxidant property of each extract was evaluated in different concentration and observed that the CRL have least antioxidant property and maximum antioxidant activity was observed by WCF and CNL both compared with vitamin C The antioxidant property of each plants extract was increased when concentration of extract was increased (Figure 1).

Hydroxyl radicals scavenging
The results indicated that the CCL and CCL have maximum hydroxyl radicals scavenging and least activity was observed in CNL extract as compared with vitamin C. The hydroxyl radicals scavenging property of each plants was increased when concentration of extract was increased (Figure 2).

Superoxide scavenging activity
The results confirmed the maximum superoxide scavenging activity was present in the CNL and least activity was observed in the CCL extract as compared with vitamin C. the superoxide scavenging activity of each plant extract was increased when concentration of extract was increased (Figure 3).

Hydrogen peroxide scavenging property
The results indicated the maximum hydrogen peroxide scavenging activity was present in the CRL and CNL and WCF have approximately same hydrogen peroxide scavenging activity. The hydrogen peroxide scavenging activity of each plant was increased when the concentration of extract was increased (Figure 4).

Reducing power Assay
The results proved that the CNL and WCF have same reducing power assay and CRL and CCL have approximately same reducing power as compared with vitamin C. The reducing power assay of each plant extract was increased when the concentration of extract was increased (figure 5).

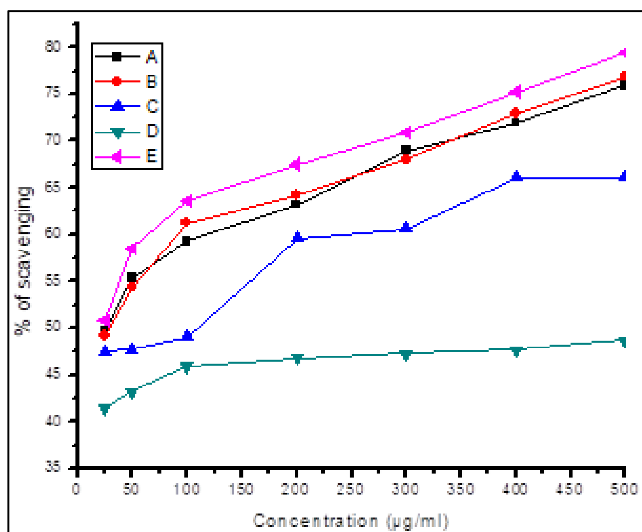


Fig 1: The image represents the antioxidant property of *Cestrum nocturnum* (A), *Withania coagulans* (B), *Catharanthus roseus* (C), *Cymbopogon citratus* (D) and Vitamin C (E).

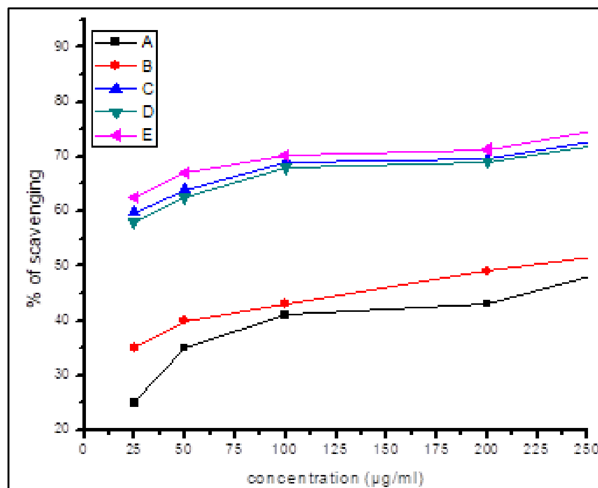


Fig 2: The image represents the hydroxyl radicals scavenging property of *Cestrum nocturnum* (A), *Withania coagulans* (B), *Catharanthus roseus* (C), *Cymbopogon citratus* (D) and Vitamin C (E).

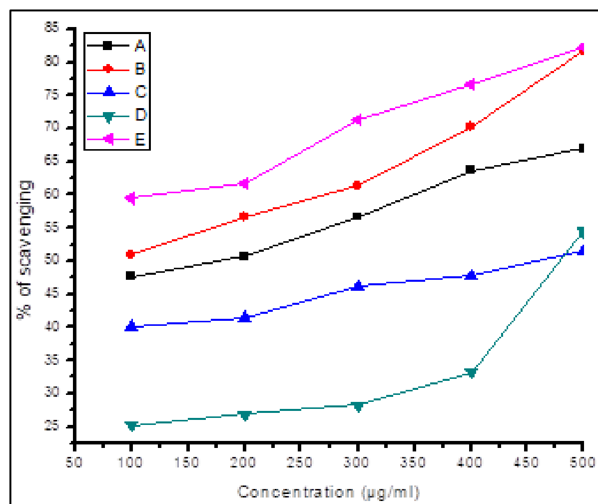


Fig 3: The image represents the superoxide radicals scavenging property of *Cestrum nocturnum* (A), *Withania coagulans* (B), *Catharanthus roseus* leaves (C), *Cymbopogon citratus* (D) and Vitamin C (E).

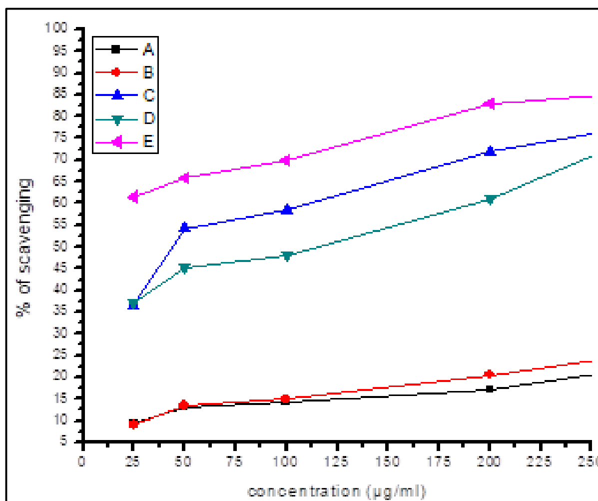


Fig 4: The image represents the hydrogen peroxide scavenging property of *Cestrum nocturnum* (A), *Withania coagulans* (B), *Catharanthus roseus* (C), *Cymbopogon citratus* (D) and Vitamin C (E).

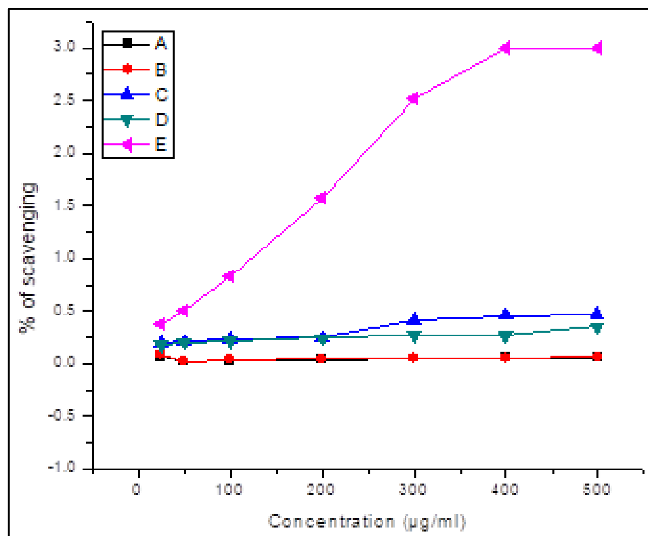
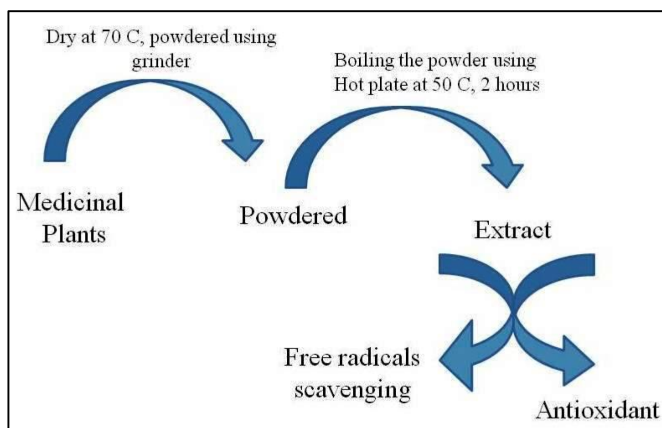


Fig 5: The image represents the reducing power assay of *Cestrum nocturnum* (A), *Withania coagulans* (B), *Catharanthus roseus* (C), *Cymbopogon citratus* (D) and Vitamin C (E).



Conclusion

The medicinal plants have bioactive compounds which provide unique property to the plants. The natural antioxidant presents in the plants such as *Cestrum nocturnum*, *Withania coagulans*, *Catharanthus roseus*, *Cymbopogon citratus* that can protect against oxidative stress. The antioxidant and free radicals scavenging property of *Cestrum nocturnum*, *Withania coagulans*, *Catharanthus roseus*, *Cymbopogon citratus* was determined using standard methods. The bioactive compounds such as proteins, phenols/tannins and flavonoids present in the *Cestrum nocturnum*, *Withania coagulans*, *Catharanthus roseus*, *Cymbopogon citratus* extract which provide them antioxidant property and benefited against the oxidative stress. The DPPH, Hydroxyl radicals, superoxide scavenging and reducing power assay confirmed the each plant have antioxidant and free radicals scavenging property.

Conflict of interest

The authors of this work were declared that the no conflict of interest.

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