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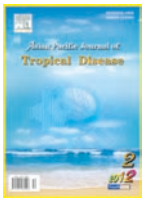

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In-vitro antioxidant activity of crataeva magna lour. dc bark extract

Sridhar N[1], Manisha Mishra S[2] , Venkataraman S[3]

[1]*Research Scholar, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Tanjore 613 401. Tamilnadu.*
[2]*Asst. Professor, Amity University, Lucknow, U.P.*
[3]*Research Director, C.L.Baid Metha College of Pharmacy & Research Foundation, Thorapakkam, Chennai – 600 096, Tamilnadu.*

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ABSTRACT

The plant *Crataeva magna* belonging to family Capparaceae is used in anti spasmodic, hypotensive, anti-inflammatory, hypoglycemic, anti protozoal, analgesic purposes. The present study was carried out to evaluate appropriate animal model. The antioxidative potential of different solvent extracts of *Crataeva magna* were evaluated using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), 2,2′-Azino-Bis(3-ethylbenzthiazoline-6-Sulphonic acid) (ABTS), superoxide radical, hydroxyl radical, nitric oxide radical scavenging activities and lipid peroxidation inhibition assay. Among those solvent extracts, ethanolic extract of C. magna exhibited highest level of antioxidant activities. The ethanolic extract also inhibited H_2O_2 mediated haemolysis and lipid peroxidation in human RBC.

1. Introduction

In living systems, free radicals are generated as part of the body′s normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection and even fasting, can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of diabetes[1-4]. Free radical damage may lead to cancer. Antioxidants interacts with radicals and may prevent some of the damage by free radicals. Laboratory evidence from chemical, cell culture and animal studies indicate that antioxidants may slow or possibly prevent the development of cancer[5]. Erythrocytes (RBC) have been extensively used to study oxidative damages. The Red Blood Cell (RBC) is unique among cells in that it combines very large concentrations of both iron (haemoglobin) and oxygen. This potentially dangerous combination of oxygen and iron within the RBC makes it a powerful promoters of oxidative processes are extremely susceptible to oxidative damages to poly unsaturated fatty acids of their membranes [6].

Methods

Plant Material: The bark part of plant *Crataeva magna* was collected from young matured plant from Alagar Koil, Madurai, Tamilnadu during the month of Nov-Dec and identified by the botanist of Department of Botany, American College, Madurai by comparing with the voucher specimen present in the herbarium. After authentication fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder were then extracted firstly with petroleum ether

*Corresponding author:Sridhar N, M.Pharm., Research Scholar,CARISM, SASTRA University, Tanjore – 613 401
E-Mail ID: Sridhar_tnr@yahoo.co.in
Cell: 09944914880

then with chloroform, ethyl acetate, ethanol and water using a soxhlet apparatus. The extracts thus obtained were used to check the antioxidant activities.

Chemicals: 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) and 2,2′-Azino-Bis (3-ethylbenzthiazoline-6-Sulphonic acid) (ABTS), Gossypin, Nitroblue Tetrazolium (NBT), naphthylethylene diamine dihydrochloride, Thiobarbituric Acid (TBA) and potassium persulfate. HPLC grade acetonitrile and water were purchased from Merck (India). All other reagents were used of analytical quality.

The extracts of C. magna in petroleum ether, chloroform, ethyl acetate were dissolved in minimum volume of DMSO and made to desired concentration with distilled water. Double distilled water is sued for dissolving ethanol and water extracts. But for the DPPH assay all the extracts were dissolved in methanol.

DPPH free radical scavenging activity

The DPPH assay measures hydrogen atom (or) one electron donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is a purple colored stable free radical it becomes reduced to the yellow colored diphenyl picryl hydrazine. A methanol and petroleum ether DPPH-solution (0.15%) was mixed with serial dilutions (1 to 50 μg/ml) of extracts and shaken vigorously. The tubes were allowed to stand at 27 ℃for 15 min. The change in absorbance of samples was measured at 517 nm using a UV spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage [7].

The hydroxyl radical scavenging activity was determined according to the method reported by Klein and co-workers. Various concentrations (5,10, 20, 40, 60, 80 and 100 μg /ml in methanol and petroleum ether) of extracts were taken in different test tubes and evaporated to dryness. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80 ℃-90 ℃ for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and made up to 1L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank [8]. Lipid peroxidation by thiobarbituric acid (TBA) assay. TBA reacts with malondialdehyde to form a pink chromogen, which can be detected spectrophotometrically at 532 nm. Normal albino rats of the Wistar strain were used for the preparation of liver homogenate. The perfused liver was isolated and 10% w/v homogenate was prepared with 0.15 M KCl. The homogenate was centrifuged at 1000 g for 15 min, and clear cell free supernatant obtained was used for the study of in vitro lipid peroxidation. Different concentrations (5,10, 20, 40, 60, 80 and 100 μg/ml in methanol and petroleum ether) of extracts were taken in test tubes and evaporated to dryness. One milliliter of 0.15 M KCl and 0.5 mL of rat liver homogenate were added to the test tubes. Peroxidation was initiated by 100 μl of 0.2 M ferric chloride. After incubation at 37 ℃ for 30 min, the reaction was terminated by adding 2 ml of ice-cold HCl (0.25 M) containing 15% trichloroacetic acid, 0.38% TBA, and 0.5% butylated hydroxytoluene (BHT). The reaction mixture were heated at 80 ℃ for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatant was measured at 532 nm. An identical experiment was performed to determine the amount of lipid peroxidation obtained in the presence of inducing agents without any extract. The percentage inhibition was calculated [9].

Statistical analysis

All the experiments data's subjected to statistical analysis using Mean±SEM and one-way ANOVA.

Results

In vitro antioxidant methods used for the evaluation of antioxidant activities of C. magna extracts includes, the DPPH radical scavenging action, hydroxyl scavenging and the lipid peroxidation assay for antioxidant activity were used to assess the antioxidant properties of methanolic and petroleum ether extracts of *crataeva magna*. All these three tests shows a better assessment of antioxidant activity.

Hydroxyl radical scavenging activity

Extracts	Concentration (μg ml)	%Hydroxyl Scavenging activity±SEM	Vitamin C
1.*Crataeva magna*	05	1.01±0.1	10.21±1.0
Methanolic	10	2.41±0.2	19.31±1.0
Extract	20	6.81±0.5	30.59±1.3
	40	10.23±1.3	38.13±1.4
	60	15.23±1.5	60.22±2.0
	80	20.20±1.4	67.10±1.2
	100	9.85±2.1	279.20±1.1
2.*Crataeva magna*	05	8.01±0.1	10.21±1.0
Petroleum ether	10	20.41±0.2	19.31±1.0
Extract	20	38.73±1.3	30.59±1.3
	40	41.23±1.3	38.13±1.4
	60	56.10±1.5*	60.22±2.0
	80	61.41±1.9*	67.10±1.2
	100	68.27±2.0*	79.20±1.1

*P<0.05, Significance Vs Standard (n=3).

Table 3

Lipid peroxidation assay

Extracts	Lipid peroxidation % inhibition±SEM (*n*=3)						
	05 μg/ml	10 μg/ml	20 μg/ml	40 μg/ml	60 μg/ml	80 μg/ml	100 μg/ml
Methanolic	1.17±1.0	09.37±1.3	17.57±1.4	21.51 ±1.3	27.11±1.2	30.13±2.1*	25.27±1.1*
Petroleum Extract	3.17±1.0	6.61±1.5	11.70±1.1	18.51±1.3	23.15±2.0	29.33±2.0*	38.13±2.0*
Vitamin C	3.11±1.1	12.11±1.3	17.25±0.1	20.82±1.1	29.22±2.4*	35.57±1.2*	32.15±0.2*

Discussion

Antioxidant potential of Methanolic extract at different concentrations was tested by the DPPH method. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to 1, 1-diphenyl-2-picrylhydrazine. The discoloration shows the free radical scavenging potentials of the antioxidant extract.

The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades with the hope of finding an effective remedy for several present day diseases and means to delay aging symptoms [10]. The disorders related to the excessive oxidation of cellular substrates (oxidative stress) include type-II diabetes, neuro degenerative diseases, and some types of cancer [11]. Secondary metabolites from medicinal plants function as small molecular weight antioxidants through direct antiradical, chain-breaking of the free radical propagation & interaction with transition metals. Other mechanisms include the inhibition of ROS-generating enzyme such as xanthine oxidase, inducing nitric oxide synthase, and improving the endogenous cellular antioxidant mechanisms such as the up-regulation of the activity of SOD [12]. It is known that free radical cause auto-oxidation of unsaturated lipids in food [13]. On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolics hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid [14]. Polyphenolic compounds such as, Flavonoids, Phenolic acids and Tannins are considered to be major contributors to the antioxidant activity. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agent, hydrogen donators and singlet oxygen quenchers[15]. The results showed that the Methanolic extract of *Crataeva magna* was active in the DPPH test with an IC_{50} value of 18.12±0.24 μg/ml and Petroleum ether extract with IC_{50} value of 26.31±0.41(Table 1). The hydroxyl radical scavenging activity of the pet ether extract showed maximum activity 68.27±2.0 at 100 μg/ml (Table 2). In the lipid peroxidation assay the methanolic extract showed the good effectiveness and inhibition values of 25.27±1.1% at 100 μg/ml (Table 3).

DPPH radical scavenging assay

The IC_{50} value is the amount of extract needed for scavenging 50% of the radical produced in the reaction mixture. DPPH radical scavenging assay shows that, the *Crataeva magna* methanolic extract IC50 Values (μg mL-1) are 18.12±0.24*, *Crataeva magna* petroleum ether extract IC50 Values (μg mL-1) are 26.31±0.41.

Conflict of interest statement

We declare that we have no conflict of interest.

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