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Original Research Article

In Vitro Antioxidant Activity of *Abelmoschus manihot* (L.) Medik. Roots

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Abstract

This study is an attempt to evaluate antioxidant potential of *Abelmoschus manihot* (L.) Medik (Malvaceae), an important medicinal plant in the Indian system of medicine. The various extracts of *A. manihot* roots includes petroleum ether, methanolic ethyl acetate soluble and ethyl acetate insoluble fraction of methanolic extracts and aqueous extract was evaluated by qualitative preliminary phytochemical test. Further study was designed to screen antioxidant potential of various extracts of *A. manihot* roots by determining Nitric oxide (NO) radical, Superoxide (SO) radical, Hydrogen peroxide (OH) radical scavenging and anti-lipid peroxidation activity. Ascorbic acid was used as a standard drug. In present study, free radical scavenging activity of various extracts of *A. manihot* roots was revealed that *A. manihot* roots has significant antioxidant potential when compared with standard. Our finding suggested that among comparative significance of various extracts, the methanolic extract of *A. manihot* roots having better efficacy and significant activity. The present study enlighten the antioxidant potential of this plant and also helps to support the traditional medicinal claim and believes of this plant in therapeutics and highlighted profound potential of *A. manihot* roots to be investigated for bioactive compounds responsible for antioxidant effect.

Keywords

Abelmoschus manihot
Antioxidant effect
Free radicals
Phytochemicals

Introduction

A large annual erect hairy herb or undershrub, represented by *Abelmoschus manihot* (*A. manihot*) (L.) Medik. (Malvaceae) Synonym: *Hibiscus manihot* Linn is medicinally important plant of the Malvaceae family commonly known as '*Jangali bhendi*' is a 1.2-1.8 m. high, commonly found on the Kokan, Western Ghats

and Western coasts of India (Anonymous, 2005). Roots of this plant having yellowish brown color with 3-6 cm long, wavy shape (Kirtikar and Basu, 2005). In the folk medicine, this plant has been known since ancient times for its curative properties and has been utilized for treatments of various ailments such as bark is considered as an emmenagogue and used to treat wounds and cuts (Kirtikar and Basu, 2005; Manandhar, 1993). Root paste and leaves useful for boils, sores, sprains, inflammations, tuberculosis and leucoderma (Manandhar, 1993; Chopra, 2001; Sharma and Mishra, 2009; Patil and Bhaskar, 2006). The juice of the flowers is used to treat chronic bronchitis and toothache (Manandhar, 2002). The leaves of this plant reported for antinflammatory activity (Jain and Bari, 2010; Winter et al., 1962). Leaves showed bone-sparing effect (Puel, 2005). Flowers reported as a neuroprotective and antiviral (Cheng et al., 2006; Lin-lin et al., 2007). Stems reported for wound healing activity (Jain and Bari, 2010). Roots of this plant reported for larvicidal activity (Virendra et al., 2006).

A review has mentioned the phytochemical properties of different parts constituted a wide range of chemical compounds such as stems of *A. manihot* reported for presence of stigmasterol and γ- sitosterol (Jain and Bari, 2009). Flowers are reported for hyperoside and flavanoids includes hibifolin, quercetin and myricetin derivatives (Lai et al., 2009). The literature survey and screening of scientific data revealed that although *A. manihot* roots are traditionally used in the treatment of various diseases for long time, preliminary screening has been done. The present investigation include free radical scavenging activity and anti-lipid peroxidation testing of *A. manihot* roots is therefore taken up to evaluate antioxidant potential of this plant.

Materials and methods

Plant material

The plant, *A. manihot* roots was collected in Trimbakeshwar Hills, Nashik District (Maharashtra) in May 2008. The plant was authenticated and herbarium deposited in Botanical Survey of India, Pune, Maharashtra, India under voucher specimen number CDSAM3 (No.BSI/WC/Tech/2008/164). The roots of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

Extraction of plant material

The air-dried roots of *A. manihot* were made into a coarse powder. The powdered material was defatted with petroleum ether. The defatted material was successively extracted with solvents i.e. methanol, ethyl acetate and distilled water by successive solvent extraction method using Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate by using separating funnel. Fraction was obtained as an ethyl acetate soluble and ethyl acetate insoluble. Then the extract was filtered through muslin cloth and the filtrate was evaporated under reduced pressure and vacuum-dried. The remaining marc after methanolic extraction was further macerated with distilled water for 48 h with intermittent shaking (Mukherjee, 2008).

Preliminary phytochemical analysis

The qualitative chemical test of various extracts of *A. manihot* was carried out using standard procedure (Kokate et al., 2009).

In- vitro antioxidant activity

Nitric oxide radical scavenging activity

The reaction mixture (3ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the drug in different concentrations (10-100 µg/ml) was incubated at 25^0C for 150 min. At intervals samples (0.5 ml) of incubation solution were removed and 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test compounds, whereas ascorbic acid was taken as standard. The % of inhibition was calculated by using equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] x 100 (Sreejayan and Rao, 1997; Acharya et al., 2004).

Superoxide anion radical scavenging activity

Superoxide radical (O_2^-) was generated from auto-oxidation of hematoxylin and was detected by an increase in absorbance at 560 nm in a spectrophotometer. The reaction mixture contains 0.1 M of phosphate buffer (pH-7.4), EDTA (0.1mM),

hematoxylin (50µM) and incubated at 25ºC for different time periods. Inhibitions of auto-oxidation of hematoxilin by crude, boiled extracts over the control were measured. The absorbance at 560 nm is measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] × 100 (Martin et al., 1987; Kono, 1978).

Hydrogen peroxide radical scavenging activity

All solutions were prepared freshly. A Solution of Hydrogen Peroxide (20 mM) was prepared in Phosphate buffer Saline (PBS) (pH 7.4).Various concentration of the extract or standard in methanol (1 ml) were added to 2 ml of Hydrogen Peroxide Solution in PBS. After 10 min the absorbance was measured at 230nm. After cooling, the absorbance was read at 230 nm against a blank (containing only buffer and deoxyribose). The absorbance read at the end of the experiment was used for the calculation of the % inhibition. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] × 100 (Shirwaikar and Somashekar 2003).

Thiobarbituric acid-reactive substance (TBARS) assay

The peroxide formation was measured in the reaction mixture contained rat liver homogenate (0.1 ml, 25%, w/v) in Tris-HCl buffer (20 mM, pH 7.0), KCl (150 mM), ferrous ammonium sulphate (0.8 mM), ascorbic acid (0.3 mM) , was incubated for 1 hr at 37 0C (14; 15). The incubated reaction mixture (0.4 ml) was treated with 0.2 ml of 8% sodium dodecyl sulphate (SDS), thiobarbituric acid (1.15 ml, 8%) and acetic acid (1.5 ml, 20%, pH 3.5). The total volume was then made upto 4 ml by adding distilled water and kept in a water bath at 100 0C for 1 hr. After cooling, 1ml of distilled water and 5ml of a mixture of n-butanol: pyridine (15:1 v/v) was added and shaken vigorously. The absorbance of the organic layer was measured at 560 nm using UV-Visible spectrophotometer after centrifugation. The % inhibition of lipid peroxide formation was determined by comparing the results of the extract and control samples. The % of inhibition was calculated by using following equation: % Inhibition= [(Absorbance control – Absorbance sample)/Absorbance control] × 100 (Ohkawa et al., 1979; Tripathi and Pandey, 1999).

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test. The values are expressed as mean ± SEM and $P<0.05$ was considered significant.

Results and discussion

Extractive values and preliminary phytochemical analysis

The extractive values of petroleum ether, chloroform, ethyl acetate and methanolic and aqueous extract were found to be 3.8%, 3.3%, 4.2%, 7.0%, 8.3% w/w respectively. Preliminary phytochemical analysis of various extracts of *A. manihot* roots revealed the presence of carbohydrates, glycosides, flavonoids, tannins, steroids and proteins.

In- vitro antioxidant activity

The results of the present study on *in vitro* antioxidant activity of *A. manihot* roots are given in Table 1 and the respective IC_{50} values are provided in Table 2. Antioxidants, such as phenolic compounds including flavonoids, chalcones, lignoids, stylbenoids, tannins, and diarylheptanoids, are distributed in the plant kingdom and may prevent oxidative damage by scavenging ROS. Therefore, the phenolic constituents of plants are of interest as potential chemo preventive agents, and plants may be an attractive alternative to currently available commercial antioxidants, because they are biodegradable to non-toxic products (Shirwaikar et al., 2004). In the present study, results of preliminary phytochemical analysis stated that the various extracts of *A. manihot* roots contains phenolic compounds such as tannins, flavonoids indicated plant extract seems to be showed antioxidant capacity.

Antioxidant screening of various extracts of *A. manihot* roots showing nitric oxide radical scavenging activity is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be

estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions (Acharya et al., 2004).

The various extracts of *A. manihot* roots showing hydroxyl radical scavenging potential. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. Ferric-EDTA was incubated with H_2O_2 and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen upon heating with thiobarbituric acid (TBA) at low pH. When the test extracts were added to the reaction mixture, they removed hydroxyl radicals from the sugar and prevented their degradation (Rai et al., 2009). TBARS were determined by an indicator phospholipid peroxdation and a measure of the extent of DNA and deoxribo damage (Sahoo et al., 2008). Measuring the color of thiobarbituric acid reactive substances (TBARS) formed at the end of the reaction. Malonaldehyde (MDA) which is formed as end product in lipid peroxidation react with thiobarbituric acid (TBA) to give TBARS which is pink in color (Chun-Ching et al., 1998).

Table 1. *In vitro* antioxidant activity of *A. manihot* roots extracts.

Group	Treatment (Conc. µg/ml)	Inhibition (%)			
		NO radical scavenging	SO radical scavenging	Hydrogen peroxide radical scavenging	Anti-lipid peroxidation
I	Petroleum ether extract	0.3615 ± 0.009**	0.4255 ± 0.012**	0.3575 ± 0.009ns	0.5066 ± 0.008*
II	Methanol extract	0.3663 ±0.010**	0.4992 ± 0.015**	0.3618 ± 0.012**	0.4989 ±0.011**
III	Ethyl acetate soluble extract	0.6285 ±0.016**	0.6051 ± 0.018***	0.6324 ± 0.016**	0.6017 ±0.015ns
IV	Ethyl acetate insoluble extract	0.5113 ±0.02**	0.4478 ± 0.017**	0.5245 ± 0.02**	0.5491 ±0.02ns
V	Aqueous extract	0.4568 ±0.024**	0.3756 ± 0.021*	0.4562 ± 0.024**	0.4964 ±0.023ns
VI	Standard Ascorbic acid	0.2562 ±0.029***	0.2050 ± 0.024***	0.2520 ± 0.028**	0.2760 ±0.028**

Values are expressed as mean ± SEM, n=6; When Group (VI) compared with Group (I, II, III, IV and V); *P<0.05, **P<0.01, ***P<0.001, ns- non significant.

Table 2. Data showing IC 50 values of various extracts of *A. manihot* roots.

Group	Treatment (Conc. µg/ml)	IC 50 values			
		NO radical scavenging	SO radical scavenging	Hydrogen peroxide	Anti-lipid peroxidation
I	Petroleum ether extract	65	121	187	60
II	Methanol extract	65	121	187	60
III	Ethyl acetate soluble extract	65	121	187	60
IV	Ethyl acetate insoluble extract	65	121	187	60
V	Aqueous extract	65	121	187	60
VI	Standard Ascorbic acid	65	121	187	60

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH which convert active oxygen molecules into non-toxic compounds. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidation mechanisms to inhibit the chain reaction of lipid peroxidation (Constantin et al., 1990).

The various extracts of *A. manihot* roots showed significant inhibition and free radical scavenging potential when compared with standard ascorbic acid and simultaneously reduced lipid peroxidation which revealed that significant decrease in MDA level in extracts groups. Among the various extracts of *A. manihot* roots, methanolic extract showed significant inhibition and better anti-lipid peroxidation property. Hence the results are comes to suggested that among the various extracts of *A. manihot* roots, methanolic

extract is better efficacious and having significant antioxidant potential.

Thus, it can be concluded that, present study gives some scientific evidences on effect of extraction solvents was made to find out the therapeutically better efficacious extract. Therefore, after screening among comparative significance of various extracts, the methanolic extract of *A. manihot* roots having better efficacy and significant antioxidant activity. The present study also support the traditional believes of this medicinal plant and highlighted profound potential of *A. manihot* roots to be investigated for bioactive compounds responsible for hepatoprotective and antioxidant effect.

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