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**EVALUATION OF *ABELMOSCHUS MANIHOT* (L.) MEDIK FLOWERS FOR
FREE RADICAL SCAVENGING ACTIVITIES USING IN VITRO ASSAYS**

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ABSTRACT

Present investigation includes evaluation of free radical scavenging potential of *Abelmoschus manihot* (*A. manihot*) (L.) Medik (*Malvaceae*), an important traditional medicinal herb in the Indian system of medicine. The dried powdered material of *A. manihot* flowers macerated to prepare total methanolic extract and solvent extraction technique prepared various fractions such as n-hexane fraction, ethyl acetate fraction and acetone fraction. Experimental design to screen extract and various fractions for its free radical scavenging potential 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. The results were revealed that *A. manihot* flowers have significant antioxidant potential when compared with standard. Our finding suggested that, the ethyl acetate fraction of *A. manihot* flowers having better efficacy and significant antioxidant activity. This work enlightens the antioxidant potential of this

plant and also helps to support the traditional medicinal claim and believes of this plant in therapeutics.

Keywords: *Abelmoschus manihot*, Malvaceae, *Jangli bhendi*, Free radicals, antioxidant effect

INTRODUCTION

The plant, *Abelmoschus manihot* (*A. manihot*) (L.) Medik. (*Malvaceae*) Synonym: *Hibiscus manihot* Linn is medicinally important plant of the Malvaceae family commonly known as 'Jangali bhendi'. A large annual erect hairy herb or undershrub of height upto 1.2-1.8 m., commonly found in Kokan, Western Ghats and Western coasts of India [1]. Flowers having yellowish brown color, erect, 3-6 cm long with small scattered prickles [2]. In the traditional medicine, this plant anciently utilized for treatments of various ailments such as bark is useful as an emmenagogue and also recommended to treat wounds and cuts [2, 3]. Leaves and root paste useful for the treatment of boils, sores, sprains, inflammations, tuberculosis and leucoderma [3 - 6]. The flowers juice is used to treat chronic bronchitis and toothache [7]. The leaves of this plant reported for antiinflammatory activity [8, 9]. Leaves showed bone-sparing effect [10]. Flowers reported for various pharmacological actions such as

neuroprotective and antiviral [11, 12]. and larvicidal activity [13]. Stems reported for wound healing activity [14].

A review has mentioned the phytochemical properties of different parts constituted a wide range of chemical compounds such as flowers of *A. manihot* reported for presence of stigmasterol and γ - sitosterol [15]. Flowers are reported for hyperoside and flavanoids includes hibifolin, quercetin and myricetin derivatives [16]. The literature survey and screening of scientific data revealed that although *A. manihot* flowers are traditionally used in the treatment of various diseases for long time, preliminary screening has been done. The present investigation therefore taken up to evaluate *A. manihot* flowers for testing of free radical scavenging potential and antioxidant property of this plant.

MATERIALS AND METHODS

Plant material

The plant, *A. manihot* flowers was collected in Trimbakeshwar Hills, Nashik

District (Maharashtra) during the month of September. The plant was authenticated and identified at Botanical Survey of India, Pune, Maharashtra, India. The flowers 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 dried powdered material of flowers (500 g) was extracted with methanol at room temperature by cold maceration techniques with shaking and stirring. Three macerates prepared and combined to get total methanolic extracts. The extract was evaporated to dryness under reduced pressure below 40°C. The some portion of total methanolic extract was further fractionated by solvent-solvent with n-hexane, ethyl acetate and acetone in the order of their increasing polarity to obtain respective fractions. The fractions were concentrated under vacuum and dried in desiccators [17].

Preliminary phytochemical analysis

The qualitative chemical test of total methanolic extracts such as n-hexane fraction, ethyl acetate fraction and n-butanol fraction of *A. manihot* flowers was carried out using standard procedure [18].

Free radical scavenging activity

Nitric oxide radical scavenging activity

The reaction mixture containing sample in methanol 100 µg/ml (3ml), and sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) was incubated at 25°C for 150 minutes. At intervals samples (0.5 ml) of incubation solution were removed and 0.5 ml of Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm [19, 20].

Superoxide anion radical scavenging activity

The reaction mixture contains sample in methanol 100 µg/ml (3ml), 0.1M of phosphate buffer (pH-7.4), EDTA (0.1mM), hematoxylin (50µM) and incubated at 25°C for different time periods. Inhibitions of auto-oxidation of hematoxylin by extracts over the control were measured. The absorbance at 560 nm is measured against blank samples. Superoxide radical (O₂⁻) was generated from auto-oxidation of hematoxylin and was detected by an increase in absorbance at 560 nm in a spectrophotometer. Decreased absorbance of the reaction mixture indicated increased

superoxide anion scavenging activity [21, 22].

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). The sample in methanol 100 µg/ml (3ml) was added to 2 ml of Hydrogen Peroxide Solution in PBS. After cooling, the absorbance was measured at 230 nm against a blank (containing only buffer and deoxyribose) [23].

Thiobarbituric acid-reactive substance (TBARS) assay

The peroxide formation was measured in the reaction mixture contained sample in methanol 100 µg/ml (3ml) and 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°C. 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°C 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 [24, 25].

Statistical analysis

The percentage inhibition 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:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100.$$

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The values are expressed as mean ± SEM (n = 6) and P<0.05 was considered significant.

RESULTS

In the present study, the powdered material of flowers was extracted and extractive values of total methanolic extract and its fractions such as n-hexane fraction, ethyl acetate fraction and acetone fraction were found to be 9.4% w/w 2.2%, w/w, 3.8% w/w 3.2% w/w respectively. Preliminary phytochemical

analysis showed total methanolic extracts of *A. manihot* flowers revealed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds. The n-hexane fraction shows positive result for the phytosterol. Ethyl acetate fraction showed positive test for shinoda test indicated presence of rich in flavonoids. Acetone fraction showed positive for FeCl₃ test indicated abundance of phenolic compounds.

The results of in vitro assays showed the total methanolic extract of *A. manihot* flowers (Group I) and its fractions includes n-hexane fraction (Group II), Ethyl acetate fraction (Group III) and Acetone fraction (Group IV) significant inhibition and free radical scavenging potential when

compared with standard ascorbic acid (Group V). Simultaneously results also indicated that total methanolic extract and its fractions of *A. manihot* flowers reduced lipid peroxidation and revealed that significant decrease in MDA level. It indicated that extract and its fractions seem to be significant anti-lipid peroxidation potential as compared to standard ascorbic acid. However comparative evaluation of total methanolic extracts and fractions, it was prominently noted that ethyl acetate fraction of *A. manihot* flowers indicated better significant free radical scavenging activity and anti-lipid peroxidation efficacy as compared to other fraction and extract (Table 1).

Table 1: Free radical scavenging activity of *A. manihot* flowers

Group	Treatment	% Inhibition			
		NO radical scavenging	SO radical scavenging	Hydrogen peroxide radical scavenging	Anti-lipid peroxidation
I	Total methanolic extract	73.67 ± 0.96**	52.09 ± 2.971*	60.90 ± 0.015**	49.64 ± 0.721*
II	n-hexane fraction	21.16 ± 0.38*	11.08 ± 0.502 ^{ns}	13.46 ± 0.017*	17.85 ± 0.23*
III	Ethyl acetate fraction	77.00 ± 0.11**	58.81 ± 1.839**	63.84 ± 1.551**	52.24 ± 0.025**
IV	Acetone fraction	34.65 ± 1.240*	42.75 ± 0.036*	38.06 ± 0.041*	43.78 ± 0.47*
V	Standard	84.73 ± 0.25	73.67 ± 0.96	75.71 ± 2.040	80.07 ± 0.64

Values are expressed as mean ± SEM, n=6. When Group (III, IV, V, VI, VII, VIII) compared with Group (IV);

*P<0.05, **P<0.01, ***P<0.001 symbols represent statistical significance; ns - not significant

DISCUSSION

Preliminary phytochemical analysis of extract and each fraction indicated presence of metabolites viz., alkaloids, tannins, flavonoid, steroid, terpenoids and phenolic compounds. It

also stated that the *A. manihot* flowers contains phenolic compounds such as tannins, flavonoids indicated plant extract seems to be showed antioxidant capacity. The preliminary phytochemical tests also support and confirmed that ethyl acetate

fraction showed positive test for flavonoid and indicated that ethyl acetate fraction having flavonoid rich portion and hence it has marked prominent significance as compared to other fractions with better efficacy when comparative with standard drug ascorbic acid.

In the present investigation, free radical scavenging screening of total methanolic extract and fractions of *A. manihot* flowers 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 [20].

The total methanolic extract and fractions of *A. manihot* flowers showed significant superoxide and hydroxyl radical scavenging potential. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. Ferric-EDTA was incubated with H₂O₂ 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 extract were added to the reaction mixture, they removed hydroxyl radicals from the sugar and prevented their degradation [26]. TBARS were determined by an indicator phospholipid peroxidation and a measure of the extent of DNA and deoxiribo damage [27]. 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 [28].

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 [29].

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 [30]. The extract and fractions of *A. manihot* flowers indicated presence of phenolic constituents and flavonoids and it claim for its better significance and bio-potential of scavenging action against free radical as well as lipid peroxidation when compared with standard ascorbic acid.

CONCLUSION

Present study concluded that the ethyl acetate fraction of *A. manihot* flowers is having better efficacious, significant antioxidant bio-potential against free radicals and lipid peroxidation. It showed better efficacy and significant antioxidant capacity. Thus, this study gives support the traditional believes of this medicinal plant.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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