EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 02
445-449
Year: 2018

ANTIOXIDANT ACTIVITIES AND TOTAL PHENOLIC AND FLAVONOID CONTENTS IN *SMILAX PERFOLIATA* LOUR. AND *FRAGARIA INDICA* ANDR. OF NORTH-EAST INDIA.

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Article Received on 30/11/2017 **Article Revised on 20/12/2017** **Article Accepted on 10/01/2018**

ABSTRACT

Traditional medicine plays an important role in primary health care in India. The objective of the present study is to find out the antioxidant potential of some wild edible plants, traditionally used by the local people of Assam and also to investigate the effect of antioxidant potential of the plants. Methanolic extract of these two medicinal plants were examined for their antioxidant activity by using DPPH and Hydrogen peroxide radical scavenging activity method. Total Phenolic and Flavonoid content was measured by using Folin Ciocalteu and Aluminium chloride reagent .Methanol extracts of the plant showed varying radical scavenging activity with *Fragaria indica* exhibiting the highest DPPH scavenging activity with an IC_{50} value of 122± 0.73µg/ml as compared to standard Ascorbic acid with an IC_{50} value of 145.18± 0.73µg/ml .*Smilax perfoliata* exhibiting the highest H_2O_2 radical scavenging activity with an IC_{50} value of 215± 0.43µg/ml as compared to standard Ascorbic acid with an IC_{50} value of 276.11± 0.63µg/ml. The methanolic extract of *Fragaria indica* had the highest total Phenolic and Flavanoid content with 114.75µgml-^{1} and 172.78µgml-^{1} respectively. The results indicate that these plants have significant antioxidant activity and Phenolic and Flavonoid content which could be utilized as potential source of natural antioxidant in the food or in pharmaceutical industry.

KEYWORDS: Antioxidant, Total Phenolic, Total Flavonoid, DPPH, Hydrogen peroxide.

INTRODUCTION

Antioxidants are the substances which delay, prevent or even inhibit the oxidation of oxidizable substrate/system in which they are present, by inhibiting the initiation or propagation of oxidative chain reactions.[1] They reduce themselves, thus acts as an antioxidant. 'Oxidizable substrate 'means very nearly everything found in the living cells counting proteins, lipids, DNA and carbohydrates.[2] Antioxidants provides a protective effect against ROS (Reactive oxygen species) such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) and free radicals, like hydroxyl radical (·OH) and superoxide anion (O_2-)[3] generated as byproducts of biological reactions such as the mitochondrial respiratory chain or from exogenous factors or environmental stresses.[4-5] Antioxidants possess free radical chain reaction breaking properties thus defend living cells against oxidative damage.[6] Thus they may function as free radical scavengers, complexing agents for pro-oxidant metals, reducing and quenchers of singlet oxygen formation.[7-8] Antioxidant activity is indispensable for life to neutralize the strongly oxidizing environment present inside our body. Plants are always been a source of medicine from ancient times. Herbal medicine is still use traditionally in health care sectors in several developing countries. The concept of developing herbal drugs from medicinal plants used in indigenous medicine is followed from time immemorial. Many drugs in ayurveda derived from medicinal plants have been reported as rich sources of antioxidants and the use of such natural resources as diet supplements will help in reducing the incidence of many diseases related to oxidative stress.[9]

Medicinal plants are abundantly found in North-east India. Some of these are used not just for the treatment of specific diseases, but also for good general health. These types of plants have not attracted much attention of researchers, but it is pertinent to study their antioxidant activities, as these are believed to enhance resistance to various diseases. In the folkloric system of medicine the genus *Smilax* is used in the treatment of venereal diseases, skin disorders, sores, swellings, and abscess and also applied for rheumatism and pain in lower extremities.[10] The genus *Fragaria* belongs to the Rosaceae family are also used in medicine .The fruits

contain salicylic acid and are beneficial in the treatment of liver and kidney complaints, as well as in the treatment of rheumatism and gout .Antioxidant properties have recently been discovered in the fruit, making them a valuable preventive for cancer. The leaves are gently astringent.[11]

The objectives of the present study were to determine the antioxidant activity, total phenol and total flavonoid content of these two plants of North-east India.

MATERIALS AND METHODS

Collection and identification of Plant materials

Whole plant of *Fragaria indica* Andr. of the family Rosaceae and leaves of *Smilax perfoliata* Lour. of the family Smilacaceae were collected from Dibrugarh and Jorhat district. The plants were authenticated by BSI, Eastern Circle Shillong .Herbariums were collected with conventional herbarium technique and preserved.

Preparation of Extract

Leaves of both the plants were cut into pieces, washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried plant parts were then ground in a mechanical grinder to coarse powder and stored in airtight containers free from moisture. Methanolic extract of powdered crude drug of plant part were extracted by soxhelation (Continuous hot extraction) with 1000 ml of methanol for 18hours at 65º C after pre-treatment with 1000ml of petroleum ether (40-600) in order to defat the material. When the powdered material had become totally exhausted on treatment with methanol, the solvent from the extract was recovered with rotary evaporator. The concentrated extract was dried and stored in a desiccators for use in subsequent experiments.

Determination of DPPH radical scavenging activity

Radical scavenging activity of the methanolic extract of *Fragaria indica* (MEFI), and methanolic extract of *Smilax perfoliata* (MESP), against stable DPPH was determined spectrophotometrically using the method as described by Blois in 1958[12], with trivial modifications in the method. The absorption maximum of a stable DPPH radical in methanol was at 517nm. The capability to scavenge the DPPH radical was calculated as the inhibition percentage of free radical by the sample/ standard using the following formula.

% Inhibition of DPPH scavenging activity
= {(Ao – At)/ Ao} x 100

The IC_{50} values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested plant extracts and the ordinate represents the average percent of scavenging capacity.

Determination of Hydroxyl radical (OH -) scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and methanolic extract of *Fragaria indica* (MEFI) and methanolic extract of *Smilax perfoliata* (MESP) for hydroxyl radical generated by Fe_3+-Ascorbate–EDTA–H_2O_2 system (Fenton reaction) according to the method of[13] in 1990 with trivial modifications in the method. Absorbance was measured spectrophotometrically at 532nm against control**.** The hydroxyl radical scavenging activity of the extract was reported as % inhibition of deoxyribose degradation and was calculated as follows.

% Inhibition of Hydroxyl radical (OH -) scavenging activity =
{(Ao – At)/ Ao} x 100

where A Control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}.

Where, A_0 was the absorbance of the control, and A_t was the absorbance of test/ standard. Antioxidant activity of extract was expressed as IC_{50} value.

Determination of Hydrogen peroxide radical scavenging activity

The ability of extracts to scavenge H_2O_2 was determined according to the method of Ruch *et al.* in 1989.[14] Absorbance was measured spectrophotometrically at 230nm. The percentage inhibition was calculated as:
{(Ao – At)/ Ao} x 100

where A Control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value is defined as the concentration (µg / ml) of dry extract that inhibits the formation of H_2O_2 radicals by 50%.

Determination of Total Phenolic content:

Total soluble Phenolics in the extracts were determined according to the method used by Mcdonald[15] with trivial modification using gallic acid as a standard phenolic compound. The phenol reacts with Phosphomolybdic acid in presence of alkaline medium to produce blue colour complex known as molybdenum blue complex. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the formula.

$$T = C \times V M$$

Where, T= Total phenolic contents, mg g-1 plant extract, in Gallic Acid Equivalent (GAE), C= Concentration (mg ml-1) of Gallic acid obtained from calibration curve, V= Volume of extract (ml), M= Weight (mg) of methanolic plant extract.

Determination of total flavonoid content

Total soluble flavonoid content of the fractions was determined with aluminium chloride using quercetin as the standard according to the method of Ebrahimzadeh[16]

with trivial modifications in the method. The total flavonoid content in the fractions was determined as µg quercetin equivalent by using the standard quercetin graph and using the formula**:**

$$T = C \times V / M$$

Where, T= Total flavonoid content, mg g-1 plant extract, in Quercetin Equivalent (QE), C= Concentration (mg ml-1) of Quercetin obtained from calibration curve, V= Volume of extract (ml), M= Weight (mg) of methanolic plant extract.

RESULTS AND DISCUSSION

The methanolic extract of *Fragaria indica* and *Smilax perfoliata* were capable of inhibiting, quenching free radicals to terminate the radical chain reaction and acting as reducing agents. The methanolic extract of the plants showed strong anti-oxidant activity by inhibiting DPPH and hydrogen peroxide scavenging activities when compared with standard ascorbic acid. *Fragaria indica* exhibiting the highest DPPH scavenging activity with an IC_{50} value of 122± 0.73µg/ml as compared to standard Ascorbic acid with an IC_{50} value of 145.18± 0.73µg/ml. *Smilax perfoliata* exhibiting the highest H_2O_2 radical scavenging activity activity with an IC_{50} value of 215± 0.43µg/ml as compared to standard Ascorbic acid with an IC_{50} value of 276.11± 0.63µg/ml (Table 1) (Fig 1 and Fig 2). The highest total flavanoid content was observed in the methanol extract of *Fragaria indica* measured by aluminium trichloride reagent in terms of quercetin equivalent (QE) is 114.75 ±0.12µgml-^{1} as compared to *Smilax perfoliata* with 56.12±0.14 µgml-^{1}(Table 2). The highest phenolic content was observed in the methanol extract of *Fragaria indica* measured by Folin ciocalteu reagent in terms of gallic acid equivalent (GAE) which was found to be 172.78 ±0.15µgml-^{1} as compared to *Smilax perfoliata* with 38±0.16 µgml-^{1}(Table 3). The results indicate that these plants have significant antioxidant activity and Phenolic and Flavonoid content which could be utilized as potential source of natural antioxidant in the food or in pharmaceutical industry.

Table 1: IC_{50} values OF DPPH radical scavenging activity and Hydrogen peroxide radical scavenging activity.

Sl. No.	Activity	Extract or references	IC $_{50}$(Mean ± S.D) (µg/mL)
1	DPPH Scavenging activity	Ascorbic acid	145.18±0.73
		MEFI	122.13± 0.73
		MESP	248.56± 0.53
2	Hydrogen peroxide radical scavenging activity	Ascorbic acid	276.11± 0.63
		MEFI	264.55± 0.61
		MESP	215.14± 0.43

Table 2: Total Flavonoid content of *Fragaria indica* and *Smilax perfoliata.*

Sl No.	Test	In Quercetin equivalent	
		MEFI(µgml^{-1})	MESP(µgml^{-1})
1	Total Flavonoid content	114.75 ±0.12	56.12 ±0.14

Table 3: Total Phenolic content of *Fragaria indica* and *Smilax perfoliata*

Sl No.	Test	In Gallic acid equivalent	
		MEFI(µgml^{-1})	MESP(µgml^{-1})
1	Total Phenolic compound	172.78 ±0.15	38 ±0.16

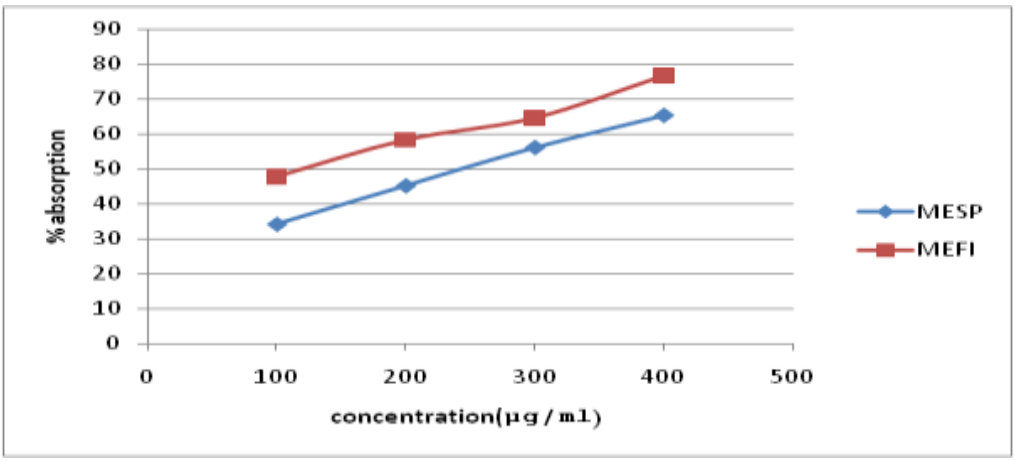

Fig 1: Concentration of methanol extract Vs. DPPH free radical scavenging activity of *Fragaria indica* Andr. and *Smilax perfoliata* Lour.

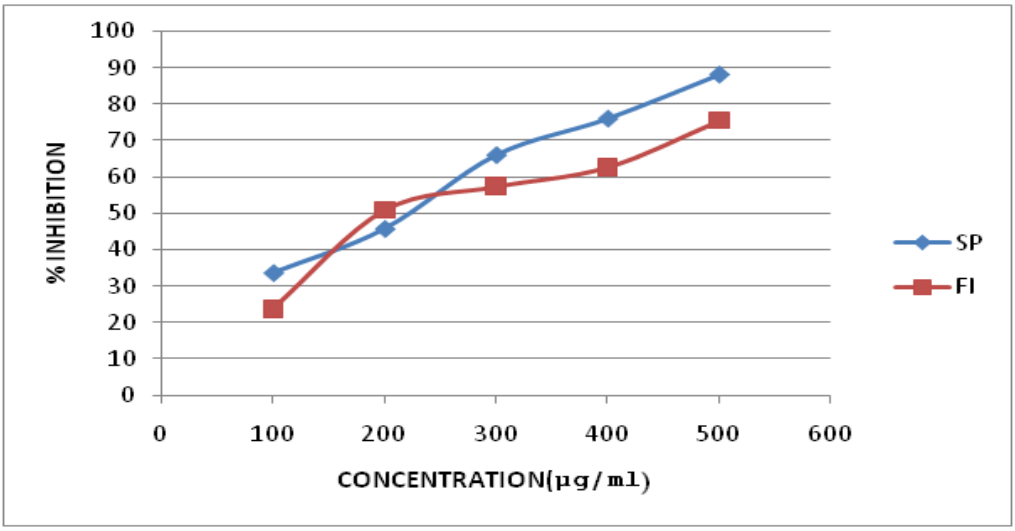

Fig 2: Concentration of methanol extract Vs. Hydrogen peroxide scavenging activity of *Fragaria indica* Andr. and *Smilax perfoliata* Lour.

CONCLUSION

The methanolic extract of *Fragaria indica* and *Smilax perfoliata* were capable of inhibiting, quenching free radicals to terminate the radical chain reaction and acting as reducing agents. The methanolic extract of the plants showed strong anti-oxidant activity by inhibiting DPPH, hydroxyl radical, and hydrogen peroxide scavenging activities when compared with standard ascorbic acid. In addition plants are found to contain a noticeable amount of total phenolics and total flavonoid contents which plays a major role in controlling oxidative damages along with good reducing power. Accordingly in this study, a significant and linear relationship was found between the anti-oxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to anti-oxidant activity . Natural antioxidants of plants origin have greater application and they also find use as nutraceuticals and phytopharmaceuticals as they have significant impact on the status of human health and disease prevention[17,18]. The present study provides scientific basis of the use of these plant extracts in traditional health care system. Detail work by using different methods will be the aim of further investigation. Moreover works are under way in elucidating the pharmacological activity with special reference to antidiabetic, antiarthritic and anti-inflammatory potential of this plant.

ACKNOWLEDGEMENT

Authors are thankful to the U.G.C. for financial support & Dibrugarh University, Assam for providing necessary facilities.

DECLARATION

The corresponding author declares that it is a original work and it is not published in any other journal.

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