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OXIDATIVE STRESS INHIBITORY APTITUDE OF *SAPONARIA OFFICINALIS* AND *ZANTHOPHYLLUM ARAMATUM*

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ABSTRACT
The present study was aimed to evaluate the antioxidant activity two traditional medicinal plants, *Saponaria officinalis* and *Zanthophyllum aramatum.* The root part of plants was used for preparation of extracts using maceration process. The extracts of plants were studied for free radical scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide free radicals and antioxidant capacity was studied using ferric thiocyanate (FTC) method. The selected plants' extracts showed the concentration dependent percentage inhibition on tested free radicals. The extracts showed the good scavenging activity on DPPH free radical and less on superoxide free radical. The ethyl acetate extract of *Z. aramatum* showed best activity and is comparable with standard drug ascorbic acid. The extracts showed the more activity at 400µg/100µL. The results of the FTC method also showed that the selected plants extracts have antioxidant capacity. The extracts of selected plants showed the lower absorbance compared to the control. The ethyl acetate extract of *Z. aramatum* reduced the amounts of peroxides from the linolenic acid oxidation with the percentage of 51.07 on 7th day of FTC method. The chloroform extract of *Z. aramatum* showed less free radical scavenging activity and less peroxide inhibition in FTC method. Finally, among two plants *Z. aramatum* have the more antioxidant capacity.

KEY WORDS: *Saponaria officinalis, Zanthophyllum aramatum,* roots, free radicals, Antioxidant activity.

INTRODUCTION

Free radicals are the molecules with unpaired electrons in their outer shell generate during the process of cellular metabolisms (Valko *et al.,* 2007). These are reactive oxygen species (ROS) include superoxide anion ($O_2\cdot^-$), singlet oxygen ($^1O_2$), hydroxyl radical (·OH), perhydroxyl radical ($H_2O\cdot$) (Ahsan *et al.,* 2003) and reactive nitrogen species (RNS) includes nitric oxide (·NO), peroxynitrite ($ONOO^-$), nitrogen dioxide ($\cdot NO_2$) etc (Rakesh *et al.,* 1999) are play very important role at their lower and higher levels with antagonistic nature. These molecules at lower levels play important role in cellular responses (Genestra, 2007; Kowald and Kirkwood, 2000) and immunity (Young and Woodside, 2001). The higher levels of these molecules lead to generate imbalance between their production and ruin, causing oxidative stress (Valko *et al.,* 2007; Chandra Kala *et al.,* 2015). This leads to the damage to cellular basic components includes proteins, lipids and DNA. The over produced free radicals in the body cleanup by the antioxidants produced in the body (Jacob, 1995). Antioxidants are the molecules prevent the free radicals reaction by donating the electrons for their stabilization. As above said, imbalance between free radicals' production and their ruin may be insufficient amount production of antioxidants in the body (Lobo *et al.,* 2010). This is may be due to different reasons like stress, pollutants, overweight, fertilizers, pesticides and harmful effects of drugs. The modern days behavioural habitats and life style of humans mainly causing the over production of free radicals in their body (Knoops *et al.,* 2004), it is not possible to know the over production of free radicals in the body (Chinwe Elochukwu, 2015). So, to control the overproduction of free radicals, the production or intake of antioxidants in the body must be increase. The naturally available defence to free radicals is enzyme produced in the body like superoxide dismutase (SOD), glutathione enzyme system, and sufficient nutrients availability for production of antioxidants in the body (Jacob, 1995; Matés *et al.,* 1999). The nutrients availability for antioxidants is overcome through consume diet which have rich in antioxidants and supply of exogenous antioxidants. In recent times, the identification of antioxidants from natural resources including medicinal plants is attracting the researchers to know from indigenous medicinal plants around the world. In this point of view, we selected two traditional medicinal plants, i.e. *Saponaria officinalis* and *Zanthophyllum aramatum* to evaluate their antioxidant activity.

Saponaria officinalis is commonly called as soapwort plant belongs to caryophyllaceae family. The different parts of *S. officinalis* has been used in traditional medicine, roots as blood purifier, diuretic, diaphoretic; roots and leaves for scrofula and skin diseases; sap for scabies, hepatic eruptions, to increase bile flow (Khare, 2007). *Zanthophyllum aramatum* commonly known as Indian prikly ash belongs to the Rutaceae family and it is used in medicine as carminative, stomachic and anthelmintic. The fruits and seeds of *Z. aramatum* were used for treatment of fever, scabies, dyspepsia, snake bite remedy, pains rheumatism, cholera and diabetes (Singh and Singh, 2011). There was very not as much of scientific evidences on their biological activities. So, we selected the roots parts of the *S. officinalis* and *Z. aramatum* for their antioxidant activity.

MATERIALS & METHODS

Chemicals and Drugs

The chemicals and solvents used in the current study were analytical grade. DPPH, Linolenic acid, Ammonium thiocyanate, Ferrous chloride, Butylated hydroxytoluene were purchased from Sigma chemicals, USA, Nitroblue tetrozolium, was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai. Riboflavin was purchased from Loba Chemie Pvt Ltd., Mumbai.

Plant Material collection and Preparation of extracts

The plant materials *Saponaria officinalis* (Voucher number: 1221) and *Zanthophyllum aramatum* (Voucher number: 1765) were collected from Tirupati region, Andhra Pradesh and authenticated by the taxonomist Dr. K. Madhava Chetty, Depart of Botany, Sri Venkateswara University. Freshly collected roots of plant materials were dried under shade and the dried material was milled to obtain a coarse powder. The powdered material was separately extracted in a Soxhlet apparatus for 6 h successively with ethyl acetate, chloroform and methanol successively. Finally, collected soxhlet solution was concentrated to dryness under vacuum by using Rota-vapor and stored in desiccators.

Free radical scavenging activity

Free radicals scavenging activity was studied for prepared extracts of selected plants using Dimethyl sulphoxide (DMSO) as vehicle on superoxide, hydroxyl and DPPH free radicals (Ganga Rao *et al.,* 2013; Mallikarjuna Rao *et al.,* 2012). The percentage inhibition and IC_{50} values were calculated.

Superoxide radical scavenging activity

Superoxide scavenging activity of the selected plant extracts were evaluated as per standard methods. It is by absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding reduction by nitroblue tetrazolium.

Hydroxyl radical scavenging activity

The scavenging activity of selected plants extracts on hydroxyl radical was measured as per established method. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe+2/ EDTA/H_2O_2 system.

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured as per methods. This method is based on measure of color absorbance of alcoholic DPPH solution (Blue color) after addition of antioxidant solution (Extract/Compound). If antioxidants present in the test compound blue color yellow color due to DPPH.

Calculation of Percentage Inhibition

The percentage inhibition of superoxide production by the extract was calculated using the formula:

Inhibitory ratio = $\frac{(Ao-A_1) \times 100}{A_0}$

Ao: Absorbance of control; A_1: Absorbance of plant extract or/and Ascorbic acid.

IC_{50} calculation form percentage inhibition

The optical density obtained with each concentration of the extract/ascorbic acid was plotted taking concentration on X-axis and percentage inhibition on Y-axis. The graph was extrapolated to find the 50% inhibition concentration of extract/ ascorbic acid.

Antioxidant activity

Ferric Thiocynate (FTC) method

Antioxidant activity of selected plants was evaluated using standard method (Saha *et al.,* 2004; Gulcin *et al.,* 2007; Elmastas *et al.,* 2007). In this method, different extracts and standard drug (4mg) were dissolved in 4mL ethanol, then mixed with 4.1mL of 2.52% linolenic acid in ethanol, 8mL of 0.02 M phosphate buffer (pH 7.0) and 3.9mL of distilled water. The mixture was placed at 40°C and then 0.1mL was mixed with 9.7mL of 75% (v/v) ethanol and 0.1mL 30% ammonium thiocyanate. Three minutes after adding the ferrous chloride (0.1mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid), the absorbance was measured at 500nm in a spectrophotometer. This step was repeated every 24 h until the control (ethanol, the mixture without added sample) reached its maximal absorbance value. The percentage (%) inhibition of lipid peroxidation was estimated by the following formula:

% inhibition= $100-((A_1/A_0) \times 100)$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts/standard.

Statistical analysis

The results of the present study expressed in mean ± SEM.

RESULTS & DISCUSSION

The selected plants part (roots) extracts showed the concentration dependent free radicals scavenging activity and good antioxidant activity. The *S. officinalis* plants extracts showed more free radical scavenging activity on DPPH free radical compared to hydroxyl and superoxide radicals at higher dose (Table 1). The percentage inhibition of ethyl acetate, chloroform, methanol extracts of *S. officinalis* and ascorbic acid on DPPH, hydroxyl and superoxide free radicals at 400µg were 76.33%, 62.0%, 80.67%, 85.67%, 34.0%, 34.33%, 59.7%, 86.0%, 37.67%, 58.0%, 72.0% and 82.33% respectively. Among three extracts of *S. officinalis* methanolic extract showed the better scavenging activity on tested free radicals. The chloroform extract showed the less activity and ethyl acetate extract showed moderate scavenging activity. The methanolic extract scavenging activity was comparable with the standard drug ascorbic acid. The IC 50 concentrations of were not detected for ethyl acetate and chloroform extracts on hydroxyl free radical and for ethyl acetate extract on superoxide free radicals because the higher dose of tested extracts' percentage inhibition not exceeded fifty percent on hydroxyl and superoxide radicals (Figure 1, Figure 2 and Figure 3). The IC 50 concentration for ethyl acetate, chloroform, methanol extracts of *S. officinalis* and ascorbic acid on DPPH, hydroxyl and superoxide free radicals were 155µg, 259µg, 128µg, 95µg, ND, ND, 248µg, 83.0µg, ND, 343µg, 255µg and 130µg respectively (Table 1).

TABLE 1. IC 50 values of *Saponaria officinalis* and *Zanthophyllum aramatum* on different free radicals

Name of the plant/ compound	Name of the extract	IC 50 value in µg on different free radicals		
		DPPH	Hydroxy l	Superoxide
Saponaria officinalis	Ethyl acetate	155	ND	ND
	Chloroform	259	ND	343
	Methanol	128	248	255
Zanthophyllum aramatum	Ethyl acetate	93.5	180	ND
	Chloroform	346	348	297
	Methanol	181	160	316
Ascorbic acid	-	95	83	130

ND: Not detected

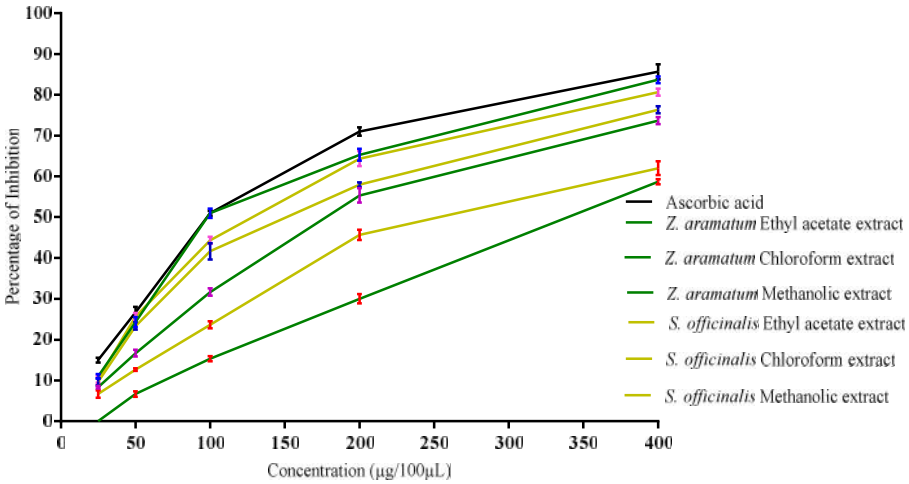

FIGURE 1. Percentage inhibition of different extracts of *Saponaria officinalis* and *Zanthophyllum aramatum* on DPPH free radical.

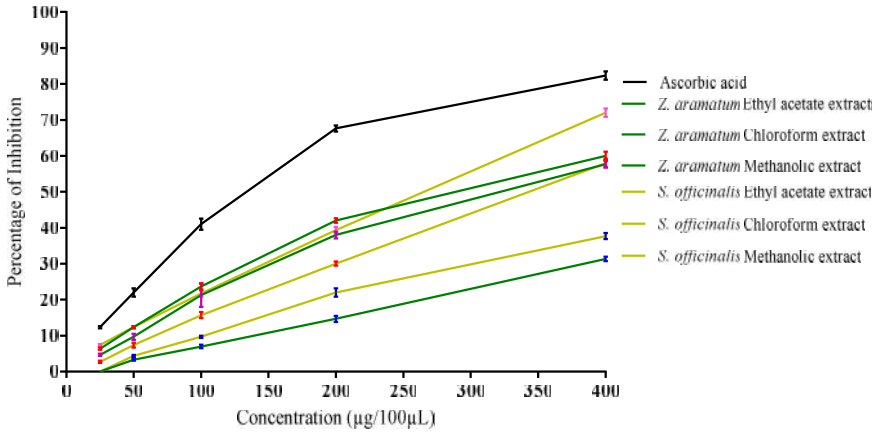

FIGURE 2. Percentage inhibition of different extracts of *Saponaria officinalis* and *Zanthophyllum aramatum* on superoxide free radical.

The different extracts of *Zanthophyllum aramatum* also showed the concentration dependent scavenging activity on tested free radicals. The percentage inhibition of ethyl acetate, chloroform, methanol extracts of *Z. aramatum* and ascorbic acid on DPPH, hydroxyl and superoxide free radicals at 400µg were 83.67%, 58.67%, 73.67%, 85.67%, 71.67%, 56.33%, 76.33%, 86.0%, 31.33%, 60.0%, 57.67% and 82.33% respectively (Figure 1, Figure 2 and Figure 3). Three extracts showed the more scavenging activity on DPPH free radical, less activity on superoxide free radical and moderate activity on hydroxyl free radicals. Among three extracts of *Z. aramatum* ethyl acetate and methanol extracts showed almost similar scavenging activity on tested free radicals and their results were comparable with standard drug ascorbic acid. The IC 50 concentration for ethyl acetate, chloroform, methanol extracts of *S. officinalis* and ascorbic acid on DPPH, hydroxyl and superoxide free radicals were 93.5µg, 346µg, 181µg, 95µg, 180, 348, 160µg, 83.0µg, ND, 297µg, 316µg and 130µg respectively (Table 1). The ethyl acetate extract did not showed the IC50 concentration at doses, but the different extracts showed good scavenging activity on tested free radicals along with ascorbic acid.

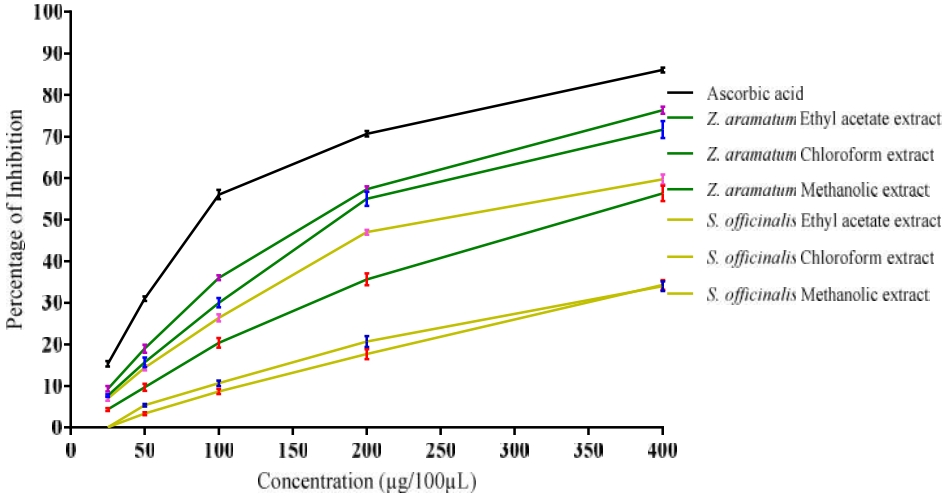

FIGURE 3. Percentage inhibition of different extracts of *Saponaria officinalis* and *Zanthophyllum aramatum* on hydroxyl free radical.

The results of the FTC method showed that the selected plants extracts have antioxidant capacity and their capacity was comparable with standard drug BHT. The FTC method was measures the amount of peroxide in lipid (linolenic acid) peroxidation. The peroxide reacts with the ferrous chloride forms the ferric ion, it combines with the ammonium thiocyanate forms the red color ferric thiocyanate. The thicker red color have the high absorbance, less color have lower absorbance. The thick color show the presence of more free radicals and less absorbance confirms the stabilization of formed free radicals due to peroxidation reaction (Sonia Sharma and Adarsh Pal Vig, 2013). The selected plants extracts showed the perceptible antioxidant capacity along with BHT during the seven day study of FTC method. The extracts of selected plants showed the lower absorbance compared to the control (Figure 4, Figure 5), the lower absorbance may be because the extracts reduced the amounts of peroxides from the linolenic acid oxidation (Table 2) (Emynur Shafekh *et al.*, 2012; Al-Naqeeb *et al.*, 2009). The antioxidant activities of the extracts were constant during the seven days of the study, the absorbance of the control increased.

TABLE 2. Percentage inhibition of *Saponaria officinalis* and *Zanthophyllum aramatum* on peroxidation in linolenic acid system in FTC method on seventh day

Name of the plant/ compound	Name of the extract	Percentage inhibition
Saponaria officinalis	Ethyl acetate	48.70±0.93
	Chloroform	36.78±0.78
	Methanol	40.15±0.79
Zanthophyllum aramatum	Ethyl acetate	51.07±0.53
	Chloroform	33.90±0.53
	Methanol	47.95±0.81
BHT	-	84.94±0.08

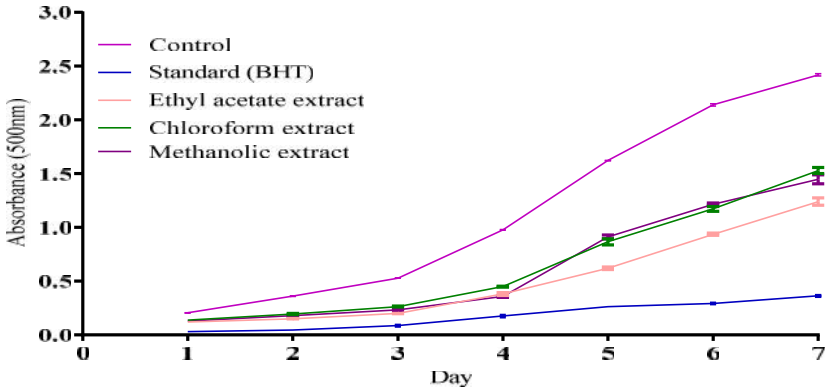

FIGURE 4. Antioxidant properties of different extracts of *S. officinalis* in FTC method.

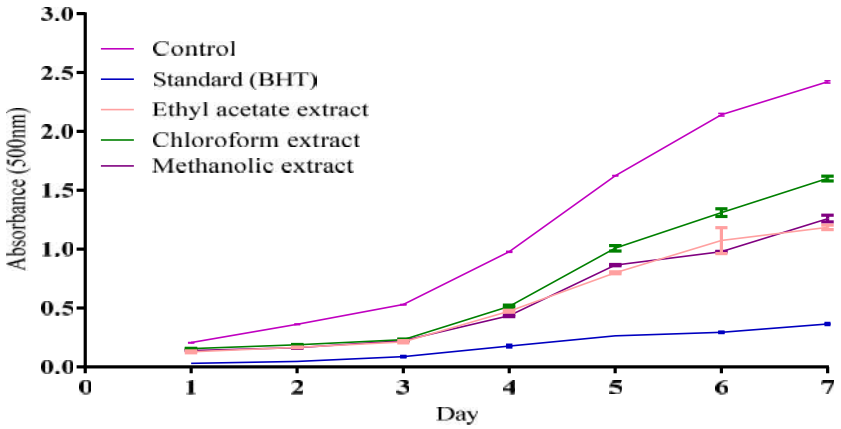

FIGURE 5. Antioxidant properties of different extracts of *Z. aramatum* in FTC method.

CONCLUSION

In this study, the selected plants' extracts showed concentration dependent free radical scavenging activity on DPPH, hydroxyl and superoxide free radicals and showed the antioxidant activity in FTC method. However, the different solvent extracts of selected plants showed variation in the activity, it may be the solvents' polarity effects the separation of compounds in the plants. The methanol extract of *S. officinalis* showed more activity compared to other extracts of it. Ethyl acetate extract of the *Z. aramatum* showed better activity compared to other extracts of it. Among two plants *Z. aramatum* showed the better activity. Therefore, form the results of the present study it is suggested that, the roots of *S. officinalis* and *Z. aramatum* have the potent antioxidant compounds and they could offer the protective from oxidative stress.

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