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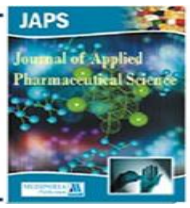

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In vitro, antioxidant and scavenging activities of *Hibiscus rosa sinensis* crude extract

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ABSTRACT

The objective of this study is to ascertain the potency of , *Hibiscus rosa sinensis* leaves extract,with 70% ethanol/ water, as potential natural antioxidant.The phytochemical screening identified the bioactive compounds of the dry extract; carbohydrates and/or glycosides, steroids and/or triterpenes, flavonoids and tannins.The total phenolic and the total flavonoids contents reached 48.4 mg catechol equivalent and 24.26 mg quercetin equivalent /g dry weight, respectively.We assayed *in vitro* total antioxidant capacity and reducing power of *H. rosa* extract (HRE), using butylated hydroxytoluene (BHT) and ascorbic acid (ASA) as references, respectively.HRE recorded two-fold stronger antioxidant capacity than that of BHT while its reducing power was less than that of ASA, with reaction time and extract concentration dependent manner. In addition we evaluated the scavenging activities of HRE for $O_2^{\bullet -}$, H_2O_2 and NO compared to that of butylated hydroxyanisole (BHA). BHA recorded up to (61.6%), (65.8%), and (37.3%), respectively, at 500 µg/ml. Scavenging ability of HRE was very closed to that of BHA, in case of $O_2^{\bullet -}$ (60.4%) and NO (36.3%) while it was lower in case of H_2O_2 (48.5%). Finally, we investigate the protection effect of HRE against lipid peroxidation (LPO) and protein oxidation(PO) using Fe^{+3}/ ascorbate oxidizing system. LPO shwed 2.5-fold increase while PO reduced 56% of –SH groups. The co-incubation of Fe^{+3}/ascorbate with Hibiscus extract inhibited lipid and protein oxidative damage nearly with 31%, at 500 µg/ml. So, we can conclude that the in vitro study emphasized HRE effective antioxidant and scavenging activities which may be due to its phenolics and flavonoids contents.

Keywords: *Hibiscus rosa sinensis*; Antioxidant capacity; Scavenging activity; Lipid peroxidation ; Protein oxidation.

INTRODUCTION

Over 50% of all modern clinical drugs are of natural product origin whereas the natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995). In recent years, focus on plant research has increased all over the world. Collected evidences showed immense potential of medicinal plants used in various traditional systems, for their biological activities and antioxidant principles (Farombi, 2003; Gilani *et al.*, 2005; Pan *et al.*,2009). Hibiscus (Malvaceae) is a genus of herbs, shrubs and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to posses various medicinal properties. Studies have shown that the plants of the Hibiscus genus have the potential to provide biologically active compounds that act as anti-oxidants and cardio protective agents. Hence, Hibiscus genus may be a great natural source for the development of new drugs and may provide a cost effective mean of treatment for cancer and other diseases in the developing world *(*Maganha *et al.*, 2009*)*.

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Hibiscus rosa sinensis (Malvaceae) is widely cultivated in the tropics as an ornamental plant. Chinese hibiscus is the english name of *Hibiscus rosa sinensis*. It is an evergreen woody glabrous showy shrub of 1.5-2.4m in height. Flowers are axillary, solitary, campanulate, red, blue, yellow or white, 10.2-15.2 cm diameter. Capsules are rotund; many seeded *(Upadhyay et al., 2011)*. The previous studies showed that its extract affects the male fertility *(Singh and Udupa, 1882), the treatment of inflammatory disease* and spermatogensis *(Reddy et al., 1997)*. The anti-diabetic activity of *H. rosa sinensis* in rural populations and in hyperglycemic rats were reported *(Sachdewa et al., 2001; Sachdewa and Khemani, 2003)*. The authors reported that the hypoglycemic activity of this extract is not mediated through insulin release and this increase the potential use of this species for human health purposes.

Moreover, There is very important evidence of the anticancer action of *H. rosa sinensis* extract against the tumor promotion stage of cancer development, in mouse skin with ultraviolet radiation.*(Sharma et al., 2004).*The crude extract of aerial parts of *H. rosa sinensis*, and its subsequent fractions, clearly showed the presence of two components that have cholinomimetic and calcium antagonist activities. So, the possible pharmacological rationale use of the plant for constipation and diarrhea was suggested. *(Gilani et al., 2005)*. On the other hand, the ancient Indian medicinal literature reported that the flowers of *H. rosa-sinensis* have beneficial effects in heart diseases, mainly in myocardial ischemic disease, due to its enhancement of the myocardial endogenous antioxidants by an adaptative response and without producing any cytotoxic effects *(Gauthaman et al., 2006)*. Recently, *Nade et al. (2011)* suggested that *Hibiscus rosa* had a protective role against age and scopolamine- induced amnesia, indicating its utility in management of cognitive disorders.

Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are generated in biological systems either as byproducts of oxygen reduction or by xenobiotics catabolism *(AK and Gülçin , 2008)*. ROS include free radicals such as superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\cdot}$) and non-free radicals such as hydrogen peroxide(H_2O_2) while RNS include non free radical nitric oxide (NO). The imbalance between the generation of ROS and the endogenous antioxidant defenses mechanisms leads to oxidative modification in cellular membrane or intracellular molecules leading to disease conditions *(Gülçin,2006)*, and exert various deleterious effects in cells and tissues that basically depend on the subcellular structure where they are generated *(Sies, 1985)*. When these species are generated within the hydrophobic domains of cell membranes, the free radical chain reaction of lipid peroxidation predominates *(Ames, 1989)* and being of particular importance in a large number of disorders such as cancer *(Emerit and Cerutti, 1981)* and degenerative diseases including Alzheimer's disease ,Parkinson's disease and Hodgkin's disease *(Jenner,1991)*.

The antioxidants have been widely used as food additives to provide protection against oxidative degradation of food. The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid (ASA). However, (BHA) and (BHT) have been suspected of being responsible for liver damage and carcinogenesis *(AK and Gülçin, 2008*).

Therefore, a need for identifying alternative natural and safe sources of food additive antioxidants, especially of plant origin, has notably increased in recent years.

Our present study is designed to evaluate the antioxidant and scavenging potential of *Hibiscus rosa sinensis* extract. For this reason, the major active compounds of its extract, which may be responsible for antioxidant activity, were determined through preliminary phytochemical screening. Total phenolic contents, total flavonoid contents and antioxidant properties were also assayed. Scavenging activity of free and non-free reactive oxygen species (ROS), including ($O_2^{\bullet-}$) and (H_2O_2) and reactive nitrogen species(RNO), including (NO) were assayed. Furthermore, *H. rosa* extract effect against lipid peroxidation and protein oxidation were also evaluated. To our knowledge, this is the first report, *in vitro*, on antioxidative and scavenging activities of *Hibiscus rosa sinensis* extract.

MATERIALS AND METHODS

Reagents and chemicals

Folin-Ciocalteu reagents, Sodium nitrite, anhydrous aluminum chloride, ammonium molybdate, potassium ferricyanide, ferric chloride, ascorbic acid, ammonium acetate, riboflavin, methionine, nitroblue tetrazolium (NBT), butylated hydroxytoluene (BHT), sodium nitroprusside ,sulfanilamide, naphylethylenediamide dihydrochloride, trichloroacetic acid, Bovine serum albumin, agarose gel, ethidium bromide, and Ellman's reagent (5,5′-dithio-bis-2-nitrobenzoic acid), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thiobarbituric acid was obtained from Fluka (Berlin, Germany). All other used chemicals and solvents were of the high analytical grade.

Plant material

The aerial parts of *Hibiscus rosa sinensis* were collected in the month of April 2011 from the faculty of agriculture - Ain Shams university, Egypt. The plant was identified with the help of available literature and authenticated by the taxonomist at Department of Botany, Faculty of Science, Menufiya University, Egypt. The leaves of the plant were washed with tap water followed by distilled water, dried in shade for 10 days prior to study and then stored in airtight glass jars, until in use.

Preparation of *H. rosa* extract (HRE)

The air-dried powdered leaves of the plant were repeatedly extracted in the room temperature with 70% ethanol/ water until exhaustion.

Post-mitochondrial supernatant preparation

The livers, of normal healthy rats, were removed quickly, and immediately perfused with ice-cold saline. The liver was

homogenized (1:10, w/v) in chilled phosphate buffer (0.1 M, pH 7.4) containing KCI (1.17%). The homogenate was filtered, centrifuged at 3000 rpm for 10 min to separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 rpm for 20 min at 4 ^{o}C to obtain post-mitochondrial supernatant (PMS).

Preliminary phytochemical screening

A small portion of the dry extract was used for the phytochemical tests for; carbohydrates *(Conalez et al.,1962),*tannins*(Wall et al., 1954),*alkaloids (*Fulton, 1932),* flavonoids *(Geissmann, 1962),* steroids *(Wall, et al., 1954),* saponins *(Conalez, et al., 1962 and Harbone, 1973)* and coumarins. *(Farnsworth, 1966).*

Determination of total phenolics and total flavonoids content

The total phenolics content was determined according to the Folin-Ciocalteu method *(Bao et al., 2005)* and the final results were expressed as mg catechol equivalent /g of dry weight of extract. While the total flavonoids content of the extract were measured by the method of *(Sultana et al., 2007)* and the final results expressed as mg quercetin equivalent /g dry weight. All tests were performed in triplicate and mean was centered.

Evaluation of total antioxidant and reducing power capacities

The total antioxidant activity of the extract was measured using a modified version of the method described by *(Prieto et al., 1999).*In which the antioxidant activity is expressed as the absorbance of samples measured at 695 nm. While its reducing power,as a reduction of Fe^{3+} to Fe^{2+}, was measured by method of *(Oyaizu, 1986)* that modified by *(Gülçin, et al., 2010).* In which, the presence of reductants such as antioxidant substances in the samples causes the reduction of Fe^{3+} / ferricyanide complex to Fe^{2+} form . therefore, Fe^{2+} can be monitored by measuring the absorbance of Perl"s Prussian blue complex at 700 nm. A higher absorbance indicated higher capacities of both antioxidant activity and the reducing power of the extract. All tests were performed in triplicate and mean was centered.

Free radical scavenging activity

Scavenging ability of the extract for free superoxide radical ($O_2^{\bullet-}$) was determined according to the method of *Beauchamp and Fridorich (1971)* described by *Zhishen et al. (1999),* while hydrogen peroxideand (H_2O_2) and nitric oxide (NO) by the methods of *(Ruch et al. 1989)* and *(Govindarajan et al.,2003)* respectively. Generally, decreased absorbance of the reaction mixture indicates increased scavenging activity.

Lipid peroxidation (LPO) and protein oxidation (PO)

In vitro, LPO was performed in hepatic Post-mitochondrial supernatant by the method of *(Wright et al. 1981*), the intensity of colored complex, thiobarbituric acid reactive substances (**TBARS**) that is formed as a byproduct of lipid peroxidation, was measured at 532 nm against a reagent blank. While (PO) was assayed by the method of *(Kaur et al., 2006)* and the produced total sulphydryl groups (TSH) were performed according to the method of ***(Sedlak and Lindsay, 1968)*** with Ellman's reagent.

Statistical analysis

All data are the average of triplicate analyses. Results are expressed as mean ± standard errors. Data were analyzed by one-way analysis of variance (ANOVA) followed by Student's t-test. P values less than 0.05 were considered statically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

In recent years, attention has been focused on the antioxidant properties of plant- derived dietary constituents of food (*Gülçin, 2006*). Our preliminary phytochemical screening for *H.rosa* leaves (**table 1**) revealed the presence of carbohydrates and/or glycosides, steroids and/or triterpenes, flavonoids, and tannins while alkaloids and/or nitrogenous bases, saponins, and coumarins were absent. On the same line, it was reported that the flowers of *H. sabdariffa* and the leaves of *H.cannabinus* contain gossypetin, glycoside, hibiscin, anthocyanin, flavonoid and polyphenol (*Amin and Hamza, 2005; Lin et al., 2007; Wu et al., 2007*).

Looking for the previous studies, the presence of these phytochemical compounds is known to support the bioactivities of medicinal plants (*Jayaprakasha et al., 2001 ; Ak and Gülçin , 2008 ; Pan et al., 2010*) and thus may be responsible to evaluate antioxidant activities for HRE in our study.

Table 1. Phytochemical screening of *H. rosa sinensis.*

Chemical constituents	*H. rosa sinensis leaves*
Carbohydrates and/or glycosides	+ Ve
Steroids and/or triterpenes	+ Ve
Flavonoids	+ Ve
Tannins	+ Ve
Alkaloids and/or nitrogenous bases	- Ve
Saponins	- Ve
Coumarins	- Ve

Total phenolics, and flavonoids content

Plant phenolics and flavonoids are a major group of compounds which have the following effects; choleretic and diuretic functions , decreasing blood pressure, reducing the viscosity of the blood and stimulating intestinal peristalsis (*Lin et al.,2007*) , as well as primary antioxidation or free radicals scavenging activities *(Shahidi and Wanasundara, 1992 ; Rathee, et al., 2007; Pan et al., 2010*).

In the present study the phenolics content of *H. rosa* extract was found to be 48.40 mg catechol equivalent /g of dry sample while the flavonoid contents was 24.26 mg quercetin equivalent/g of dry sample (**table 2).**

The most important phytochemicals in plant foods are phenolics whereas there are more than 8000 phenolic

Table 2. Total phenolics, and flavonoid contents in *H. rosa sinensis*.

Total phenolic contents (mg catechol equivalent /g)	**Total flavonoid, contents** (mg quercetin equivalent /g)
48.4± 1.03	24.26 ± 1.1

Table 3. Scavenging of superoxide , hydrogen peroxide, and nitric oxide by *H. rosa sinensis* extract.

Group	Superoxide radical	Hydrogen peroxide	Nitric oxide
Control	100 ± 2.09	100 ± 1.01	100 ± 0.2
H. rosa sinensis			
25 µg/ml	15.2 ± 3.37	7.8 ± 2.02	5.2 ± 1.93
50 µg/ml	32.7 ± 2.1*	17.2 ± 2.02	10.7 ± 1.87
100 µg/ml	40 ± 1.05*	30.3 ± 3.03*	17.4 ± 4.29
250 µg/ml	51.5 ± 3.69**	33.3 ± 1.75*	24.9 ± 4.26*
500 µg/ml	60.4 ± 2.19**	48.52 ± 3.03**	36.3 ± 2.47*
BHA			
200 µg/ml	61.6 ± 3.15**	65.8 ± 2.21**	37.3 ± 3.6*

(*) 0.05 > P < 0.01 (**)

phytochemicals (*Kuti,2004*). These phenolic compounds interrupt chain oxidation reactions by donation of a hydrogen atom or chelating metals. Moreover, their bioactivities may be related to their ability to inhibit lipoxygenase and scavenge free radicals *(Decker 1997; Kessler et al., 2003).*

Probably the most important natural phenolics are flavonoids, which contain hydroxyl functional groups, because of their broad spectrum of chemical and biological activities, responsible for antioxidant effect of the plants *(Vundac et al.,2007)*. So, the true antioxidant potential is often more accurately revealed by expressing antioxidant activity in terms of phenolics and flavonoids content (*Pan et al., 2010).*

Therefor, in this study, the obtained level of phenolics and flavonoid in *H. rosa* extract may be a sign to suggest that the extract may possess antioxidant activity. Our suggestion is in close agreement with previous reports that there is a strong correlation between the total phenolic and flavonoids content and antioxidant activity of extract from plant (*Cai et al., 2004; Pan et al., 2007*).

Total antioxidant and reducing power capacities

In the recent years, attention has been focused on the antioxidant properties, of plant-derived dietary constituents of food that has an important role in the prevention of disease. So, the antioxidant capacity is widely used as a parameter for medicinal bioactive components (*Cai et al., 2004; Pan et al., 2004; Pan et al., 2009*).

From our results, Fig. (1), the total antioxidant capacity, of *H. rosa* extract (500 µg/ml), was of high level and has reaction time dependent manner. It recorded nearly twofold higher than that of butylated hydroxytoluene (BHT) that used as a standard with the same concentration.

On the other hand reducing power capacity of the extract serves as a significant indicator of its potential antioxidant activity. Fig. (2) showed that the reducing power of *H.rosa* extract (HRE) increased gradually in concentration dependent manner. Although the power

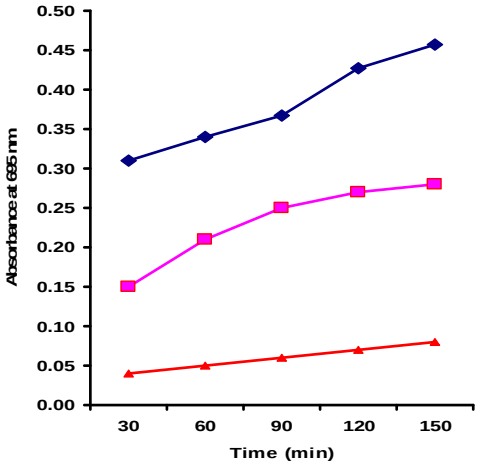

Fig. 1: Total antioxidant capacities of *H. rosa sinensis* compared to BHT. 500 ug/ml HRE (♦), 500 ug/ml BHT (■), Control (▲). Results are mean of three parallel measurements.

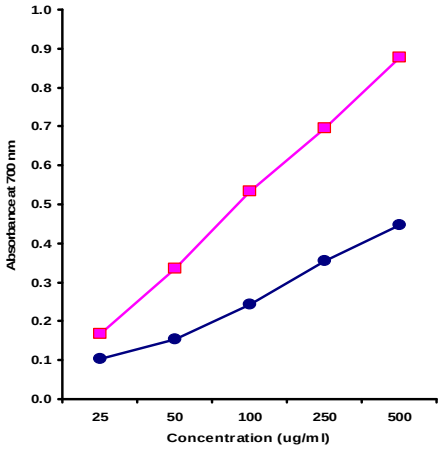

Fig. 2: Reducing power capacities of *H. rosa sinensis* compared to ascorbic acid. HRE (●), ascorbic acid (■). Results are mean of three parallel measurements.

was, nearly, one fold lesser than that of ascorbic acid (ASA) standard, at the all concentrations, HRE recorded its considered absorbance at 500µg /ml concentration.

In general, the reducing power of plant extract was reported to be directly correlated with its antioxidant activity and is based on the presence of reluctant, which exert antioxidant activity by breaking the free radical chain and donating a hydrogen atom. *(Gordon, 1990; Duh et al., 1999*). It is well documented that the antioxidant activity of putative antioxidants have been attributed to various mechanisms among which are : prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging power (*Gülçin, et al., 2003a*).

Ak and Gülçin (2008) reported that phenolic antioxidants usually scavenge free radicals by an electron-transfer mechanism. The authors added that the electron donating capacity reflecting the reducing power of bioactive compounds, phenolics and flavonoides, which serve as a significant indicator of its potential antioxidant activity (*Vundac et al., 2007; Pan et al., 2010*) Herein, in the reducing power assay, we can suggest that the

presence of antioxidants in the extract would result in the reduction of F^{3+} to F^{2+} by donating an electron.So, our results support the notion that the *H. rosa* extract performed its antioxidant activity and reducing power due to its functional components, phenolics and flavonoides that act as reluctant.

ROS and RNS scavenging activity

It is known that free radical cause auto-oxidation of unsaturated lipids in food *(Haslam. 1996; Kaur et al., 2006; AK and Gülçin, 2008).* On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of lipid *(Jain, et. al., 2008).* As shown in **table (3**), free radical scavenging ability of the *H. rosa* extract was found to decrease in the order : superoxide > hydrogen peroxide > nitric oxide, at various concentrations of the extract (25-500 µg/ml). Moreover the scavenging ability of the *H.rosa* extract, of 500 µg/ml concentration , for superoxide (60.4 ± 2.19%) and nitric oxide (36.3± 2.47%) nearly reached that of BHT standard (61.6 %± 3.15) , (37.3 ± 3.6:%), respectively, at the concentration (200µg/ml). While the extract scavenging power was lower in case of hydrogen peroxide (48.52 ± 3.03%) than BHA (65.8 ± 2.21%) at the same concentrations. All values were statistically significant ($P < 0.05$).

Superoxide anion radical is not only one of the strongest reactive oxygen species among the generated free radicals but also a precursor to other active free radicals such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which play an important role in the oxidative damage in lipids, proteins, and DNA and thereby inducing tissue damage *(Pietta, 2000 ; Gülçin,2006).* It has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical in vitro (*AK and Gülçin, 2008).* Also, *in vivo*, the up to date study suggested that the flavonoids may involve the dismutation of superoxide anion radical (*Demir et al., 2011).*

Our results showed that *H. rosa* extract inhibited gradually, in a concentration dependent manner, the superoxide radicals in the reaction mixture. It scavenged up to 60.4% superoxide radicals at a concentration of 500 µg/ml. This scavenging activity of the extract was equal to that of standard antioxidant BHA suggesting that *H. rosa* is a potent scavenger of superoxide.We thought that this consumption of superoxide anion radical, in our study, was due to the antioxidative power of *H. rosa* extract that gained from its flavonoids content.

For some extent, hydrogen peroxide itself is not very reactive but has the ability to penetrate biological membranes and it may be toxic to cell because it may give rise to hydroxyl radical which mediates oxidative DNA damage *(Gülçin et. al., 2003). H. rosa* extract was capable of scavenging hydrogen peroxide in a concentration dependent manner. The extract significantly scavenged up to 48.5% hydrogen peroxide radicals at a concentration of 500µg/ml which is lesser than that of BHA (65.8%) at a concentration of 200 µg/ml. Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water *(Akinpelu et. al., 2010).* It is reported that the antioxidative action of flavonoids consisted of an initial significant decomposition effect on H_2O_2 followed by a subsequent phase of very slow decomposition *(Sadowska-Woda et al., 2010).*

On the other hand, nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical *(Lee et al., 2007).* It is well documented that NO plays a crucial role in the pathogenesis of inflammation where it is secreted as inflammatory mediator, this may explain the use of *H. rosa* and *H. cannabinus* extracts for the treatment of inflammatory disease *(Moncada et. al., 1991; Lee et al., 2007).*The authors reported that *H. cannabinus* has some various active compounds including tannins, polyphenolics, alkaloids, essential oils and steroids which inhibited NO production by radical scavenging activity (*Lee et al., 2007).* Herein, the data recorded that the level of nitric oxide was significantly reduced by *H. rosa* extract whereas it scavenged up to 36.3% nitric oxide radicals at a concentration of 500 µg/ml. Consequently, we can link the recent findings of anti- inflammatory properties of *H. rosa* and the herein results of its NO reduction, as a results of radical scavenging activity due to its preliminary phytochemical screening contents; steroids, tannins, and flavonoids.

Effects on lipid peroxidation(LPO), and protein oxidation (PO)

In this study *H. rosa sinensis* is suggested to possess a significant radical quenching activity. To confirm this postulation, we investigated whether the extract prevented ROS mediated damage to lipids and protein *in vitro*. Incubation of post mitochondrial supernatant (PMS) with Fe^{3+}/ascorbate oxidizing-system for 60min led to about 2.5-fold increase in lipid peroxidation, as evidenced by enhanced level of thiobarbituric acid reactive substances (TBARS), compared to the control. While with bovine serum albumin the same system oxidized about 56% of -SH groups of protein. Co-incupation with *H. rosa* extract inhibited significantly, dose dependently, the oxidative damage of lipid and protein nearly with 31% at extract concentration 500 µg/ml.

Lipid peroxidation is thought to proceed by radical mediated abstraction of the hydrogen atom from a methylene carbon on a polyunsaturated fatty acid side chain (*Oboh and Shodehinde, 2009*). In general, our results recorded high potent $O_2^{\bullet-}$ scavenging (60.4%) and lipid peroxidation inhibition (31%) with *H. rosa* extract whereas the former being probably responsible for the latter. This is in agreement with (*Kaur et al., 2006 ; Demir et al., 2011)* who found *in vivo* that the scavenging of $O_2^{\bullet-}$ may contributed to protection obtained against induced LPO and PO.The ability of extract to scavenging these radicals suggests that it contains compounds that are electron donors, which can react with free radicals to convert them to more stable products and radical chain reaction. *(Singh et al., 2002 ; Kaur et al.2006 ; AK and Gülçin, 2008).* It is well documented, that there is a strong correlation between the total phenolics and flavonoids content and antioxidant activity (*Cai et al., 2004*; *Pan et al., 2007a*)

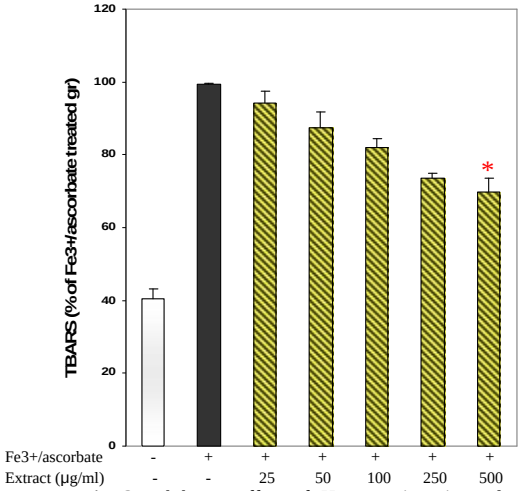

Fig. 3: Inhibitory effect of *H. rosa sinensis* on lipid peroxidation in PMS induced by Fe^{3+}/ascorbate system. Results are expressed as % of Fe^{3+}/ascorbate treated group. Each value is mean ± S.E. (n = 3). ***(*)P<0.05***.

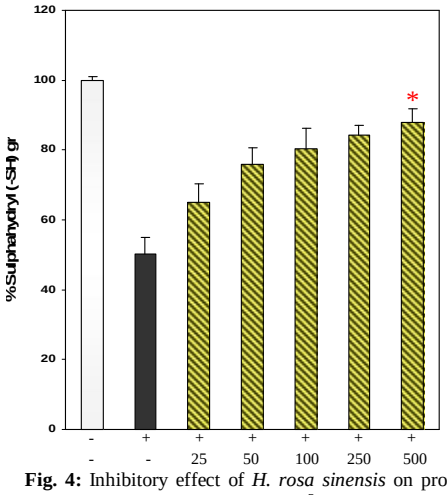

Fig. 4: Inhibitory effect of *H. rosa sinensis* on protein (BSA) oxidation induced by Fe^{3+}/ascorbate system. Results are expressed as % of non treated group. Each value is mean ± S.E. (n = 3). ***(*)P<0.05***.

However, we can emphasize that *H. rosa* extract scavenging of $O_2^{\bullet-}$ and inhibition of lipid peroxidation are attributed to its phenolics and flavonoids contents because of their best-described property is the inhibition of lipoprotein oxidation *(Jiang et al., 2007).*

CONCLUSION

Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy. In this study the crude water-ethanolic extract of *Hibiscus rosa sinensis* leaves were investigated with various antioxidant systems. The results indicated that *H. rosa sinensis* possessed abundant phenolic and flavonoids contents and exhibited excellent antioxidant activities comparing to synthetic antioxidants (BHT, BHA). The results of the present study would help to ascertain the potency of the crude extract from *H. rosa sinensis* as potential source of natural antioxidants. It can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of food and pharmaceuticals. Therefore, the extract from *H. rosa sinensis* is worthy of further studies on definitive mechanisms of its chemotherapeutic activities and potential effects *in vivo* are needed.

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