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Original Article

COMPARATIVE STUDIES OF ANTIOXIDANT ACTIVITY FROM THE PETALS OF SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT

Objective: The present study was to evaluate the *in vitro* antioxidant activity from the petals of four different Indian medicinal plants (*Punica granatum, Hibiscus rosasinensis, Cassia auriculata* and *Moringa oleifera*).

Methods: The phytochemical screening of the methanol extract of petals of four different Indian medicinal plants was performed for flavanoids using standard procedures. The methanol extracts were also evaluated for the antioxidant and free radical scavenging potential by employing different *in vitro* assays such as reducing power assay, metal chelating activity, hemoglobin-induced linoleic acid peroxidation assay, H_2O_2 (Hydrogen peroxide), NO (Nitric oxide), Superoxide anion scavenging assay, ABTS (2, 2'-Azino–bi's (3-ethyl benzothiazoline–6–Sulfonic acid) assay and Hydroxyl radical scavenging assays. The total phenolic and flavonoid contents were assessed by Folin-Ciocalteau and aluminium chloride reagents.

Results: The preliminary phytochemical screening for petals of four different medicinal plants revealed the presence of flavanoids. From the above study, the results indicated that the methanolic extract of *Hibiscus rosasinensis* petals showed highest radical scavenging activities in all assay techniques at 20 µl/ml and also possessed abundant phenolic (62.4%) and flavanoid contents (73.2%) respectively when compared to other Indian medicinal plants.

Conclusion: The results obtained in the present study indicate that the petals of *Hibiscus rosasinensis* are a potential source of natural antioxidants which may be due to its abundant phenolic and flavonoid contents.

Keywords: Medicinal plants, Antioxidant activity, Flavanoid.

INTRODUCTION

Medicinal plants have been a therapeutic source for a long time and plant products played an essential role in ancient medicine. The plants are rich in secondary metabolites such as flavanoids, carotenoids, phenols, alkaloids, terpenoids and steroids, etc., that have been found to have an excellent antioxidant as well as antimicrobial properties [1]. According to World Health Organization 65-80% of the world population rely on traditional medicine to treat various diseases and 50% of all modern clinical drugs are of natural product origin, whereas the natural products play an important role in the drug development program in the pharmaceutical industry [2]. Medicinal plants are used traditionally to prevent or cure diseases all over the world [3]. The use of medicinal plants is becoming popular worldwide, because of their lesser side effects and low resistance towards microorganisms [4].

Hibiscus rosasinensis (Malvaceae) is a genus of herbs, shrubs and trees. Studies have been shown that the plants of *Hibiscus* genus have the potential to provide biologically active compounds that acts as antioxidants and cardioprotective agents. Hence, *Hibiscus* genus can be a great natural source for the development of new drugs and can provide a cost effective mean of treatment for cancer and other diseases in the developing world [5]. The ancient Indian medical literature reported that the flowers of *Hibiscus rosasinensis* have beneficial effects in heart diseases, mainly in myocardial ischemic disease, through enhancement of the myocardial endogenous antioxidants by an adaptive response and without producing any cytotoxic effects [6].

Cassia auriculata, Linn (Caesalpiniaceae) commonly known as Tanners *Cassia* [Avaram] is a shrub with large, bright yellow flowers, growing wild in Central Provinces and Western peninsula parts of India [7]. It is one of the main constituents of 'Kalpa herbal tea 'and has proven antidiabetic action [8]. The five parts of the plant roots, bark, leaves, flowers, and unripe fruits taken in equal quantity, dried and powdered known as 'Avarai panchaga choornam' has the beneficial effect in diabetes [9]. The flowers are used in urinary discharges, nocturnal emissions, diabetes, throat troubles. The roots are alexeteric, useful in urinary discharges, tumors, skin diseases, asthma. The leaves are used as anthelmintic, in ulcers, leprosy, skin diseases [10]. The plant has been reported to possess hepatoprotective [11], antiperoxidative and antihyperglyceamic activity [12], and microbicidal activity [13].

Moringa Oleifera Lam (MO) (Family: Moringaceae), commonly known as a drumstick tree or horseradish tree. Drumstick has been claimed in traditional literature to be valued against a wide variety of diseases. Indian Materia Medica describes the use of roots of *M. Oleifera* in the treatment of a number of ailments, including asthma, gout, lumbago (pain in the muscles and joints of the lower back), rheumatism, an enlarged spleen or liver and internal deep seated inflammations [14]. In recent decades, the extracts of leaves, seeds and roots of *M. Oleifera* have been extensively studied for many potential uses, including hypotensive [15], anti-tumor [16], hepatoprotective [17], analgesic activity [18] and antioxidant [19] The juice of the leaves is believed to stabilize blood pressure, flowers are used to cure inflammations, pods are used for joint pain, and the roots are used to treat rheumatism.

The pomegranate, *Punica granatum* L., belongs to Punicaceae family. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine, the pomegranate is considered "a pharmacy into itself " and is used as an antiparasitic agent [20] a "blood tonic, "[21] and to heal diarrhea, and ulcers [22]. Pomegranate also serves as a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India [23]. The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and obesity.

The objective of the present investigation is to determine the antioxidant potential of petals of 4 different Indian medicinal plants.

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh petals of different medicinal plants were collected from the Sri Sairam Siddha Medical College and Research Centre, Herbal Garden, Tamilnadu, India and authenticated by Dr. S. Sankaranarayanan, taxonomist, Department of Medicinal Botany, Sri Sairam Siddha Medical College, West Tambaram, Chennai.

Processing of plant material and solvent extraction

The shade dried petals of different medicinal plants were ground to fine powder and sieved. 20g of the finely grounded petals were soaked in 70% methanol at room temperature for 24 hrs. The extract was filtered using Whattmann filter paper No.1 and then concentrated in vacuum at 40 °C-50 °C (overnight) using a rotary evaporator. The residue was mixed with methanol and used for further studies.

Phytochemical screening

Test for Flavanoids

The preliminary phytochemical studies were conducted on methanolic crude extracts of different medicinal plants to find out the presence of flavonoids using standard analytical procedures [24].

Determination of total phenolics and total flavonoids content

The total phenolic content was determined according to the Folin-Ciocalteu method [25] and the final results were expressed as mg catechol equivalent/g of dry weight of extract. While the total flavonoids content of the extract was measured by the method of [26] and the final results expressed as mg quercetin equivalent/g dry weight. All tests were performed in triplicate and mean was centered.

Reducing capacity assessment

The reducing capacity assessment was determined using the modified method [27]. The different concentration of methanolic extract (5-20µl) mixed with 2.5 ml of phosphate buffer (200 mM, and pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM). The mixture was incubated at 50 °C for 20 min. Then 2.5 ml of trichloroacetic acid (600 mM) was added and centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 6 mM) and the absorbance was measured at 700 nm. Ascorbic acid was used as a positive control.

Metal chelating activity

The chelating of ferrous ions of the methanolic extract was estimated by the method [28]. The different concentration of methanolic extract (5-20 µl) was mixed with 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+}complex formation was calculated as $[(A_0\text{-}A_s)/A_s] \times 100$, where A_0 is the absorbance of the control, and As is the absorbance of the extract/standard.

EDTA was used as positive control.

Antioxidant activity in hemoglobin-induced linoleic acid peroxidation test

The antioxidant activity of methanolic extract was determined by a modified spectrophotometry assay [29]. The methanolic extract (5-20 µl/ml) was mixed with1 mmol/l of linoleic acid emulsion, 40 mmol/l of phosphate buffer (pH 6.5), and 0.0016% hemoglobin, was incubated at 37 ºC for 45 min. After the incubation, 2.5 ml of 0.6% HCl in ethanol was added to stop the lipid peroxidation. Then, 100 µl of 0.02 mol/l $FeCl_3$ and 100 µl of ammonium thiocyanate (15g/50 ml) was added and vortexed thoroughly. The total antioxidant activity determination was performed in triplicate using the thiocyanate method by reading the absorbance at 480 nm.

Scavenging of hydrogen peroxide

The hydrogen peroxide scavenging assay was carried out following the procedure [30]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The different concentration of methanolic extract (5-20 µl) was added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mm). The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contains sodium phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of extract and standard compounds were calculated using the following equation

$$H_2O_2 \text{ scavenging effect (\%)} = (1-A^s/A^{c)} \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample extract or standards.

Assay of nitric oxide-scavenging activity

The sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of methanolic extract (5-20 µl) and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Vitamin C was used as positive control [31]. The mixture was recorded at 230 nm. The blank solution contains sodium phosphate buffer without H_2O_2. The percentage of nitric oxide scavenging of extract and standard compounds were calculated using the following equation

$$\text{Nitric oxide scavenging effect (\%)} = (1-A_s/A_c) \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample extract or standards.

Superoxide anion scavenging (NBT reduction) assay

The superoxide anion scavenging activity was determined by spectrophotometric method [32]. The different concentration of methanolic extract (5-20 µl/ml) was mixed with 1.4 ml of 50 mM KH_2PO4-KOH, pH 7.4 containing 1 mM EDTA, 0.5 ml of 100 µM hypoxanthine, 0.5 ml of 100 µM NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 µl of phosphate buffer and 0.5 ml of test extract fractions in saline. The xanthine oxidase was added last. The subsequent rate of NBT reduction was determined on the basis of spectrophotometric determinations of absorbance at 560 nm. Ascorbic acid was used as standard. The percentage inhibition of superoxide anion scavenging activity was calculated as

$$\text{\% Superoxide anion scavenging activity} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample extract or standards.

ABTS radical scavenging activity

ABTS (2, 2'-Azino–bi's (3-ethyl benzothiazoline–6–Sulfonic acid) radical scavenging activity of methanolic extract was determined according to method [33]. ABTS radical was freshly prepared by adding 5 ml of a 4.9 mM potassium persulfate solution to 5 ml of a 14 mM ABTS solution and kept for 16 h in the dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Vitamin C. Similarly, in the test group 1 ml reaction mixture comprised 950 µl of ABTS solution and different concentration of extract solutions. The reaction mixture was vortexed for 10 Sec and after 6 min absorbance was recorded at 734 nm against distilled water by UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and plant extract for hydroxyl radicals generated by Fe^{3+}-ascorbic acid-ethylenediamine tetra acetic acid (EDTA)-H_2O_2 system (Fenton reaction) according to the

method of [34]. The reaction mixture of 1.0 ml consisted of 100 µl of 2-deoxy-D-Ribose (28 mM in 20 mM KH_2PO_4-KOH buffer, pH 7.4), different concentration of methanolic extract (5-20 µl/ml), 200 µl EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM); it was incubated at 37 °C for 1 h. One milliliter (1.0 ml) of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added and incubated at 100 °C for 20 min. After cooling, absorbance of pink color was measured at 532 nm. Gallic acid was used as a positive control.

RESULTS AND DISCUSSION

Phytochemical screening of different medicinal plants

In recent years, attention has been focused on the antioxidant properties of plant-derived dietary constituents of food [35]. Our preliminary phytochemical screening of different medicinal plants (table 1) revealed the presence of flavonoids. It was reported that the petals of *Hibiscus also* contain gossypetin (gossypetin-8-glucoside) and gossypetin-7-glucoside [36]. In the previous report, the presence of the flavanoid is known to support the bioactivities of medicinal plants [37-39].

Total phenolics and flavonoids content

Plant phenolics and flavanoids are secondary metabolites that are commonly found in medicinal plants and are reported to have multiple biological effects, including antioxidant activity. In the present study, the total phenolic content of petals of *H. Rosasinensis* was found to be 62.4% in terms of catechol equivalents, while the flavanoid content 73.2% was of quercetin equivalents was the highest TPC and flavanoid content than other medicinal plants *(Moringa oleifera, Cassia auriculata and Punica granatum)*

The most important natural phenolics are flavanoids, which contain hydroxyl functional groups, because of their broad spectrum of chemical and biological activities, responsible for antioxidant effect of the plants [40]. Therefore, in this study, the obtained level of phenolics and flavonoid in *H. rosasinensis* extract may be a sign to suggest that the extract may possess antioxidant activity.

Reducing power capacity

Reducing power capacity of the extract serves as a significant indicator of its potent antioxidant activity. Fig.1 showed that the reducing power of *H. rosasinesis* extract increased gradually in a concentration dependent manner. 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 exerts antioxidant activity by breaking the free radical chain and donating a hydrogen atom. [41, 42]. In previous literature, reported that phenolic antioxidants usually scavenge free radicals by an electron-transfer mechanism [43].

In the reducing power assay, the presence of antioxidants in the methanolic extract would result in the reduction of $Fe^{3+}$to $Fe^{2+}$by donation of electrons. The maximum reducing property was found at the 20 µl/ml of the methanolic extract of petals of *H. Rosasinensis* which was higher than the other medicinal plants (fig. 1).

Table 1: Phytochemical constituents of methanol extracts of *petals* of different medicinal plants

S. No.	Constituents	*Hibiscus rosasinensis*	*Moringa oleifera*	*Cassia auriculata*	*Punica granatum*
1	Flavanoids: Alkaline reagent	++	++	++	++
	Shinoda test	++	++	++	++
	Lead acetate	++	++	++	++

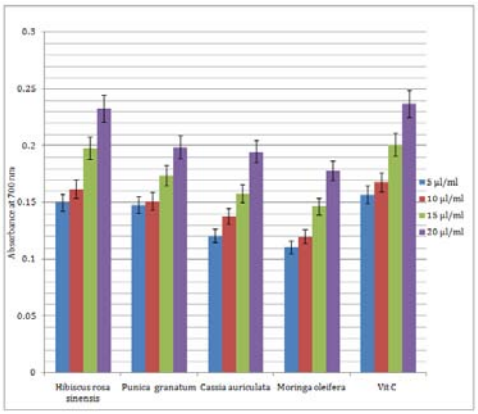

Fig. 1: Effect of methanolic extract on reducing power activity of petals of four different medicinal plants

Metal chelating activity

The transition metal ion, Fe^{2+}possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [44]. Ferrozine can quantitatively form complexes with Fe^{2+}. The absorbance of Fe^{2+}–ferrozine complex was decreased dose dependently, i.e. the activity was increased with increasing concentration from 5 to 20 µl. The chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [45]. The result proved the methanolic extract possessed Fe^{2+}chelating activity & may play a protective role against oxidative damage induced by metal catalyzed decomposition reactions. *Hibiscus rosasinensis* showed very strong chelating activity might be due to high concentration of phenolic compounds that can chelate metal ions when compared to other medicinal plants. (fig. 2)

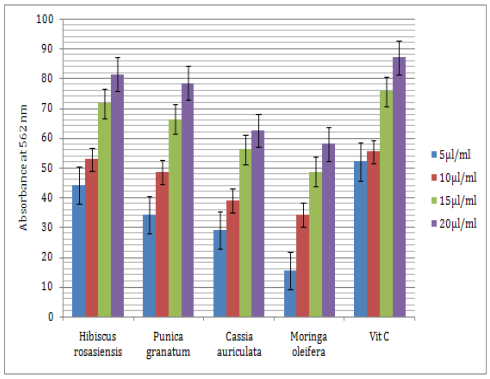

Fig. 2: Effect of methanolic extract on Metal chelating activity of petals of four different medicinal plants

Antioxidant activity in a hemoglobin induced linoleic acid

The antioxidant potential of petals of 4 different medicinal plants was evaluated employing linoleic acid peroxidation system (fig. 3). During the linoleic acid peroxidation, linoleic acid hydroperoxides, generated from the peroxidation of linoleic acid and subsequently decomposed to many secondary oxidation products, or the intermediate products may be converted to stable end products and the substrate was exhausted [46]. Among the 4 medicinal plants, the maximum inhibitory activity was found in 20µl/ml of the methanolic extract of *Hibiscus Rosa sinensis* petals.

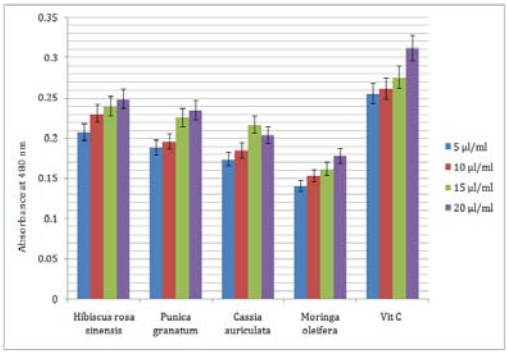

Fig. 3: Effect of methnolic extract hemoglobin-induced linoleic acid of petals of four different medicinal plants

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with and Fe^{2+}possibly Cu^{2+}ions to form hydroxyl radicals and this may be the origin of many of its oxide effects. The maximum hydrogen peroxide scavenging activity was found in 20µl/ml of the methanolic extract of *Hibiscus rosasinensis* petals (fig. 4)

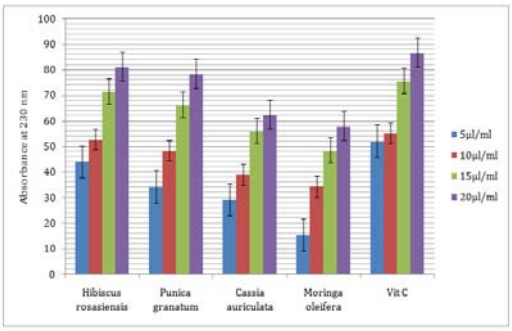

Fig. 4: Effect of methanolic extract on Hydrogen peroxide activity of petals of four different medicinal plants

Nitric oxide radical scavenging activity

The nitric oxide scavenging assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions that can be estimated using Griess reagent. In the present study, the methanolic extract of petals of *Hibiscus rosasinensis* scavenged the nitric oxide radical and/or inhibited the nitrite formation, but at much lower levels when compared with standard ascorbic acid. (fig. 5)

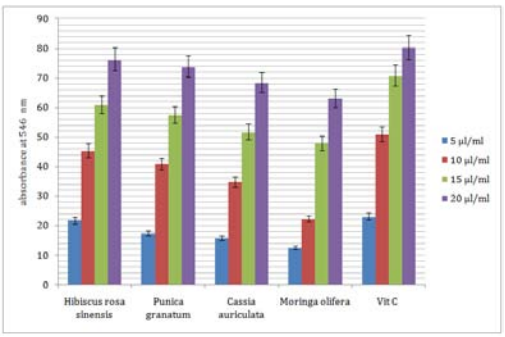

Fig. 5: Effect of methanolic extract on nitric oxide scavenging activity of petals of four different medicinal plants

Superoxide anion radical scavenging activity

Superoxide anion is a precursor to active free radicals and plays an important role in the formation of other ROS, such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA [47]. In addition, it has been reported that antioxidant properties of some flavanoids are effective mainly via scavenging of superoxide anion radical. Superoxide anion derived from dissolved oxygen riboflavin/methionine/illuminate system and reduces NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation (Parejo *et al.*, 2002) and the decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. Among the 4 medicinal plants, methanolic extract of *Hibiscus rosasinensis* shows maximum activity at 20 µl (fig. 6).

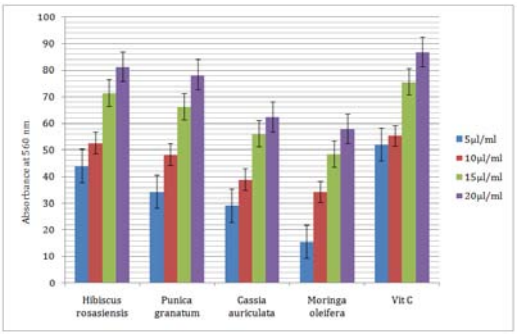

Fig. 6: Effect of methanolic extract on Superoxide anion radical scavenging activity of petals of four different medicinal plants

ABTS radical scavenging activity

Generation of the $ABTS^{+}$ radical cation(s) forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of pure substances solutions, aqueous mixtures and beverages [48]. A more appropriate format for the assay is a decolorization technique, in that the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{+}$ involves the direct production of the blue/green $ABTS^{+}$ chromophore through the reaction between $ABTS^{+}$ radical cation(s) and potassium persulfate. The methanolic extract of *Hibiscus rosasinensis* has effective $ABTS^{+}$ radical scavenging activity in a concentration-dependent manner (5 to 20 µl) (fig. 7)

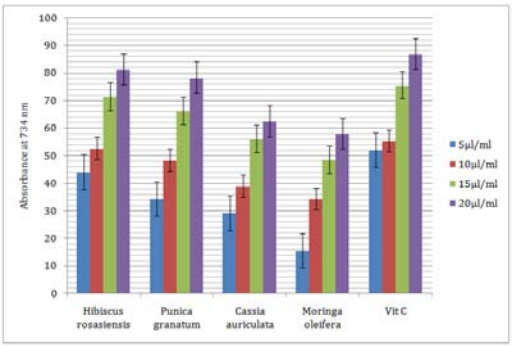

Fig. 7: Effect of methanolic extract on ABTS radical scavenging activity of petals of four different medicinal plants

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging

species in free radical pathology, capable of damaging almost every molecule found in living cells [49]. This radical has the capacity to cause strand breakage in DNA, which contributes to carcinogenesis, mutagenesis and cytotoxicity [50]. The maximum hydroxyl radical scavenging activity was found in 20µl/ml of the methanolic extract of *Hibiscus rosasinensis* petals (fig. 8)

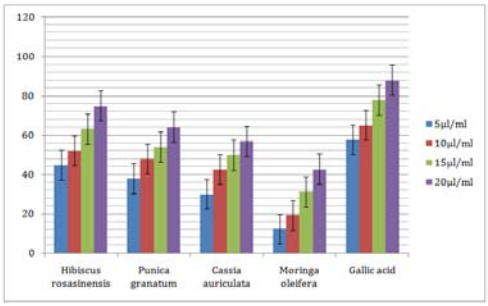

Fig. 8: Effect of methanolic extract on Hydroxyl radical scavenging activity

CONCLUSION

In this study, the methanolic extract of petals of four different Indian medicinal plants was investigated with various antioxidant systems. The results indicated that *H. rosasinensis* petals possessed abundant phenolic and flavonoid contents and exhibited excellent antioxidant activities. The results of the present study would help to develop new drug candidates for antioxidant therapy. Further investigations on the isolation and identification of bioactive compounds from the petals of *Hibiscus rosasinensis* would help to ascertain its potency.

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CONFLICT OF INTERESTS

Declared None

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