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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF Hibiscus rosa sinensis and Azadirachta indica (NEEM) LEAVES EXTRACT

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ABSTRACT

The aim of the present study was to investigate the phytochemical screening and in vitro antioxidant activity of aqueous and ethanolic leaf extract of Hibiscus rosasinensis and Azadirachta indica. Phytochemical screening was carried out on extract by standard procedure. The qualitative phytochemical analysis revealed that the presence of alkaloids, phenol, saponins, flavonoids, tannins, steroids and glycosides. The qualitatively phytochemical analysis shows that presence of alkaloids, phenols, flavonoids, tannin and saponins respectively. The total phenol and flavonoid content was 38.0 to 126.7 mg gallic acid equivalent of both aqueous and ethanol extracts of both plant extracts and from 10.5 to 66.l mg quercetin equivalent. The antioxidant activity of aqueous and ethanolic extract of Hibiscus rosasinensis and Azadirachta indica was evaluated by DPPH free radicals scavenging assay. Ascorbic acid was used as reference compound. Aqueous and ethanolic extract of Hibiscus rosasinensis and Azadirachta indica exhibited high % of free radical scavenging activity as compared with ascorbic acid. The present study concludes that aqueous and ethanolic extract of Hibiscus rosasinensis and Azadirachta indica possess significant role in antioxidant properties and could be exploited as source of antioxidant additives.

Keywords: Phytochemical, antioxidant activity, Hibiscus rosa sinensis, Azadirachta indica.

I. INTRODUCTION

Medicinal plants have been used for centuries before the initiation of traditional medicine. Leaves, fruits, flowers, stem, roots and bark can all be ingredient of herbal medicines. The medicinal values of these plants lie in their component of phytochemicals, which produce a definite physiological action on the human body (Akinmoladun, *et al*., 2007). The therapeutic efficiency of these medicinal plants has been attributed to the presence of various phytochemicals such as alkaloids, phenols, saponins, flavonoids, steroids and terpenoids and other secondary metabolites. Studies have demonstrated that many of these phytochemicals contribute as antioxidant, anti-inflammatory, antitumor, anti-mutagenic, anti-carcinogenic, antiviral and antibacterial agents (Sala, *et al*., 2002). Oxidative stress takes place, as soon as there is extreme free radical production and low antioxidant defense, which leads to chemical modification of biomolecules causing structural and functional disruption (Hoake, 2002). Oxidative damage plays a major pathological role in human diseases like arthritis, diabetes and inflammation (Halliwell, 1991). Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Living cells generate free radicals and other reactive oxygen species (ROS) by-products as a result of physiological and biochemical processes. The potentially reactive derivatives of oxygen are O_2 $\bullet^-$, H_2O_2 and $^\bullet OH$, are continuously generated inside the human body as a consequence of exposure to a plethora of exogenous chemicals in our ambient environment and or a number of exogenous metabolic processes involving redox enzymes and bioenergetics electron transfer. Under normal circumstances, the free radicals are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and detoxified by the antioxidants present. However, overproduction of ROS and inadequate antioxidant defense can easily affect and persuade oxidative damage to lipids, proteins and DNA which may eventually lead to many chronic diseases, such as aging, cancer, diabetes and other degenerative diseases in humans (Harman, 1998).

In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. Natural antioxidants such as α tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidant butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens (patel, *et al*., 2010). Therefore, there is considerable interest in finding safer antioxidants from natural sources to replace the synthetic ones.

Hibiscus rosa sinensis L (Malvaceae) is an ornamental plant often planted as a fence or hedge plant. It is native to china and also occurs in India and Philippines. The flowers have been reported to possess anti-implantation and antispermatogenic activities (Murthy, *et al*., 1997). Traditionally the plant is attributed to ant fertility activity in ayurvedic literature. Leaves and flowers also

possess hypoglycaemic activity (Sachdewa, 2003). The extracts of *Hibiscus rosa sinensis* have also been shown a protective effect against the tumor promotion stage of cancer development (Sharma, 2004).

Azadirachta indica A, commonly known as Neem is widely distributed in Indian subcontinent and its neighboring countries for more than a thousand years as one of the most versatile medicinal plant having wide spectrum biological activity. It has been an inherent part of folklore and the traditional medicinal system. Every part of the tree is used as medicine for household remedies against various human ailments and even as bio-pesticides for agricultural purpose. Neem oil, leaves, bark, stem products have been therapeutically used for the treatment of respiratory disorder, inflammation, constipation, skin infection (Sithisarn, et al., 2006) arthritic disorder, fever and diabetes etc.

Therefore, our present study is to investigate the preliminary phytochemical screening and *in vitro* antioxidant and free radical scavenging potential was studied.

II. MATERIALS AND METHODS

Plant collection

The plant material *Hibiscus rosa sinensis*, and *Azadirachta indica* were collected from a village near Hosur, local government area of Tamil Nadu, India, in the month of February, 2015. Sample identification was carried out in the department of Herbal science, Faculty of science, CHRIST University, Bangalore using a voucher specimen (ABCD-102).

Chemicals

DPPH was purchased from Sigma- Aldrich, USA. Ascorbic acid, methanol and all other solvents and chemicals were purchased from Hi Media Pvt. Ltd., unless stated otherwise

Phytochemical screening

A small portion of the plant extract was used for the phytochemical tests for compounds which include alkaloids, phenols, tannin, saponin, flavonoid and steroids with the methods. (G Trease, SM Evans, 2002)

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

One milliliter of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of plant extract dissolved in distilled water ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. the absorbance was measured spectrophotometer at 517 nm. The scavenging ability of the plant extract was calculated using this equation.

DPPH Scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/ (Abs_{control}) \times 100$
Where $Abs_{control}$ is the absorbance of DPPH + methanol; Abs_{sample} is the absorbance of DPPH radical + sample (i.e. the plant extract or standard)

Nitric oxide (NO) radical scavenging assay

3.0 ml of sodium nitroprusside in phosphate buffer (10 mM) was added to 2.0 ml of plant extract. The resulting solution was then incubated at 25ºC for 60 min. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1%, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. (Sreejayan and Rao, 1997) NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Ferric reducing antioxidant power (FRAP) assay

0.2 ml of the extract was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20 mM $FeCl_3$. 6H2O solution) and the reaction mixture was incubated at 37ºC for 30 min and the increase in absorbance at 593 nm was measured. (Oyaizu, 1986) The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g).

Hydrogen peroxide (H_2O_2) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch. (Ruch, et al., 1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution is added to 1 ml of plant extract. The absorbance at 230 nm is determined after 10 min. H2O2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

III. RESULTS AND DISCUSSION

Our preliminary phytochemical analysis for *Hibiscus rosa sinensis* and *Azadirachta indica* shown in (Table 1) revealed that the presence of alkaloids, phenols, tannins, saponins, flavonoids, steroids, glycosides, carbohydrates, proteins and starch were found in the both plant extract. In qualitative phytochemical analysis the presence of phenol, tannins, saponins, flavonoids and alkaloid identified in all the two plants.

Antioxidants exhibit many biological responses to inflammation and immunity, they function as signaling mechanisms for redox regulation, even minimal levels of oxidative stress is highly sensed and the protective anti-oxidant mechanism is set into action which is essential for the maintenance of the structural integrity of proteins. In recent years, attention has been focused on the antioxidant properties of plant derived constituents of food. Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. It is known that free radical cause auto-oxidation of unsaturated lipids in food. 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. Ascorbic acid, tartaric acid and oxalic acid are natural antioxidants present in hibiscus extracts. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Aqueous leaf extract displayed striking DPPH radical scavenging activities then ethanol leaf extract (Table 2) that might be attributed to their hydrogen donating ability. Presence of cyclopropenoids, taraxeryl acetate attributes to their reducing capability.

The toxicity and damage caused by NO and O2 is multiplied as they react to produce reactive peroxynitrile ($ONOO^-$), which leads to serious toxic reactions with bio molecules. In our study the aqueous and ethanolic extract of *H. rosa sinensis* showed a remarkable nitric oxide radical scavenging activity(Table 2). It is well documented that NO plays a crucial role in the pathogenesis of inflammation where it is secreted as a mediator, this may explain the use of *H. rosa sinensis* extract for the treatment of inflammatory diseases.

FRAP assay directly measured antioxidant or reductants in a sample that react with ferric tripyridyltriazine ($Fe3^+$ TPTZ) complex and produce colored ferrous tripyridyltriazine ($Fe3^+$ TPTZ). The antioxidant ability of hibiscus varied significantly (Table 2) the ethanolic leaf extract showed higher FRAP antioxidant activity. The phenolic compounds exhibited reduction properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers.
Hydrogen peroxide through a weak oxidizing agent is important because of its ability to penetrate biological membranes, once inside the cell it can probably react with Fe2+ and Cu2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Halliwell B, Gutteridge JMC, 1999). The extract also scavenged H2O2, however compared to O_2; H2O2 was scavenged weakly by the extract.

Table.1. Phytochemical analysis of aqueous and ethanolic extract of *Azadirachta indica* and *H. rosa sinensis*

S. No	Phytocompounds	Aqueous extract of *Azadirachta indica*	Ethanolic extract of *Azadirachta indica*	Aqueous extract of *H. rosa sinensis*	Ethanolic extract of *H. rosa sinensis*
1	Alkaloids	+	+	+	+
2	Phenols	+	+	+	+
3	Tannin	+	+	+	+
4	Saponin	+	+	+	+
5	Flavonoid	+	+	+	+
6	Steroids	+	+	+	+
7	Glycosides	+	+	+	+
8	Carbohydrates	+	+	+	+
9	Proteins	+	+	+	+
10	Starch	+	+	+	+
11	Phlobatannins	+	+	+	+

+, present, -, absent

TABLE:2 Antioxidant Activity Of Aqueous And Ethanolic Extract Of *Azadirachta Indica* And *H. Rosa Sinensis* (mg/g)

S.No.	Assays	Aqueous extract of *Azadirachta indica*	Ethanolic extract of *Azadirachta indica*	Aqueous extract of *H. rosa sinensis*	Ethanolic extract of *H. rosa sinensis*
1	Dpph Scavenging Assay	50.48	34.5	49.48	11.8
2	Nitric Oxide Assay	0.98	12.3	0.82	66.8
3	Frap Scavengingassay	8.04	6.01	7.89	15.4
4	H_2o_2 Radical Scavening Assay	43.86	32.7	36.47	23.04

IV. CONCLUSION

Results of our study suggested the great value of the species of Neem and Hibiscus leaves for use in phytotheraphy and pharmacy. Based on this, it could be concluded that these plants are natural sources of antioxidant substances of high importance. Considering the observed antioxidant potential of the investigated aqueous and ethanol extract of Neem and Hibiscus and the potent DPPH radicals, OH^- radicals, NO^- radicals and FRAP radical scavenging activity, it could be presumed that high contents of high phenols and high antioxidant activity were found in both the extracts. Neem showed higher antioxidant activities than Hibiscus. Results indicated that the neem and hibiscus have significant phenol and flavonoid contents may be extracted by modern processing and used as potential source of free radical scavengers in food preservations, pharmaceuticals and in human health.

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