INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X

Impact factor: 4.295

(Volume3, Issue1)

Evaluation of Antioxidant Activity of Phenol, Hibiscus Rosasinensis, Neem and Leaves Extract at Different Infusion Times

R. Prasanna

PRIST University, Tamilnadu.

prasanna16.bt@gmail.com

Abstract: ***The present study reveals that the selected plants would exert several benefits by virtue of their antioxidant activity and could be harnessed as antimicrobial, anti-inflammatory and anti-corrosive agents. The aqueous and Ethanol crude extract of Neem, Hibiscus leaves and the mixture of both leaves' extract were screened for their free radical scavenging properties. Free radical scavenging activity was evaluated using DPPH, NO, FRAP and H2O2 free radicals. The aim of this study was to evaluate the anti-oxidant activity of Phenol, Flavonoids and Tannins content in Neem extract (Azadirachta Indica), Hibiscus leaves and the mixture of both leaves' extract at different infusion times and determination of the protective effect of metallic corrosion caused by soil microbes.***

Keywords: Hibiscus rosasinensis, neem, DPPH (1, 1-diphenyl-2-picrylhydrazyl), Nitric oxide, FRAP (ferric reducing antioxidant power), H_2O_2 free radicals, Phenol, Flavonoids, Tannins.

I. INTRODUCTION

Plants contain a wide variety of free radical scavenging molecules, such as flavonoid, anthocyanins, cartenoids, dietary glutathionine, vitamins and endogenous metabolites and such natural products are rich in antioxidant activities.

Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of many disorders such as chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others (Young and Woodside, 2001). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is ant oxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Plant-derived antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Epidemiological studies have shown that consumption of plant foods containing antioxidants is beneficial to health because it regulates down many degenerative processes and can effectively lower the incidence of cancer and cardio-vascular diseases (*Arabshahi- Delouee, S)*. Electron acceptors, such as molecular oxygen, react easily with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS). The ROS include superoxide anions (O^{+2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (+OH). There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to bimolecular (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action and anti-corrosive agents. Antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). The methods are typically based on the inhibition of the accumulation of oxidized products, since the generation of free radical species is inhibited by the addition of antioxidants and this gives rise to a reduction of the end point by scavenging free radicals.

This study was carried out to classify the leaves of the two plants, *Hibiscus rosasinensis, A. indica (Neem)* and both leaves extract into their scale of antioxidant capacity using modified antioxidant capacity scale and to investigate the relationship between total phenolics, flavonoids and tannins and antioxidant activity of the two different plants.

A NEEM (AZADIRACHTA INDICA)

Neem is a natural medicine since ancient times in Ayurveda even each and every parts of Neem plant acts as medicinal values. Meliaceae or the Mahogany family is a flowering woody family of plants Species in the genus *Azadirachta* are closely related to and sometimes confused with species of the genus *Melia* and widely distributed throughout the tropics and subtropics, with only slight penetration into temperate zones.

Neem has effect on degenerative diseases such as diabetes, cancer, TB, bronchitis, conjunctivitis, allergies, stress, insomnia, etc. The Phytochemical substances present in Neem are alkaloids, quinines, resins, tannins, biochemical substances etc. Nimbin is the bitter compound isolated from Neem leaves. Bitterness is due to presence of terepenes. The most important bioactive compound is Azardirachtin which is an insect repellent. The flavonoids and Azadirachta act as anti-corrosive agents and also anti-microbial agents which prevent the corrosion caused by soil microbes in acidified medium of soil on buried metal in soil and also adsorption behavior properties on metal to prevent the corrosion.

Several studies worked out on the different parts of Neem plant for the various activities such as anti-inflammatory, antipyretic, antifertility, analgesic, immune stimulant, anticancer activity, anti-diabetic, antimicrobial, anti malarial, etc. Neem extract has been only involved very occasionally in environmental studies and research with the analysis of adsorption of Pb (II) from aqueous extract of Neem leaves by Bhattacharya and Sharma, Studies of Cu corrosion by Neem leaves extract in H_2SO_4 by Oguzie 2006, adsorption activity by Sanjay Sharma and Corrosion inhibitive properties of Neem in acid solution by Valek, 2007 and more.

B HIBISCUS ROSASINENSIS

Hibiscus is a genus of flowering plants in the mallow family, Malvaceae. The genus is quite large, containing several hundred species that are native to warm-temperate, subtropical and tropical regions throughout the world. *Hibiscus rosa-sinensis*, known colloquially as Chinese hibiscus, China rose, Hawaiian hibiscus, and shoeblack plant, is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family Malvaceae, native to East Asia.

Hibiscus is used as anti-inflammatory, demulant, aphrodisiac, refrigerant, menorrhagic, anti-diarrhetic, anticomplementary activity, antifertility, etc. The Phytochemical substances present in Hibiscus are steroids, flavonoids, tannins, biochemical substances, alkaloids, resins, vitamin B complex, terepenes etc. Flavonoids are present which gives bitter taste to leaves and used as anti-corrosive and anti-microbial activities which is equal to Neem.

Several studies worked out on different species of Hibiscus plant for various activities such as anti-diabetic, anti-pyretic, anti-microbial and anti-inflammatory etc. Several workers worked on this plant to be effective in the treatment of arterial hypotension by Dwivedi, 1977; antifertility effect of Hibiscus by Sethi,1986; Singh, 1982; corrosion inhibition for metal in acid medium by Rajendra, 2009, Anuradha,2007.

II. MATERIALS AND METHODS

A AQUEOUS EXTRACTION

10 grams of air dried powder *Hibiscus rosasinensis* and N*eem* leaves were placed in different distilled water bath and boiled for 6 h. At intervals of 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant of different samples was collected. After 6 h, the supernatant of different samples was concentrated to make the final volume one-fourth of the original volume. Finally 10 g of each material was extracted in 25 ml of distilled water giving a concentration of 40 mg/0.1 ml. It was then autoclaved at 121 °C and 15 lbs pressure and stored at 4 °C.

B SOLVENT EXTRACTION

10 grams of air dried powder *Hibiscus rosasinensis* and *Neem* leaves were placed in 100 ml of organic solvent (ethanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant of different samples was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 ml. It was stored at 4 °C in airtight bottles for further studies.

C EVALUATION OF ANTIOXIDANT ACTIVITY

1. α, α-DIPHENYL-β-PICRYL-HYDRAZYL (DPPH) RADICAL SCAVENGING ASSAY

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2-picrylhydrazyl by the method of ***McCune and Johns***. The reaction mixture consisted of 1 ml of DPPH in methanol (0.3 mM) and 1 ml of the each extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g). Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1\text{-As/Ac}) \times 100$$

As is the absorbance of the sample at t =0 min.
Ac is the absorbance of the control at t=30 min.

2. NITRIC OXIDE (NO) RADICAL SCAVENGING ASSAY

3ml of sodium nitroprusside in phosphate buffer (10 mM) was added to 2ml of each extract (1:200 dilutions). The resulting solution was then incubated at 25°C for 60 min. To 5 ml of the incubated sample, 5ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

3. FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

0.2 ml of the extract (1:20 dilution) 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 mM TPTZ solution and 1 part of 20 mM FeCl3. 6H2O solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm was measured. The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g).

4. HYDROGEN PEROXIDE SCAVENGING ACTIVITY

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al. (1989). For this aim, a soluti 20 µg/ml concentration in 3.4 ml phosphate buffer was added to 0.6 ml of H2O2 solution (0.6 ml, 43 mM). The absorbanc phosphate buffer without H_2O_2. The concentration of hydrogen peroxide (mM) in the assay medium was determined using a H2O2 scavenging of crude extract and standard compounds were calculated using the following equation: H_2O_2 scavenging absorbance in the presence of the sample extract or standards (Gulcin, 2006b; Elmastas et al., 2005).

D ESTIMATION OF ANTIOXIDANTS

1. ESTIMATION OF TOTAL PHENOL CONTENT (TPC)

The total phenol content was determined by Folin-Ciocalteu reagent method. 0.5 ml of extract (1:5 dilution) and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance was measured at 760 nm. The total phenol content was expressed in terms of Gallic acid equivalent (mg/g).

2. ESTIMATION OF TOTAL FLAVONOIDS (TFC)

The total flavonoids content was determined by Aluminum chloride method. The reaction mixture (3ml) that comprised of 1ml of extract (1:10 dilution), 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoids content was expressed in

terms of quercitin equivalent (mg/g).

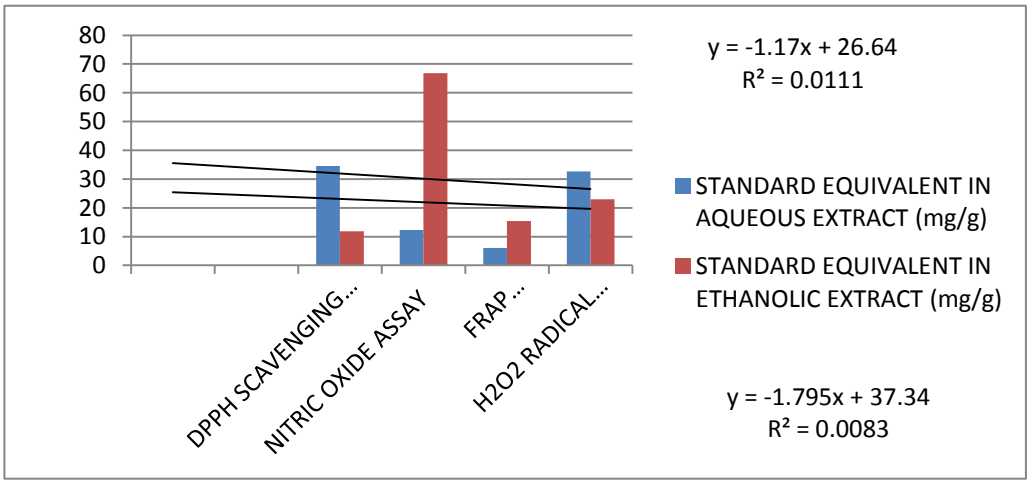
3.ESTIMATION OF TANNINS

The tannin content was determined by Folin-Ciocalteu reagent method. 1:10 diluted extract was added to Folin-Ciocalteu reagent (0.5 N), mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance measured at 760 nm. Tannins were expressed in terms of tannic acid equivalent (mg/g).

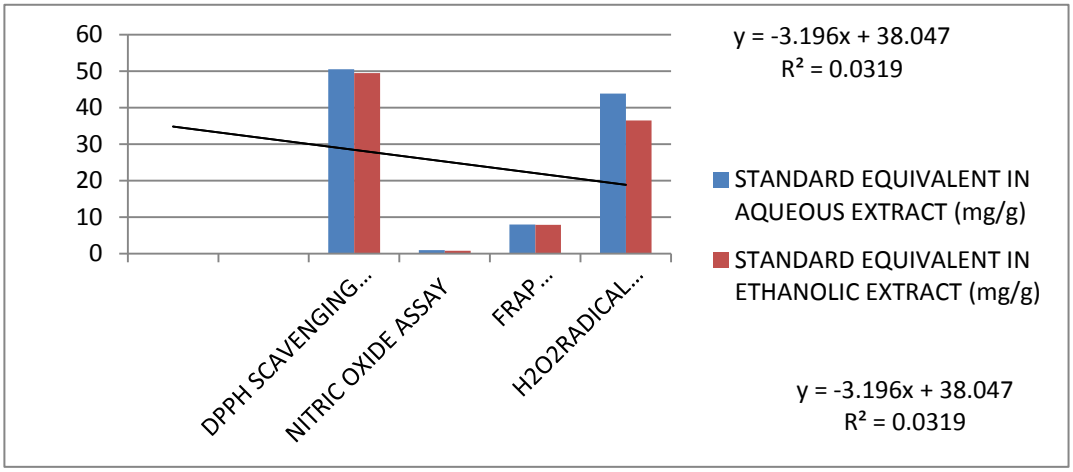
III. RESULTS AND DISCUSSION

Antioxidants are compounds that protect cells against reactive oxygen cells or free radicals in the body. Although they are created as part of the body's normal metabolic functions, free radicals react with other cells and may interfere with their ability to function. Free radicals are believed to play a role in many health conditions, ranging from cancer and atherosclerosis to wrinkles caused by too much sun. Free radicals are constantly generated resulting in extensive damage to tissues and bimolecular leading to various disease conditions. So the medicinal plants are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress.

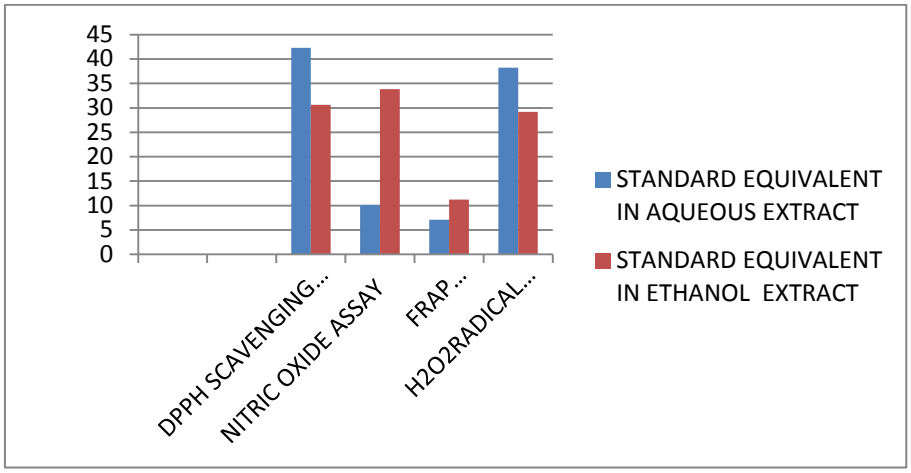
ANTIOXIDANT ACTIVITY OF HIBISCUS ROSASINENSIS LEAF EXTRACT



ANTIOXIDANT ACTIVITY OF NEEM LEAF EXTRACTS

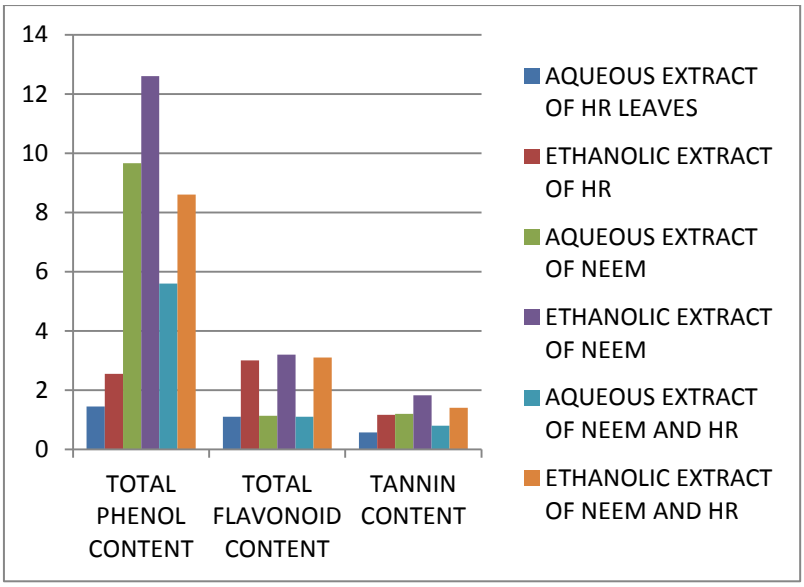


ANTIOXIDANT ACTIVITY OF BOTH LEAF EXTRACTS



The graph shows the class of antioxidant capacity of the different plant parts of Hibiscus rosasinensis, A. *indica* and both plant mixture. The order of antioxidant capacity for *A. indica* is as follows: Ethanolic extract > Aqueous extract. The order of antioxidant capacity for Hibiscus rosasinensis is as follows: Ethanolic extract > aqueous extract. The order of antioxidant capacity for both plant mixture extract is as follows: Ethanolic extract > aqueous extract. In this research, the modified method of scale of antioxidant capacity of Katalinic et al. (2006) was employed.

ESTIMATION OF TOTAL ANTIOXIDANT CAPACITIES



IV. TOTAL PHENOLICS, FLAVONOIDS AND TANNIN CONTENT

Plant phenolics, flavonoids and tannins 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).

The most important Phytochemical in plant foods are phenolics whereas there are more than 8000 phenolic Phytochemical (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, flavonoids and tannins 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, flavonoids and tannins content (Pan et al., 2010).

Therefore, in this study, the obtained level of phenolics, flavonoids and tannins in *H. rosasinensis, A. indica* and both leaves extract may be a sign to suggest that the extract may possess antioxidant activity.

The antioxidant capacity of ethanolic extract of Hibiscus rosasinensis leaves was slightly greater than that of the Aqueous extract of Hibiscus rosasinensis, and the difference was found to be significant ($p<0.05$). The antioxidant capacity of the ethanolic extract of *A. indica leaves was* higher than the antioxidant capacity of the aqueous extract of *A. indica leaves* and the difference was significant ($p< 0.05$). Also, the antioxidant capacity of ethanolic extract of *A. indica and mixed leaves* was slightly higher than the antioxidant capacity of the aqueous extract of *A. indica leaves* and the difference was not significant ($p> 0.05$).

CONCLUSION

The leaves of HR, Neem and both leaves mixture have proved to be the presence of antioxidant and phenol, flavonoids and tannin. Ethanol extract of Neem and both leaves mixture is proved to be of higher antioxidant potential in comparison to HR. Ethanol extract of all the sample have more effective than aqueous extracts.

REFERENCES

1. *Arabshahi-Delouee, S*., Young and Woodside, 2001, Cook and Samman, 1996.
2. R.A. Larson,The antioxidants of higher plants, Phytochemistry 4 (1988) 969/978.
3. M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Nederlands, J. Agric. Food Chem. 40 (1992) 2379/2383.
4. G. Cao, E. Sofic, R.L. Prior, Antioxidant capacity of tea and common vegetables, J. Agric. Food Chem. 44 (1996) 3426/3431.
5. G.A.A. Kivits, F.J.P. Vam der Sman, L.B.M. Tijburg, Analysis of catechin from green and black tea in humans: a specific and sensitive colorimetric assay of total catechins in biological fluids, Int. J. Food Sci. Nutr. 48 (1997) 387/392.
6. M.B. Grisham, J.M. McCord, Chemistry and cytotoxicities of reactive oxygen metabolites, in: A.E. Taylor, S. Matalon, P. Ward (Eds.), Biology of Oxygen Radicals, American Physiological Society, Bethesda, 1986, pp. 1 /18.
7. N.J. Miller, C.A. Rice-Evans, Factors influencing the antioxidant activity determined by the ABTS radical cation assay, Free Radic. Res. 26 (1997) 195/199.
8. C. Sa´nchez-Moreno, J.A. Larrauri, F. Saura-Calixto, A procedure to measure the antiradical efficiency of polyphenols, J. Sci. Food Agric. 76 (1998) 270/276

