International Journal of Phytopharmacy
Vol. 1 (1), pp.08-14, Sep 2011-Oct 2011

Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF FLAVONOID AND PHENOLIC CONTENTS OF *LUFFA ECHINATA* ROXB. FRUITS AND *NYCTANTHUS ARBOR-TRISTIS* LEAVES

Anuj Modi [1] **,** Vimal Kumar[2], Prateek K. Jain[1], Sunil K. Jain[1], Vaibhav P. Uplanchiwar[1]

[1] *Department of Pharmacognosy, ADINA Institute of Pharmaceutical Sciences, Sagar*
[2] *Department of Phytopharmaceutical and Natural Products, Nirma University, Ahmedabad*

Corresponding Author: pharmaanuj@gmail.com

ABSTRACT

Objective: To obtain a systematic record of the relative antioxidant activity of flavonoid and phenolic contents of *Luffa echinata* Roxb fruit and *Nyctanthus arbor-tristis* leaves extracts.
Methods: Phenolic and flavonoid content were determined by using gallic acid and quercetin as standard, antioxidant properties were evaluated by the methods, namely the Trolox equivalent antioxidant capacity assay (TEAC), Oxygen radical absorbance capacity (ORAC) Assay and DPPH free radical scavenging activity assay.
Results: Total phenolic content were found 76.34±0.44 and 45.53±0.65 mg/g, while flavonoid contents were found 65.98±0.83 and 34.92±0.76 mg/g in *Luffa echinata* Roxb and *Nyctanthus arbor-tristis* respectively. In the Trolox equivalent antioxidant capacity assay, TEAC value were found 0.34 mmole/g and 0.28 mmole/g, while in Oxygen radical absorbance capacity assay, ORAC value was found 253.7µ moles TE [#]/ g and 221.6 µ moles TE [#]/ g, where as DPPH free radical scavenging activity assay showed IC_{50} value 188±0.87 and 176±0.68 for *Luffa echinata* Roxb and *Nyctanthus arbor-tristis* extracts.
Conclusion: The present studies suggest that both the plants have moderate to potent antioxidant activity.
Keyword: Antioxidant; ORAC; TEAC; DPPH*; Luffa echinata Roxb; Nyctanthus arbor-tristis*

1. INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and functions as natural antioxidants in human body. Free radicals cause depletion of immune system antioxidants, causes change in gene expression and induce abnormal proteins. Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or prompt negative health effects[1]. It has been suggested that natural antioxidants are more safe and healthy than synthetic antioxidants[2]. Antioxidants or molecules

with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. These biomolecules contribute to prevention of coronary and vascular diseases and tumor formation by inhibiting oxidative reactions[3]. The many plant species had established as antioxidants and large number of species are still in ongoing process to prove its antioxidant potential but still there is a need to explore new antioxidant potentials. It has been mentioned the antioxidant activity of plants might be due to their phenolic or flavonoid compounds[4]. Flavonoids are a group of poly phenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests the biological actions of these compounds are related to their antioxidant activity. Typically the relative moieties in both i.e. natural and synthetic antioxidant, have an aromatic ring with at least one hydroxyl group[5]. Consumption of natural antioxidants reduces risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging[6]. Present study is carried out to investigate and to compare (I) their phenolic and flavonoids contents, (II) the free radical scavenging activity and (III) analyses of correlation between them. The antioxidant properties were evaluated by the methods, namely the Trolox equivalent antioxidant capacity assay (TEAC), Oxygen radical absorbance capacity (ORAC) assay, DPPH free radical scavenging activity assay. The Folin–Ciocalteu and Colorimetric aluminum chloride method were used to determine the total phenolic and flavonoids contents of each samples.

2. MATERIAL AND METHODS

2.1 Chemicals

1, 1-Diphenyl -2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma chemicals Co, Vadodara, Gujarat. Ascorbic acid, Folin Ciocalteu reagent and Methanol were purchased from Merck India Ltd, Mumbai.

2.2 collection and authentication of plant material

The plant specimens were collected from local area of Sagar division, Madhya Pradesh, India, during the month of July 2010. It was authenticated by Dr. Pradeep Tiwari, Department of Botany, Dr. Hari Singh Gour Vishwavidhyalaya, Sagar (M.P.). A voucher specimen no. Bot/2010/76 has been deposited at Adina Institute of Pharmaceutical Sciences, Sagar (M.P.).

2.3 Preparation of extracts

Shed dried powdered material of *Luffa echinata* Roxb. fruit and *Nyctanthus arbor-tristis* leaves were exhaustively extracted with methanol: water extract (80:20) using Soxhlet's apparatus. The extracted were concentrated under vaccum and semisolid mass obtained were used for further study.

2.4 Total phenolic determination

Total phenolics were determined by Folin Ciocalteu reagent. A dilute extract of each plant extract (0.5ml of 1:20g/ml) or Gallic acid (Std. phenolic compound) were mixed with Folin Ciocalteu reagent (10ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (10ml, 1M), mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765nm. The standard curve was prepared using 25 to 200 μg/ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.5 Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination. Each plant extracts (0.5ml of 1:20g/ml) in methanol were separately mixed with 11.5ml of methanol, 0.1ml of 10% Aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It kept at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by using quercetin solutions at concentrations 10 to 100 μg/ml in methanol[1].

2.6 Determination of antioxidant activity

2.6.1 Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay depends on the free radical damage to a fluorescent probe through the change in its fluorescence intensity. In the present assay, AAPH is used as free radical generator to reduce the fluorescence characteristics of fluorescien, which is used as the fluorescence probe. The change of fluorescence intensity (reduction in fluorescence) is an index of the degree of free radical damage. In the presence of an antioxidant, there is decrease in the change of fluorescence induced by AAPH. In the ORAC assay, the antioxidant activity of a sample is expressed relative to TROLOX, a derivative of Vitamin - E [6, 7].

2.6.2 Trolax equivalent antioxidant capacity (TEAC) assay

The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2, 2-Azino-di-[3-ethylbenzthiazoline sulphonate) to ABTS radical cation by metmyoglobin. The amount of ABTS produced can be monitored by reading the absorbance at 600nm to a degree which is proportional to their concentration. The capacity of the antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox and is quantified as millimolar Trolox equivalents [6, 7].

2.6.3 DPPH radical scavenging assay

DPPH (1, 1-diphenyl-2-picryl hydrazyl) is a stable free radical with purple color, the intensity of which is measured at 517 nm spectrophotometrically. Antioxidants reduces DPPH to 1, 1-diphenyl-2-picryl hydrazine, a colorless compound. Gallic acid was used as a standard in this assay[6].

3. RESULTS

3.1 Extraction of dried powdered crude drug

Table No. 1. Percentage yield of crude drug extract

S. No.	Crude Drug	% Yield(w/w)
1	*Luffa echinata* Roxb.	15.0
2	*Nyctanthus arbor-tristis*	11.2

3.2 Determination of total flavonoid and total phenolic

Table No. 2. Total flavonoids and total phenolic content of extracts

S. No.	Tested Extracts	Flavonoid (mg/g± SEM)	Phenol (mg/g± SEM)
1	*Luffa echinata Roxb.*	*65.98*±0.83	*76.34*±0.44
2	*Nyctanthus arbor-tristis*	34.92±0.76	*45.53*±0.65

*****Average of six determinants

3.3 Antioxidant activity by Oxygen Radical Absorbance Capacity (ORAC) Assay, Trolox Equivalent Antioxidant Capacity (TEAC) and DPPH assay

Table No. 3. ORAC, TEAC and IC_{50} value of the extracts

S. No.	Tested Extracts	*TEAC value (mmol/g)	ORAC value µmoles TE[#] µ mol/ g of substance	IC_{50} value± SEM
1	*Luffa echinata* Roxb.	0.34	253.7	188±0.87
2	*Nyctanthus arbor-tristis*	0.28	221.6	176±0.68

[#]TE: TROLOX equivalent, * TEAC – TROLOX equivalent antioxidant capacity, IC_{50}±SEM
*Average of six determinants

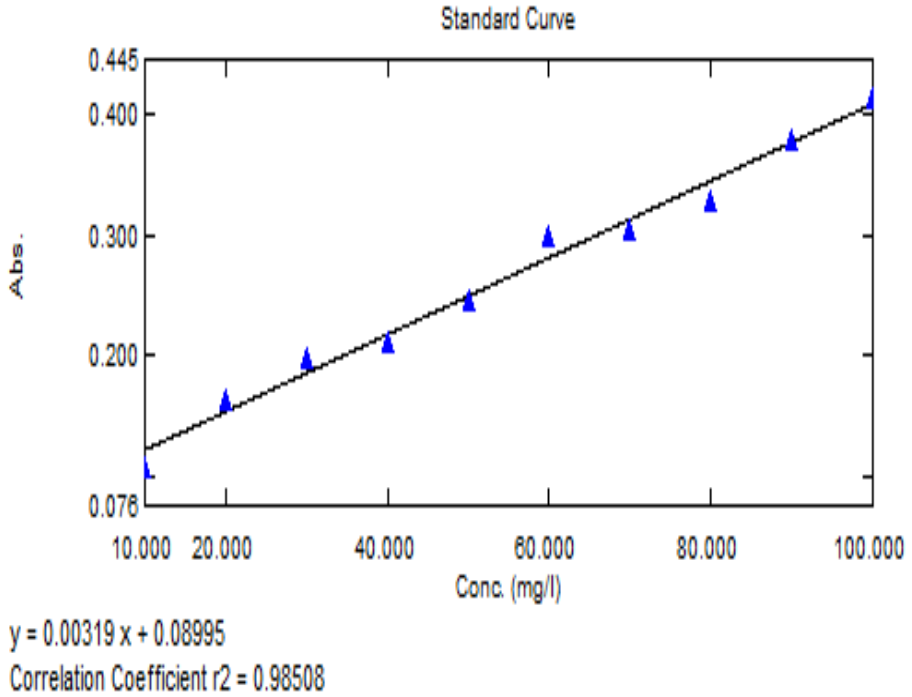

Figure No.1. Standard curve of Quercetin for estimation of total flavonoid

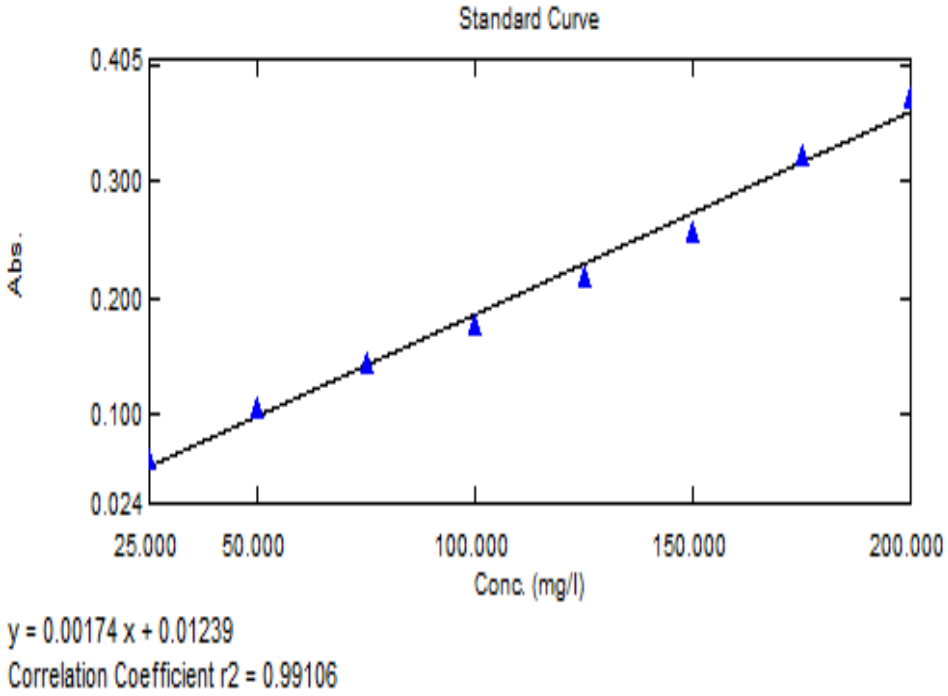

Figure No. 2. Standard of Gallic acid for estimation of total phenol

4. DISCUSSION

4.1Total flavonoid and total phenol contents of the extracts

The mechanisms of action of flavonoids are through scavenging or chelating process[8]. Phenolic compounds are a class of antioxidant agents, which act as free radical scavengers[9].The Table No.2 also show the contents of total flavonoid contents in terms of quercetin equivalent (The std curve equation: y =0.00319X + 0.08995, r2 = 0.985) (Figure No.1) and phenols that were measured by Folin Ciocalteu reagent in terms of Gallic acid equivalent (The std. curve equation: y = 0.00174X +0.01239, r2 =0.991) (Figure No.2). The flavonoid and phenolic content were found 65.98±0.83mg/g, 34.92 ± 0.76mg/g and 76.34 ±0.44 mg/g, 45.53± 0.65mg/g in *Luffa echinata and Nyctanthus arbor-tristis* respectively. According to our study, the high contents of these phytochemicals in *Luffa echinata* Roxb. can explain its high radical scavenging activity than *Nyctanthus arbor-tristis*.

4.2 Antioxidant activity determination

In recent years there has been growing interest in understanding the role of free radicals in many diseases such as cancer, arteriosclerosis, ageing and their prevention using antioxidants[10]. DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to access the *in-vitro* antioxidant activity of crude plant extracts [7, 11, 12]. Literature confirms the antioxidant activities of *Luffa echinata and Nyctanthus arbor-tristis* in various *in- vitro* models but there are no validated reports on trolox equivalent antioxidant capacity and oxygen radical absorbance capacity assays for *Luffa echinata and Nyctanthus arbor-tristis*. In the present content, we have evaluated in three in-vitro tests viz., Trolox equivalent antioxidant capacity and oxygen radical absorbance capacity, DPPH free radical scavenging assay for antioxidant properties of

methanol: water (80:20) extracts of each plant. Together the entire three assays provide a better assessment of antioxidant activity.

4.2.1 Oxygen radical absorbance capacity (ORAC) assay

In Oxygen radical absorbance capacity (ORAC) assay, the AAPH is used as free radical generator to reduce the fluorescence characteristics of fluorescien. ORAC value of *Luffa eschinata* Roxb.was found 253.7µmoles TE#/ g while *Nyctanthus arbor-tristis* showed 221.6µ moles TE#/ g.

4.2.3 Trolox equivalent antioxidant capacity (TEAC) assay

This assay measures the antioxidants in the sample and is compared with that of trolox, water soluble tocopherol analogue and is quantified as millimolar trolox equivalents. TEAC value of *Luffa eschinata* Roxb. was found 0.34 mmoles/ g while *Nyctanthus arbor-tristis* shows 0.28 mmoles/ g.

4.2.4 DPPH radical scavenging assay

In DPPH test, the ability of a compound to act as a donor for hydrogen atoms or electrons is measured spectrophotometrically. Hydroxyl radicals are the major active species that cause lipid oxidation and significant biological damage. The ability of the tested extracts to quench hydroxyl radicals seems to be directly related to inhibiting the process of lipid peroxidation[13]. IC_{50} value of *Luffa eschinata* Roxb. fruit was found 188±0.87 while *Nyctanthus arbor-tristis* shows 176±0.68. In all three in-vitro model antioxidant activity of *Luffa echinata* Roxb. was found more potent than *Nyctanthus arbor-tristis.*

CONCLUSION

On the basis of it can be conclude that, *Luffa echinata* Roxb. fruit was found to be have high content of phytoconstituents (total flavonoid and total phenol) and posses better antioxidant activity in ORAC, TEAC and DPPH assay as compared to *Nyctanthus arbor-tristis*. Finally both the extracts were found to have potent antioxidant activity.

ACKNOWLEDGEMENT

Author wishes their gratitude for **The Director, Natural Remedies, Bangalore** for providing analytical facilities and **Prof. R. K. Jain, AIPS, Sagar (M.P.)** for their valuable support.

REFERENCES

1. Saravana AK, Mazumder A, Vanitha J, Venkateshwaran K, Kamalakannan K, Sivakumar T. Evaluation of antioxidant activity, phenol and flavonoid contents of some selected Indian Medicinal Plants. Phcog Mag 2008; 4(13): 216-9.
2. Xiao-Yu Su, Zhen-Yu Wang, Jia-Ren Liu. In vitro and in vivo antioxidant activity of Pinus koraiensis seed extract containing phenolic compounds. Food Chem 2009; 117: 681-6.
3. Gursoy N, Sarikurkcu C, Cengiz M, Halil M. Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. Food and Chem Toxicol 2009; 47:2381-8.
4. Scholar P. Natural antioxidants exploited commercially, In B.J.F Hudson, Food antioxidants. Elsevier 1990; 99: 170-76.

5. Kulomaa A, Sirrn H, Riekkola M L. Identification of antioxidative compounds in plant beverages by capillary electrophoresis with the marker index technique. J of Chromat 1997, 781: 523-32.
6. Jain PK, Ravichandran V, Agrawal RK. Antioxidant and free radical scavenging properties of traditionally used three Indian Medicinal Plants. Curr Trends in Biotech and Pharm 2008: 2(4): 538-47.
7. Jain P K, Ravichandran V, Sharma S, Agrawal RK. The Antioxidant activity of some Medicinal Plants. Turk J Biol 2008; 32: 197-02.
8. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol 2003; 55: 131-42.
9. Frankel E. Nutritional benefits of flavonoids. International conference on food factors: Chemistry and cancer prevention, Hamamatsu, Japan: Abstracts. C6-2(1995).
10. Finkel T, Holbrook NJ, Oxidants, oxidative stress and biology of Ageing. Nature 2000, 408: 239-47.
11. Navarro MC, Montilla MP, Martin A, Jimenez J, Utrilla MP. Free radicals and antihepatotoxic activity of *Rosmarinus tomentosus*. Planta Medica 1992; 59:312-14.
12. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J of Biology 2006, 30: 177-83.
13. Aruoma OI, Kaur H, Halliwell H. Oxygen free radicals and human diseases. J Royal Soc Health 1991, 111: 172-77.