Research Article

Bioprospecting of *Adenoon indicum*: An Endemic Plant of Asteraceae from Western Ghats of India

R. P. Mane[1,2], P. V. Anbhule[1], S. V. Patil[1], V. B. Shimpale[1,2]*
[1]Department of Agrochemicals and Pest Management, Shivaji University, Kolhapur, Maharashtra, India.
[2]Department of Botany, The New College, Kolhapur, Maharashtra, India.
***Corresponding author's E-mail:** shimpale@yahoo.com

Accepted on: 21-10-2015; **Finalized on:** 30-11-2016.

ABSTRACT

Now a day there is a growing interest of pharmaceutical industries to replace synthetic chemicals by natural products with bioactive properties that are of plant origin. The aim of present study was to assess the endemic medicinal plant *Adenoon indicum* Dalz. of Western Ghats of India for its Phytochemicals, antioxidant properties and to determine phenolic compounds by reverse-phase high performance liquid chromatography in three different solvent systems. It is noted that solvents significantly affects the quantity of Phytochemicals and antioxidant properties. In *A.indicum* total phenol content (TPC) was highest in methanol extract and lowest in acetone extract and same trend was observed for the total flavonoid content (TFC). All the extracts of the species showed 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Reducing power and Hydrogen peroxide free radical scavenging activity (H_2O_2). The results showed that among studied antioxidant activities acetone extract of the species showed highest Hydrogen peroxide free radical scavenging activity (82.43 %). The results provide justification for the use of this plant as a source of vegetable and in folk medicine to treat various diseases.

Keywords: *Adenoon indicum*, Bioprospecting, endemic, plant W.G.

INTRODUCTION

Medicinal plants are extensively valuable as they provide basic raw materials for different industries especially for pharmaceutical industries. Among the flowering plants, the family. Asteraceae is one of the dominant families, that comprising about 1528 genera and 22750 species.[1] While in India family Asteraceae possess 900 species.[2] The bio resources of Western Ghats are quite rich. In this unique region almost all groups of economically important plants grows that include numerous life saving drug plants, nutraceuticals, metal tolerant plants, wild aromatic plants and so many others. Still many species are await for their discoveries, the flora of this region is getting depleted in an alarming rate, therefore not just conservation of these bioresources but also their sustainable utilization for human welfare should be the priority agenda. Western Ghats harbor about 490 medicinal plants of which 308 are endemic and medicinally important and its utilization ranges from cancer to rheumatism treatments. This region possess many endemic medicinal plants, as their occurrence is restricted to a narrow geographical range, it's our prime duty to investigate such medicinal plants for their chemical potentials.

Genus *Adenoon* is belonging to family Asteraceae. This genus is endemic and mainly distributed in the forests of Northern Western Ghats.[3]

During explorations of flowering plants from the Western Ghats it is observed that some of the endemic plants of family Asteraceae are used as source of vegetables and also to cure various chronic diseases mainly by local folklore. The species is used in treatment of ulcer, insect bites, as an anti-migraine and tender leaves as a source of vegetables (*pers. commu.*).

Therefore to understand the important secondary metabolites of this species, the plants were analyzed for its Phytochemicals including total phenol (TP), total flavonoids (TF), Tannin, Saponnin and antioxidant potentials.

Sample Collection and Preparation of Extracts

The plant material of *Adenoon indicum* were collected from forest of Northern Western Ghats (*A. indicum*-Panchagani-GPS N 17° 56.350' E 073° 42.330' 4500 feet MSL).

The species were authentically identified with the relevant literature and deposited at Herbarium, Department of Botany, The New College, Kolhapur (Vouch. No. *A. indicum* VBS-1278). Whole plant was washed with water and dried under shade at room temperature.

The dried plant sample was used for further screening. One gram of dried material was pulverized in a mortar and extracted with 25 ml of solvent (acetone, ethanol and methanol). The contents were mixed on orbital shaker with constant stirring at 150 rpm for 24 hrs and centrifuged at 10,000 rpm for 10 min.

The supernatant was collected and filtered through Whatman filter paper (No. 1) and was used for further analysis.

All the chemicals used were analytical grades. Methanol, acetic acid and HPLC grade water purchased from Merck (Darmstadt, Germany) while gallic acid and quercetin

were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

Phytochemical Analysis

Total Phenolic Content (TPC)

The total phenolic content was estimated by the method of Bray and Thorpe with Folin–Ciocalteu reagent (FCR).[4] 1 gm of dried plant sample ground with pestle and mortar in 80% solvents (ethanol, methanol and acetone), centrifuged at 10,000 rpm for 20 min. and supernatant was evaporated to dryness, the residue was dissolved in 5 ml water then different aliquots (0.2-2 ml) were kept in test tubes and diluted it up to 3 ml with d/w, 0.5 ml of Folin–Ciocalteu reagent was added, after 3 min. 2 ml of 20% Na_2CO_3 was mixed, then tubes were placed in boiling water bath for 1 min. and cooled. The absorbance was measured at 650 nm against reagent blank. A calibration curve was done for standard phenol (gallic acid) using different concentration (R^2 =0.958) (Fig. 1A). The total phenolic content was expressed in mg of gallic acid equivalents (GAE)/g DW of extract.

Total Flavonoid Content (TFC)

Estimation of total flavonoid content was carried out by the $AlCl_3$ colorimetric method as given by.[5] 0.5 ml of extract of different solvents (ethanol, methanol and acetone) was mixed with 1.5 ml of methanol, to this mixture 0.1 ml 10% $AlCl_3$ was added and then 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water was added and kept for 30 min. at room temperature. The absorbance was measured at 415 nm. A calibration graph was performed for quercetin which was used as standard flavonoid (R^2 =0.979) (Fig.1B). The total flavonoid content was expressed in mg of quercetin equivalents (QE)/g DW of extract.

Determination of Saponnin

The total saponnin content was estimated by the method of Obadoni & Ochuko method.[6] About 5gm of plant sample was placed into a conical flask. And 50 ml 20% ethanol was added and then sample was heated for 4 hrs. With continuous stirring at 55°C. The mixture was filtrate and extracted. The concentration transfer into separating funnel and 10 ml diethyl was added and shaken vigorously. The aqueous of ether layer was discarded.

The decontamination method was frequent and 20 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath for 40°C. After the evaporation the samples were dried and calculate the percentage of saponnin.

Total Tannin Determination

About 5gm of sample was weighed into a conical flask and 50 ml of D/W was added and then shaken thoroughly for 30 min in a mechanical shaker. Solution was centrifuge and filtered into a 50 ml volumetric flask. Take the supernatant and dilute to 100 ml D/W. This diluted supernatant was used for tannin estimation. 5 ml of the diluted extract was pipette out into a test tube and mixed with 1ml Folin-Ciocalteu reagent. Then addition of 2.5 ml saturated Sodium carbonate. Make up the volume of 50 ml incubate at room temperature (RT) for 90 min then read the absorbance at 760 nm by using Spectrophotometer (Chemito UV 2100) at 760 nm.

Identification and Quantification of the Phenolic Compounds by RP-HPLC

The analysis of phenolic compounds was carried out by using High performance liquid chromatography (HPLC-DAD). The HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC20AD reciprocating pumps connected to the degasser DGU $20A_{5R}$, UV-VIS detector DAD (Diode array detector) SPD-M20A and software LC solution.

Reverse phase chromatographic analysis were carried out under gradient conditions using C18 column, the mobile phase was water containing 0.1% acetic acid (A) and methanol (B) and the composition gradient was 5% (B) for 1 min.; 10% (B) for 5 min.; 15% (B) for 10 min.; 40% (B) for 25 min.; 95% (B) for 45 min.; 5% (B) until 65 min. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration of 20 ppm. Quantification was carried out by integration of the peaks using the external standard method, at 272 nm for gallic acid and 370 nm for quercetin.

The flow rate was 1ml/min and the injection volume was 20µl.

The chromatographic peaks were confirmed by comparing their retention time and DAD-UV spectra with those of the reference standards. All chromatographic operations were performed at room temperature.

Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The antioxidant activity of the plant extracts in various solvent such as ethanol, methanol and acetone was estimated by using the DPPH radical scavenging protocol as given by Sutharsingh[7]. The ability of scavenging DPPH radical was calculated using the following formula:

DPPH scavenged (%) = ($A_{control} - A_{test}$ / $A_{control}$) x 100

Hydrogen Peroxide Scavenging Activity

Phosphate buffer saline (PBS) was used to prepare 20 mM hydrogen peroxide solution.

Different concentrations of plant extracts and ascorbic acid as a standard (1ml) in ethanol, methanol and acetone solvents were added to 2 ml of H_2O_2 solution in PBS. The absorbance was measured after 10 min. at 230 nm against a blank.[8] The hydrogen peroxide scavenging percentage was calculated using the following formula:

% scavenged [Hydrogen peroxide] = A_0 A_1 / A_0 x 100

Where, A_0 was the absorbance of the control, A_1 was the absorbance in the presence of the sample in different solvents and standard as ascorbic acid.

Reducing Power Assay

Different concentrations of plant extracts in various solvents were mixed with phosphate buffer of P^H 6.6 (2.5 ml) and 1% potassium ferricynaide (2.5 ml). The mixture was incubated at 40°C for 20 min., after cooling 10% trichloroacetic acid (2.5 ml) was added and centrifuged for 5 min. From this reaction mixture 2.5 ml of solution mixed with 2.5 ml distilled water and 0.1% ferric chloride (0.5 ml). Ascorbic acid was used as standard and control was prepared in the same manner but lacking of sample. The absorbance was measured at 700 nm. Higher is absorbance greater is the reducing power.[9]

Statistical Analysis

The statistical analysis was carried out by using Microsoft Excel 2010 software. The determination was repeated at least three times for each sample.

RESULTS AND DISCUSSION

Total Phenol and Flavonoid Content

It is reported that the antioxidant activity of plant origin components can be ascribed mainly due to the presence of phenolic compounds.[10] The results obtained in the present study for quantitative investigation for phenols and flavonoids are presented in Table 1. The observations showed that in the species total phenolic content was highest in methanol solvent extract followed by ethanol and acetone extract (Table-1). Total phenolic content was ranged from 2.25±0.1 mg GAE/g DW to 3.94±0.2 mg GAE/g DW (Table 1). In present study the result exposed a considerable diversity in the total phenol after change in the solvent.

It is proved that flavonoids are plant nutrients that when consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body.[11] It is also noted that most of the flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritic, antioxidant and immune-modulatory properties.[12,13] The amount of total flavonoid content in different solvent of plant extract assorted broadly ranging from 2.3±0.1 to 7.5±0.3 mg QE/g DW. The result exposed that higher level of flavonoid was found in ethanolic extract (7.5±0.3 mg QE/g DW) and lower in acetone extract (2.3±0.1 mg QE/g DW). High level of flavonoids justifies the use of species as a source of vegetable since flavonoids are biological antioxidants and good for management of cardiovascular diseases. Total saponnin content was maximum 03±0.034 (% W/W) and total tannin content was found maximum 02±0.34 (%W/W) in *A. indicum* plant extracts.

Table 1: Phytochemical Analysis of different Solvent Extracts of *A. indicum.*

S No.	Solvent	Total Phenol Content (mg/gm)	Total Flavonoid Content (mg/gm)	Total Tannin Content (% w/w)	Total Saponnin Content (% w/w)
1.	Methanol	3.94 ± 0.2	4.9 ± 0.2	_	_
2.	Ethanol	2.86 ± 0.6	7.5 ± 0.3	02 ± 0.34	03 ± 0.034
3.	Acetone	2.25 ± 0.1	2.3 ± 0.1	_	_

[a]Measurement are mean ± SE of three parallel determination and expressed as Gallic acid equivalent per gram dry weight.

[b]Measurement are mean ± SE of three parallel determination and expressed as quercetin equivalents per gram dry weight.

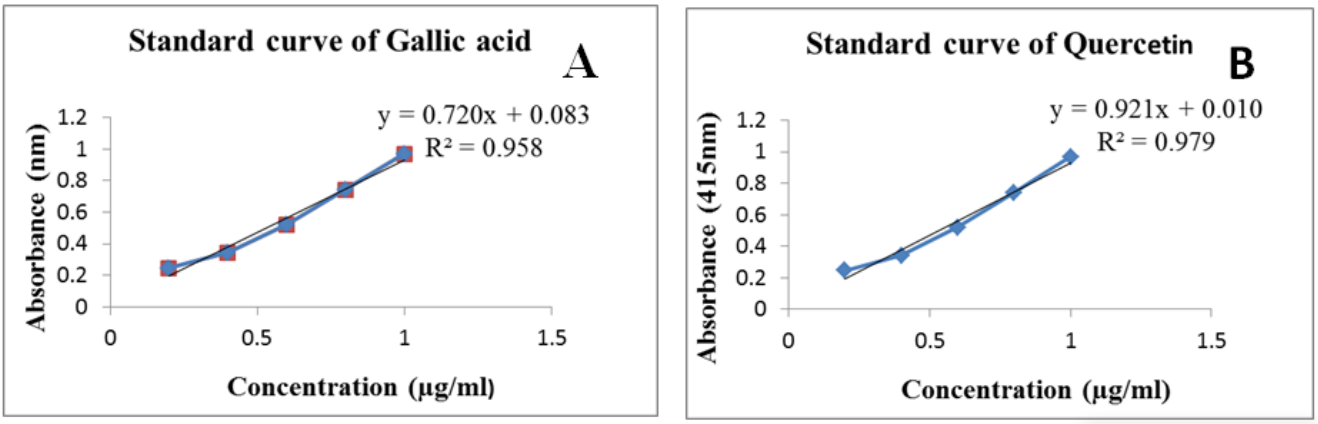

Figure 1: Standard curves: A- Standard curves of gallic acid, B- Standard curves of Quercetin.

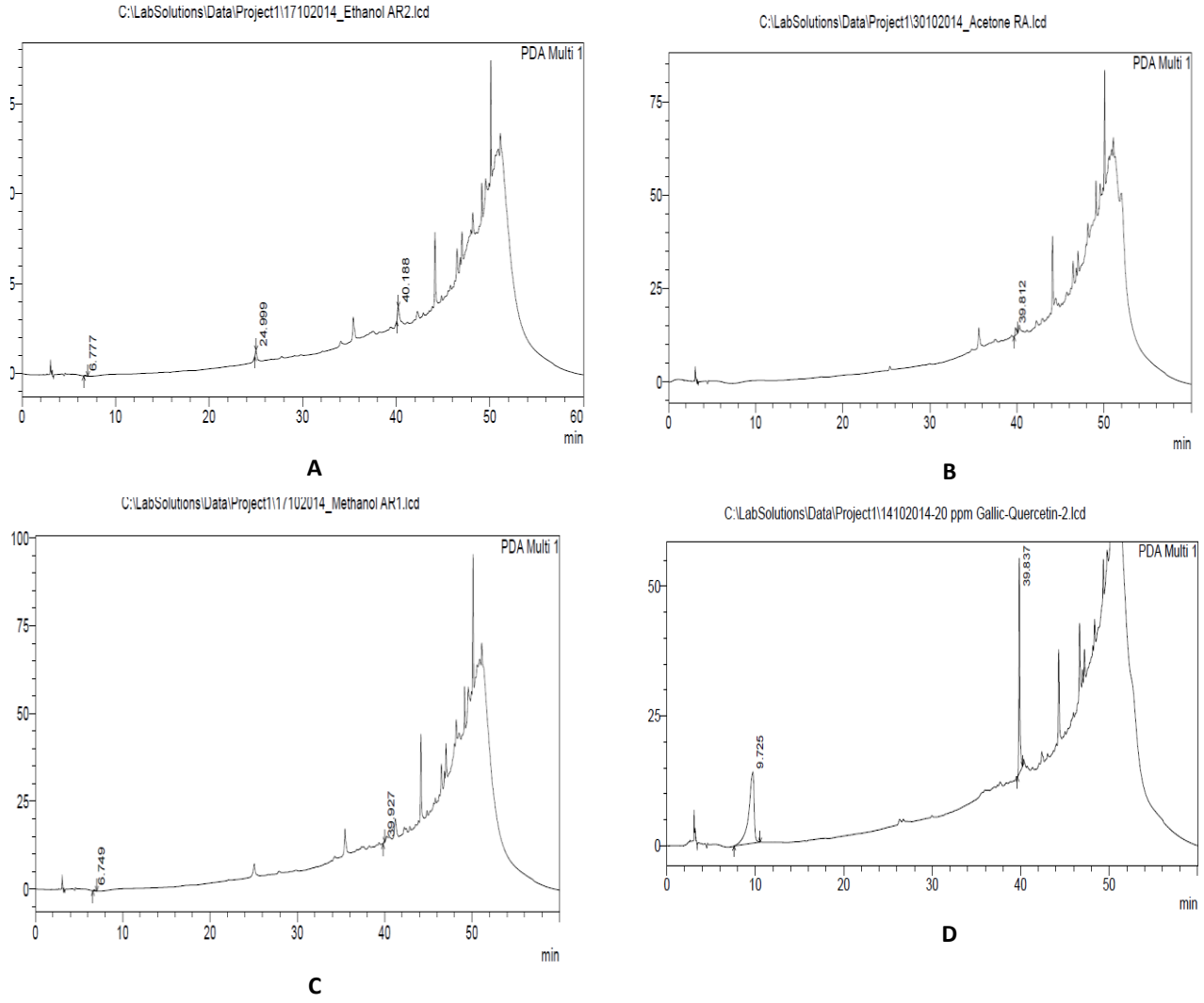

Figure 2: RP–HPLC Chromatograms of plant extract- *A. indicum*: A-Ethanolic extract B-Acetone extract C-Methanolic extract D - Standard phenolic compound at 20 ppm.

RP-HPLC Analysis of Phenolic Compounds

In present days standardization and characterization of herbal drugs is a topic of scientific interest especially in the herbal drug industry. Reversed phase HPLC has been used in a number of occasions for the analysis of phenol and flavonoids in plants. In present investigations endemic plant showed the considerable total phenolic content and therefore considered for RP–HPLC analysis. The HPLC profile in different solvent extracts like methanol, ethanol and acetone were acquired based in the reference standards (Fig. 1 A and 1B), in that gallic acid and quercetin was obtained with retention time (t_R) of 9.0 min. & 39.9 min. respectively (Fig. 2 D). Quercetin content in *A. indicum* was highest in acetone extract (6.515 ppm) followed by ethanol extract (2.01 ppm) and it was lowest in methanol extract (0.48 ppm) (Fig. 2 A-C). The sample was also screened for their gallic acid content but it was not detected in any studied sample in any solvent extract.

They also screened many aromatic plant species from Greece for gallic acid content by using Reversed phase HPLC and noted that some aromatic plants do not detect the gallic acid.[14]

DPPH Free Radical Scavenging Activity

The hydrogen atoms or the electron donation ability of the extracts was measured from the bleaching of purple-colored ethanol solution of DPPH. As a very stable organic free radical with a deep violet color, DPPH gives maximum absorption at the range of 515 to 528 nm.[15]

DPPH antioxidant property of *A. indicum* were studied in different solvent extracts like methanol, ethanol and acetone and it was observed in all the extracts.

In the results, *A. indicum* showed the highest DPPH free radical scavenging activity 72.90 ± 1.4% in methanolic extracts & lowest in acetone extracts (45.41 ± 0.97%).

The DPPH free radical scavenging activity for the plant sample is shown in Figure 3-A.

Reducing Power

Reducing power has been used as an antioxidant capability indicator of medicinal herbs.[16]

The reducing power of different solvent extracts was performed and showed solvent and concentration dependant manner.

In the present investigation *A. indicum* showed highest reducing power activity (61.44 ± 0.08%) in ethanol extract and acetone extracts showed lower reducing power activity (46.63 ± 0.15%).

The reducing power activity for the plant sample is shown in Figure 3-B.

Hydrogen Peroxide Free Radical Scavenging Activity

The extracts were capable of scavenging hydrogen peroxide in a concentration dependant manner.

The radical scavenging activity of *Adenoon indicum* plant extracts increased with increasing concentration shown in Figure 3-C.

A. indicum showed highest hydrogen peroxide activity for methanol extract (66.48 ± 0.04 %) and it was lowest in ethanol extract (52.42 ± 0.16 %).

This indicates that the plant species exhibited effective antioxidant activity.

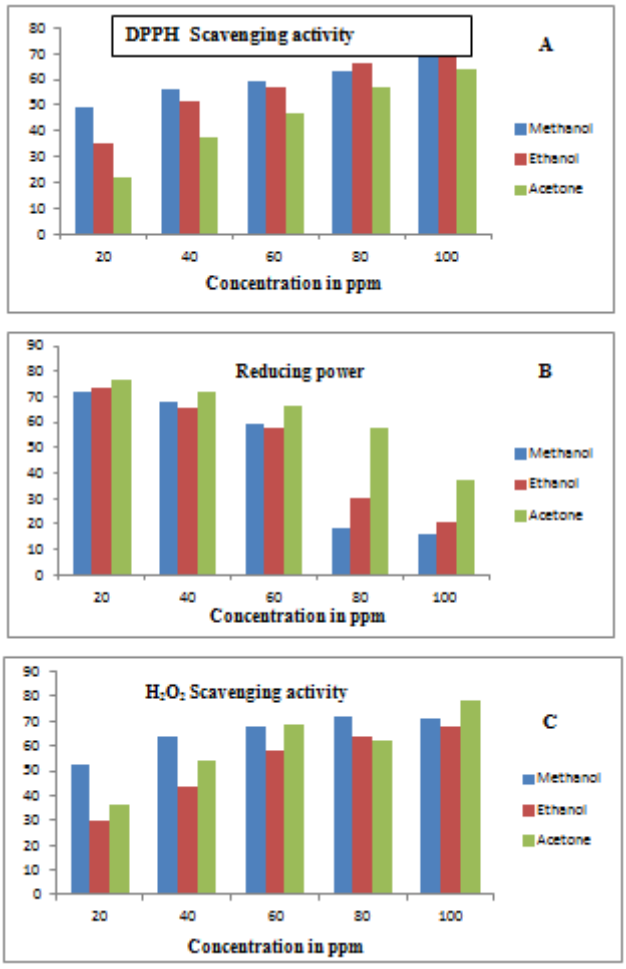

Figure 3: Antioxidant activity of various extract of *A. indicum* A: DPPH Assay, B: Reducing power assay, C: Hydrogen peroxide assay.

CONCLUSION

From the above results it revealed that the different solvent extracts of *A. indicum* shows elevated amount of phytochemicals such as phenolic and flavonoids, saponnin and tannin and also possesses good antioxidant activities.

Finally we conclude that *A. indicum* could be considered as potential source of vegetable which contains good amount of essential bioactive compounds.

The present data adds valuable information to the existing knowledge on *A. indicum* and can be used in the formulation of supplementary food & in various pharmaceutical preparations.

Acknowledgement: The authors are thankful to the University Grants Commission, New Delhi for financial assistance (42-977/2013/SR), thanks are also extended to Ms R. Sabale, Department of AgPM, Shivaji University, Kolhapur for providing RP-HPLC analysis.

REFERENCES

1. Bremer K. Asteraceae: Cladistics and classification Nord. J. Bot, 1994, 14-462.
2. Hajra PK, Rao RR, Singh DK, Uniyal BP. Flora of India Vol. 12 Botanical Survey of India, Calcutta. 1995, 454.
3. Irwin JS, Narasimhan D. Endemic genera of Angiosperms in India; Areview Rheedea, 21(1), 2011, 87-105.
4. Bray HG, Thorpe W. Analysis of phenolic compounds of interest in metabolism; Meth. Biochem. Anal, 1, 1954, 27-52.
5. Chang C, Yang M, Wen H. Chem. J. Estimation of total flavonoid content in propolis by two complementary colorimetric method. J.Food Drug Anal, 10, 2002, 178-182.
6. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of the crude extracts of some Homostatic plants in Edo and Delta states of Nigeria. Glob J Pure Apple Sci, 8b, 2001, 203–208.
7. Sutharsingh R, Kavimani S, Uvarani M, Thangathivupathi A. Quantitative phytochemical estimation and antioxidant studies on aerial parts of Naravelia zeylanic DC. International journal of pharmaceutical studies and research, 2(2), 2011, 52-56.
8. Chand T, Bhndari A, Kumawat BK, Sharma A, Pareek A, Bandsal VK. *In vitro* antioxidantactivity of aqueous extract of seed of Cucumis callosus (Rottl.) Cogn.Der Pharmacia Letter, 4, 2012, 840-844.
9. Oyaizu M. Studies on products of browning reaction: antioxidant activities of product of browning reaction prepared from glucose amine. JPN J Nutr. 44, 1986, 307-315.
10. Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci, 74, 2004, 2157-2184.

11. Taiz L. and Ziegler E. Plant Physiology 4th edn., ch. Sinauer Associates, Publishers, Massamchusetts, 13, 2006, 315-344.

12. Gill NS, Arora R, Kumar SR. Evaluation of antioxidant, anti-inflammatory and analgesic potential of the Luffa acutangula Roxb. var. amara. Res. J. Phytochem, 5, 2011, 201-208.

13. Wang M, Li K, Nie Y, Wei Y, Li X. Antirheumatoidal, Arthrities and chemical compositions of phenolic compounds-rich fraction from Urtica atrichocaulis, an endemic plant to China. Evidence-Based complementary and alternative medicine, 2012.

14. Proetos CN, Chorianopoulos, Nuchas GJ, Komaitis EM. RP-HPLC analysis of the Phenolic compound of plant extracts. Investigation of their antioxidant capacity & antimicrobial activity. J.Agric. food chem, 53, 2005, 1190-1195.

15. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of barks of Azadiracta indica, Terminalia arjuna acacia nilotica and Eugenia jambolana Lam. trees. Food chem, 104, 2007, 1106-1114.

16. Hsu CL, Chen W. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem, 83, 2003, 85-92.

Source of Support: Nil, **Conflict of Interest:** None.