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In vitro Anti-oxidant activities and phytochemical Content of aqueous Extracts of *Vernonia anthelmintica* (Bitter cumin) seed

Shivaralli S Spandana[1], Sulugodu R Nutan[2], Nethravathi BT[3], Aladahalli S Giresha[4*]
[1-3] Department of Studies in Botany, Jnana Kavery Campus, Mangalore University, Chikka Aluvara, Kodagu, Karnataka, India
[2] Department of Studies in Biochemistry, Jnana Kavery Campus, Mangalore University, Chikka Aluvara, Kodagu, Karnataka, India

Abstract
Various free radicals and related species such as reactive oxygen species/ROS and reactive nitrogen species/RNS were generated by various endogenously, physicochemical exposure or pathophysiological states able to alter lipids, proteins and DNA. Plants are the low-cost source of anti-oxidants with desirable nutrients. In this context, sequential solvent extracts of *Vernonia anthelmintica* (Kahi Jeerige/Bitter cumin) seed, was subjected to estimation phytochemical and evaluate antioxidant property. Among the extracts, aqueous *Vernonia anthelmintica* seed extract of (AqVA) exhibit higher levels of Phenolics, tannins, flavonoids, alkaloids and saponins followed by ethanol and water extract. The DPPH, nitric oxide, superoxide free radical scavenging activity and reducing power capacity of AVA exhibit increased activity by increased concentration. IC_{50} values of AqVA ranged from 80.25µg/ml to 191µg/ml.

Keywords: antioxidants activity, free radicals, phytochemicals, *Vernonia anthelmintica*, total phenols

1. Introduction
Antioxidant therapy has gained an immense importance in treatment of several oxidative diseases originated by oxidative stress due to free radicals [1]. These are radical scavengers; protect body against free radicals that may cause pathological conditions such as ischemia, neuro-degeneration, Parkinson's diseases, ageing process, asthma, arthritis, anaemia, and inflammation [2]. Free radicals such as superoxide anion, hydroxyl radicals and non radical species such as hydrogen peroxide, singlet oxygen are different forms of activated oxygen are main type of Reactive oxygen species (ROS) [3-4]. Overproduction these ROS from activated neutrophil and macrophages leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. ROS propagate inflammation by stimulating the release of the cytokines such as tumour necrosis factor α, interleukins [5], which stimulate recruitment of additional neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [6]. Inflammation is a complex process associated with pain and involves occurrences such as, the increase of vascular permeability, increase of protein denaturation and membrane alteration [7].
Phytochemicals with antioxidant properties are great interest due to their beneficial effects on human health as they provide protection against oxidative deterioration [8]. Many researchers reported that flavonoids are mainly responsible for the antioxidant activity [9] and antioxidant activities of dietary polyphenols are considered to be much greater than that of the essential vitamins [10]. *Vernonia anthelmintica* Wild. [kahi jeerige in Kannada) widely spread throughout Africa and Asia is an annual herb belongs to Asteraceae family [11-12]. The *V. anthelmintica* seed powder is used as a diuretic, tonic, anthelmintic, purgative and to treat snake bites [13-14]. According to Ayurveda, seeds are hot, acrid, astringent, anthelmintic; cure ulcers, vata and kapha also to cure skin disease, leucoderma and fever. The powdered seeds are applied externally in paralysis of the legs at Mundas of Chota Nagpur. The juice of the leaf is given to cure phlegmatic discharges from the nostrils [15-16]. Although the plant is of traditional and pharmacological relevance yet it has not been much explored. Thus, the goal of present research was to evaluate pharmacognostical parameters and antioxidant activity of *V. anthelmintica* fruits.

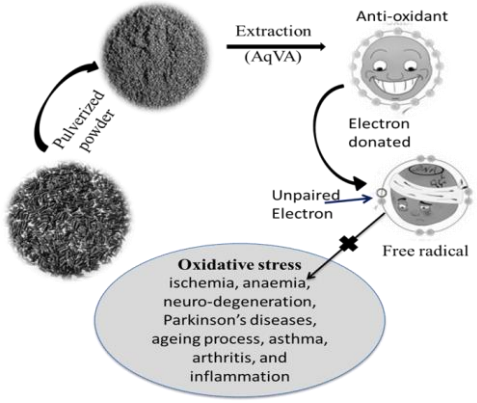

Fig 1

Kingdom:	Plantae
Order:	Asterales
Family:	Asteraceae (sunflowers)
Genus:	*Vernonia*
Species:	*Vernonia anthelmintica*

Fig 2

2. Methods

2.1 Materials

Diphenyl Picryl Hydrazyl radical (DPPH˙), Thiobarbituric acid, Quercetin, Gallic acid, were purchased from Sigma-Aldrich Chemical Laboratories, St. Louis, MO, USA. Aluchrosep silica Gel 60/UV254 TLC plates were obtained from S D FINE CHEM. Limited, Mumbai. Dimethyl sulphoxide (DMSO) and other chemicals purchased from Merck. All other chemicals and reagents used in this study were of analytical grade or better.

2.3. Collection of samples and preparation of extracts

Vernonia anthelmintica seeds were collected from Kodagu in November, 2017 and washed thoroughly under running tap water and then dried at 45°C for 48hrs. The 10g of pulverized sample was shaken separately in selected solvent in polarity wise for 72 hrs on an orbital shaker at room temperature. Extracts were filtered through Whatman No 1 filter paper, concentrated under reduced pressure at 40ºC using a rotary evaporator. The solid powder obtained was weighed to calculate the percentage yield from the initial weight. For the further studies the extracts were suspended in respective solvent to make 50 mg/ml stock solution.

The dried extracts were weighed to deter-mine the percentage yield of the soluble constituents using the formula:

% Yield: Weight of dry extract/ sample Weight taken for extraction × 100.

2.4 Phytochemical Estimation:

The photochemical analysis of different solvents extracts of *V. anthelmintica* were done by the method described in Yusuf *et., al* [17].

2.5 Estimation of Total Polyphenolic Content*:*

The total polyphenolic content of extracts was determined using folin ciocalteau's by colorimetrically [18]. Aliquotes (25-100µg) of Gallic acid was taken in test tubes and made up to 1ml with distilled water. Extract was also diluted accordingly; 1 ml of 1:1 FC reagent and 2ml of 10% sodium carbonate was added to each of the tubes. After 30 minutes absorbance was read at 760 nm against a blank. Concentration of polyphenols in extract was calculated using standard curve and expressed as gallic acid equivalents (mg of gallic acid/ g of dried extract).

2.6 Total Flavonoid Content

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to previous method [19]. Total mixture contains 1.5 ml ethanol (95%), 0.1 ml aluminium chloride (10%), 0.1 ml potassium acetate (1M) and 2.8 ml of distilled water was incubated at room temperature for 30 min. The absorbance was measured at 415 nm using Labman UV-Vis spectrophotometer. Total flavanoid content was calculated as quercetin equivalents (QE).

2.7 Anti-Oxidant Activity

2.7.1 DPPH Free Radical Scavenging Assay

Radical scavenging activity of aqueous extract of *V. anthelmintica* was measured according to the method of Blois with slight modification [19]. Briefly, 20-100µg Extracts were mixed with 5 ml of 0.1 mM methanolic solution of DPPH and incubated at 20°C for 20 min in complete dark. The DPPH alone serves as control and methanol was used for the base line correction. Gallic acid was used as standard. The absorbance of the samples was measured at 517 nm and radical scavenging activity was expressed as percentage activity using the following formula.

$$\% I = \{(Ao - A1)/Ao\} X\ 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against concentration.

2.7.3 NO Scavenging Activity

The scavenging effect of AqVA on NO was measured according to published [20]. Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) (pH 7.4) was mixed with different concentrations of the test sample (50-500 µg/ml) and incubated at 25^0C for 150 minutes. After incubation, nitrite produced from sodium nitroprusside was measured by Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% 1- naphthyl ethylenediamine dihydrochloride in water). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with 1-naphthylethyelenediamine dihydrochloride was measured at 570 nm. Catechin was used as a positive control. The percentage of NO scavenging activity was calculated using the following formula:

$$\{(Ao - A1)/Ao\} X\ 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of NJE or the standard sample.

2.7.4 Superoxide Radical Scavenging Activity

The reduction of NBT was assayed according to previously described method [19]. Superoxide radicals generated by non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system often reduce nitro blue tetrazolium (NBT) to a purple formazan. The 1 ml reaction mixture contains phosphate buffer (20 mM, pH 7.4], NBT (50 µM), NADH (73 µM), PMS (15µM) and extract (50-500µg/ml). Incubate for 5 min at room temperature and the absorbance was measured at 562nm against an appropriate blank to determine the quantity of formazan generated. Quercetin was used as positive control

$$\text{Percent inhibition (\%)} = (A_{control}\text{-}A_{test})/A_{control} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction at 560 nm and A_{test} represents the absorbance of a test reaction at 560 nm.

2.8 Determination of Reducing Power Assay

The reducing power ability of extracts was determined by the method as described in previous method [19]. The reaction mixture contains extracts (50- 500µg/ml) with equal volume of 0.2M phosphate buffer pH 6.6 and potassium ferricyanide was incubated at 50º C for 20 min. Centrifuged at 3000 rpm for 10 min by adding equal volume of 10% TCA to the mixture. To the upper layer, distilled water and 0.1% ferric chloride in a ratio of 1:1:2(v/v/v) were added. The absorbance was measured at 700 nm and increased absorbance of the reaction mixture compare to blank indicates increased reducing power activity. BHT was used as standard.

2.9 Anti-lipid peroxidation

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid rich medium [20]. Egg homogenate (0.5ml of 10% v/v) and 0.1ml of extract were added to a test tube and made up to 1ml with distilled water. 0.005ml of FeSO4 (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5ml of 0.8% (w/v) TBA in 1.1%sodium dodecyl sulphate and 0.5ml 20% TCA were added and the resulting mixture was vortexed and then heated at 95ºC for 60 min. if the sample have high amount of anthocyanin then to eliminate this non-MDA interference, another set of samples were treated in the same way, incubating without TBA. After cooling, 5.0ml of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532nm.Incubation of lipid peroxidation (%) by the extract was calculated according to

$$[1\text{-}Abs_{532}\text{+}TBA - Abs_{532}\text{-}TBA)/C] \times 100$$

Where C is the absorbance value of the fully oxidized control

2.10 Statistical Analysis

All the experiments were conducted with at least 3 independent repeats and the results were expressed as mean ± SD. Student's t-test was used to analyze the results and p value below 0.05 was considered as significant. For IC_{50} calculation and Graphs were plotted using GraphPad Prism software 5.0 (San Diego, CA, USA).

3. Result and Discussion

The damage of tissues and bio molecules leading to several disease conditions, mainly degenerative diseases and extensive lyses mainly mediated by free radicals that are constantly generated in living systems. The antioxidants work in repairing oxidative cell damages and thus prevent a lot of physical ailment [21]. Many synthetic drugs like non steroidal anti-inflammatory drugs (NSAIDs) protect against oxidative damage, but they have adverse side effects.

Consumption of natural antioxidants from food supplements and traditional medicine is the best way to overcome this problem. Photochemical/ biomolecules present in aromatic, medicinal, spices, vegetable, fruits and other plants contain exhibits antioxidant properties [22]. Various researchers also reported high antioxidant activities of different plant extracts using different assaying methods [23].

3.1 Phytochemical Content

Among the phytochemicals, total phenolics and total flavonoids contents are the widest secondary metabolite in plant kingdom. Because of ability of radical scavenging and metal chelating activities, these diverse groups of compounds have received much attention as potential natural antioxidant. Many researchers reported the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [22]. Flavonoids are a class of secondary plant phenolics with powerful antioxidant properties [23]. Qualitative estimation of phytochemical contents of *V. anthelmintica* extracts were exhibits greater total Phenolics and flavonoid contents followed by terpenoids, saponins, glycosides, alkaloids and tannins. Phytochemical content of all six extracts was found to decrease in the order AqVA > MVA > EVA > CVA >HVA > AVA (TAB.1).

Several Activity *In vitro* model systems have been used for assessing the scavenging activity of all extracts at 50µg concentration (Fig.3). AqVA exhibited the strongest radical scavenging activity compared to other extracts. Further, AqVA was subjected to check antioxidant activity by concentration dependent manner.

3.2 DPPH scavenging activity

DPPH free radical is stable nitrogen centred free radical commonly used for testing radical scavenging activity of the compound or plant extracts. The violet colour of the DPPH radical reduced to yellow colored diphenylpicrylhydrazyl radical after accepting an electron from the antioxidant compound, which is measured spectrometrically. Substances which are able to perform this reaction will be considered as antioxidants and therefore radical scavengers [24]. DPPH radical scavenging activity of AqVA was denoted in Fig. 4 over the range of 20–100 µg/mL concentration and the IC_{50} value was found to be 80.25µg/mL(Tab.2). Gallic acid used as standards.

3.3 Superoxide scavenging activity

Lot of research reveals that superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, ^{-}OH, per-oxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation [25-26]. The superoxide anion radical scavenging activity of AqVA was assayed by PMS-NADH system. The superoxide scavenging activity of AqVA increased markedly with the increase in concentrations (Fig. 4). The result suggested that AqVA is potent superoxide radical scavenger and the IC_{50} value found to be 173µg/mL (Tab.2).

3.4 NO Scavenging activity

NO is responsible for many physiologic and pathologic events because of its toxic nature with a free radical character [27]. Incubation of a sodium nitroprusside solution in PBS at 25^0C for 150 minutes resulted in linear time-dependent nitrite production, which was reduced by MCF in a concentration-dependent manner (Fig. 4). The moderate NO scavenging activity was observed by AqVA is 61.28% at 98.60µg/ml (Tab.2)

3.5 Reducing power activity

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action [28]. The reducing ability of a phytochemical generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [29]. Reducing power of the extracts increases with the increase in concentration and showed good reducing power ability in a dose dependent manner (Fig.4) with IC_{50} of 123µg/ml (Tab.2). The antioxidant principles present in AqVA cause the reduction of Fe3+/ferricyanide complex to the ferrous form and thus proved the reducing power ability.

3.6 Lipid peroxidation activity

Lipid peroxidation is widely recognized as primary toxicological event, which caused by the generation of free

radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds. In present work the TBARS assay was used to measure the degree of lipid peroxidation. TBA reacts specifically with malondialdehyde (MDA), a byproduct of lipid peroxidation to give a red chromogen, which can be read by spectrophotometrically [30]. All *V. anthelmintica* extracts capable to prevent the formation of MDA at 50µg/ml. But the highest anti-lipid peroxidation activity was revealed by the AqVA at dose dependent manner than other extracts (Fig. 5) and IC_{50} found to be 191µg/ml (Tab.2). These data suggest that, the extracts might prevent reactive radical species from damaging biomolecules such as lipoprotein, DNA, amino acids, sugar, proteins and PUFA in biological and food systems.

Figure and Table

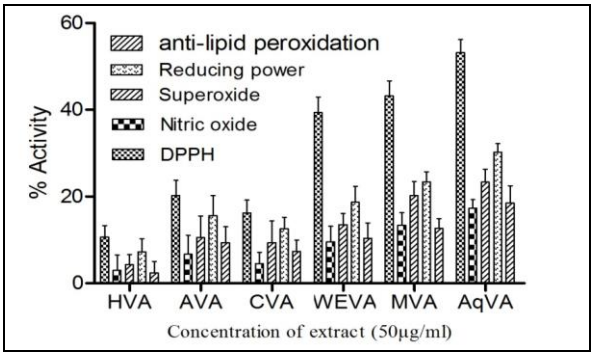

Fig 3: Anti-oxidant activities- DPPH, Superoxide, Nitric oxide radical scavenging and reducing power activity of *V. anthelmintica* seed extracts (50µg/ml).

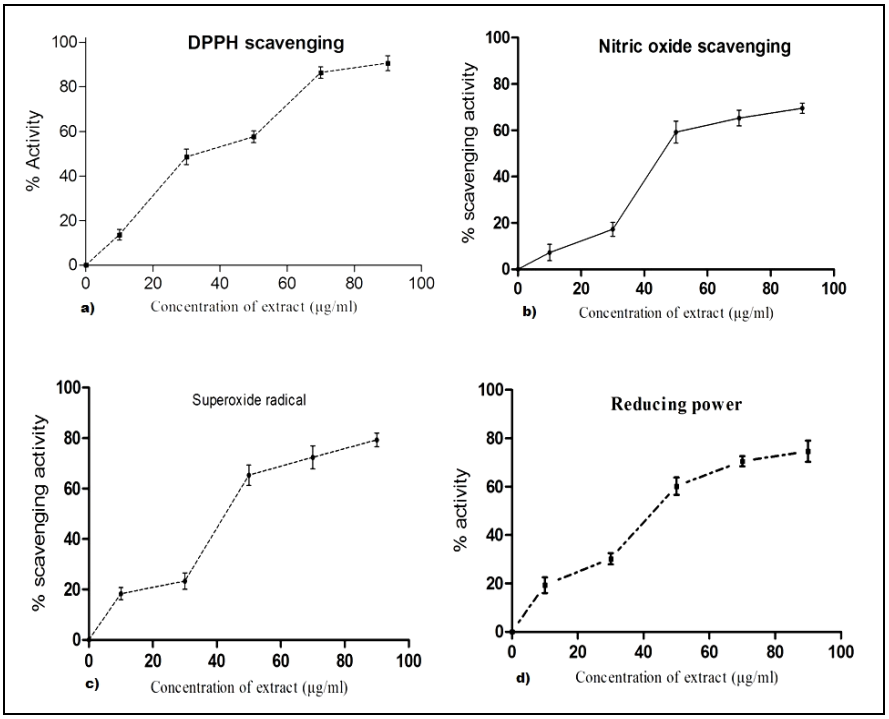

Fig 4: Anti-oxidant activity of AqVA. a) DPPH, b) Nitric oxide,c) superoxide scavenging and d) reducing power activity at concentration dependent manner (µg/ml). Data values are mean ± SD with significent from each other ($p \leq 0.05$)

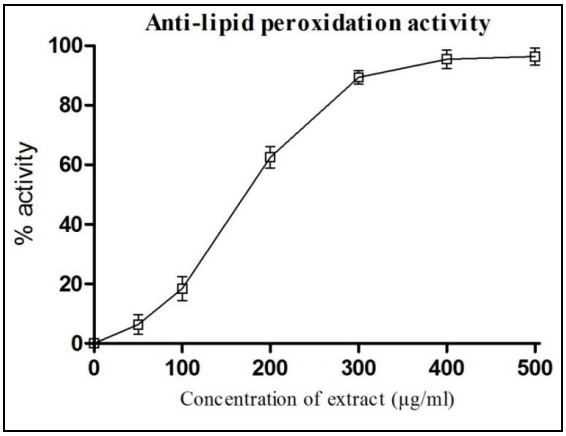

Fig 5: Anti-lipid peroxidation activity of AqVA at concentration dependent manner (µg/ml). Data values are mean ± SD with significant from each other ($p \leq 0.05$).

Table 1: Photochemical analysis of *V. anthelmintica* extracts

V. anthelmintica extracts	Phytochemicals						
	Tannins	**Alkaloids**	**Glycosides**	**Saponin**	**Phenolics**	**Terpenoids**	**Flavonoids**
Hexane	-	-	-	+	+	+	-
Acetone	-	-	-	-	+	-	-
Chloroform	+	-	+	+	+	+	-
Ethanol	+	+	+	+	++	+	+
Methanol	+	+	+	++	++	+	++
Water	++	+	+	++	+++	+	+++

Table 1: The different solvents extracts of *V. anthelmintica* were dried using flash evaporator. The 100µg of extracts were re-dissolved in the respective solvents and used for analysis. (+) present; (-) absent

Table 2: IC_{50} values of AqVA for DPPH, Nitric oxide, superoxide and reducing power activity.

S. No.	Scavenging activity	IC_{50} values of MEC µg/ml
1	DPPH	80.25
2	Nitric oxide	98.60
3	Superoxide	173.0
4	Reducing Power	123.0
5	ALP	191.0

7. Conclusion

Measurement of antioxidant capacity of plant sources is important to quantify the intake of natural antioxidants in a dietary supplement. Additionally, these studies will helps to select natural source of antioxidant and in the use of health relevant food industry. Although we have very rich history of their medicinal use in 'Ayurveda' limited antioxidant containing drugs and cosmetics was manufactured. Antioxidant can be used to produce medicines and cosmetics so that, the drug and cosmetic industries in our county can have their own herb based formulation. Hence, AqVA is rich source of total phenolics and flavonoids with good free radicals scavenging capacity may contribute a protective effect against oxidative damage induced to cellular macromolecules. The AqVA seems to be the most promising source of natural antioxidant compounds. Further, isolation of bioactive compounds required for identifying the unknown compounds to establish their pharmacological properties.

8. Acknowledgement

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9. References

1. Mahantesh SP, Gangawane AK, Patil CS. Free Radicals, Antioxidants, Diseases and Phytomedicines in Human Health: Future Perspects. World Research Journal of Medicinal & Aromatic Plants. 2012; 1(1):06-10.
2. Oke JM, Hamburger MO. Screening of Some Nigerian Medicinal Plants for antioxidant activity using 2, 2, Diphenyl- Picryl-Hydrazyl Radical. African Journal of Biomedical. Research. 2002; 5:77-79.
3. Halliwell B, Grootveld M. The measurement of free radical reactions in humans. FEBS Letters. 1987; 213:9-14.
4. Yildirim A, Mavi A, Oktay AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activity of tilia (Tilia argenta Desf. Ex. D.C.), sage (Salvia triloba L.) and black tea (*Camellia sinensis* L.) extracts. Journal of Agricultural and Food Chemistry. 2000; 48:5030-5034.
5. Scalbert A. Williamson G. Dietary intake and bioavailability of polyphenols. Journal of Nutrition. 2000; 130:2073-85.
6. Lavanya R, Maheshwari SU, Harish G, Raj JB, Kamali S, Hemamalani D, *et al.* Investigation of *In vitro* anti inflammatory, anti plate let and and arthritic activities in the leaves of Anisomeles malabarica L. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2010; 1:745-752.
7. Giresha AS, Pramod SN, Sathisha AD, Dharmappa KK. Neutralization of inflammation by inhibiting *In vitro* and *In vivo* secretory phospholipase A2 by ethanol extract of Boerhavia diffusa L. Phcognacy Research. 2017; 9:174-181.
8. Lin PY, Lai HM. Bioactive compounds in legumes and their germinated products. Journal of Agricultural Food Chemistry. 2006; 54:3807-3814.
9. Segev A, Badani H, Kapulnik Y, Shomer I, Oren-Shamir M, Galili S. Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). Journal Food Science. 2010; 75:115-119.
10. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L.) seed extracts. Food Chemistry. 2007; 101:10-19.
11. Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J Ethnopharmacol. 2013; 146:681-723.
12. Hua L, Li Y, Wang F, Lu D, Gao K. Biologically active steroids from the aerial parts of *Vernonia anthelmintica* Willd. Fitoterapia. 2012; 83:1036-1041.
13. Jaiswal V. Culture and ethnobotany of Jaintia tribal community of Meghalaya, northeast India- a mini review. Indian J Tradit Know. 2010; 9:38-44.
14. Parekh J, Chanda SV. Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turk J Biol. 2008; 32:63-71.
15. Adkarni KM. Indian Materia Medica. Mumbai: Popular Prakashan, 1982.
16. Mashelkar RA editor. Wealth of India, First supplement series (Raw Materials). New Delhi: Council of Scientific and Industrial Research, 2008.
17. Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA. Phytochemical analysis of the methanol leaves extract of Paullinia pinnata linn. J Pharm Phytother. 2014; 6:10-6.

18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16:144-158.
19. Giresha AS, Anitha MG, Dharmappa KK. Phytochemical composition, antioxidant and in-vitro anti-inflammatory activity of ethanol extract of Ruta graveolens L. Leaves. Int. J. Pharm. & Pharma Sci. 2015; 7:272-276.
20. Ohkowa H, Ohisi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction Anal. Biochem. 1979; 95(2):351-358.
21. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture. 2000; 80:561-566.
22. Aruoma OI. Free radicals, oxidative trace and antioxidants in human health and diseases. Journal of the American Oil Chemists' Society. 1987; 75:199-212.
23. Cotelle N, Bernier JL, Catteau JP, Pommery J, Walle JC, Gaydou EM. Antioxidant properties of hydroxyl flavones. Free Radical Biology and Medicine. 1996; 20:35-43.
24. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxi-dant activity of methanol extract of Ferula assafoetida and its essential oil composition, Grasas y Aceites. 2009; 60:405-412.
25. Marcocci L, Maguire JJ, Droy-Lafaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract. Biochemical and Biophysical Research Communications. 1994; 201:748-755.
26. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. *In vitro* Antioxidant and Free Radical Scavenging Activity of Diospyros lotus and Pyrus boissieriana growing in Iran. Pharmacognosy Magazine. 2009; 5:122-6.
27. Meir S, Kanner J, Akiri B, Hadar SP. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. Journal of Agricultural Food Chemistry, 1995; 43:1813-1817.
28. Yen G, Duh P. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. Journal of Agricultural Food Chemistry. 2008; 42:629-632.
29. Sharma. SK, Gupta VK. *In vitro* antioxidant studies of Ficus racemosa Linn Root. Pharmacognosy Magazine, 2008; 4:70-74.
30. Coppen PP. Rancidity in foods In: Allen JC, Hamilton RJ (Eds.), (Applied Science Publishers, New York, USA, 1983; 67.