ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 4, 2014

ISSN - 0974-2441

Research Article

STUDIES ON PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND EXTRACTION OF ACTIVE COMPOUND (SWERTIAMARIN) FROM LEAF EXTRACT OF *ENICOSTEMMA LITTORALE*

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Received: 22 May 2014, Revised and Accepted: 10 June 2014

ABSTRACT

Objective: The objective of the present study was to evaluate the phytochemical constituents, total phenol, total terpenoid, anti-oxidant activity and high-performance liquid chromatography (HPLC) analysis of swertiamarin compound from the leaf extract of *Enicostemma littorale.*

Methods: Preliminary screening involved the qualitative methods to detect the presence of terpenoids, flavonoids, phenols, tannins, steroids, quinones, saponins, cardiac glycosides and alkaloids. Total phenol and terpenoid contents were quantitatively estimated. Total phenolic content was estimated by Folin–Ciocalteau method. *In vitro* antioxidant activity of petroleum ether, chloroform, acetone, aqueous and ethanol extracts was evaluated by studying 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity using the standard procedure. The leaf extract was screened for a major metabolite namely swertiamarin compound using HPLC.

Results: The phytochemical analysis of leaf extract of *E. littorale* revealed the presence of significant secondary metabolites such as steroids, quinones, cardiac glycosides, saponins, tannins, phenols, flavonoids, terpenoids and alkaloids. The total phenol and terpenoid content in leaf extract were found to be 16.32 mg gallic acid equivalents/g and 71.0 mg/g respectively. The acetone leaf extract of *E. littorale* had showed significant radical scavenging activity. The results of -HPLC analysis in the leaf extract of *E. littorale* proved the presence of the active principle namely swertiamarin.

Conclusion: It can be concluded that *E. littorale* leaf extract can be used as a potent source of natural antioxidant and thus could prevent many free radical mediated diseases. The validated HPLC method can be used for routine quality control analysis.

Keywords: *Enicostemma littorale*, Antioxidant activity, Phenols, Terpenoids, Phytochemical screening.

INTRODUCTION

Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years, and they continue to be an important therapeutic aid for alleviating the ailments of humankind [1]. The therapeutic benefits are generally traced to specific plant compounds; specifically due to the active constituents of the plants [2]. Phytochemical screening of various plants has been reported by many workers [3,4]. These studies have revealed the presence of numerous chemicals, including alkaloids, flavonoids, steroids, phenols, glycosides, and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [5]. A number of studies have focused on the biological activities of phenolic compounds, which are antioxidants and free radical scavengers [6-8]. The crude extracts of herbs, spices and other plant materials rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [9].

The terpenoids form a group of compounds, the majority of which occur in the plant kingdom; a few terpenoids have been obtained from other sources. More terpenes have been discovered as an efficacious compound in human disease therapy and prevention. Terpenoid compound have been used to treat cancer, malaria, inflammation and a variety of infectious disease (viral and bacterial). Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body can cause oxidative damage of amino acids, lipids, proteins and DNA [10,11]. It has been established that oxidative stress is the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [12,13]. The screening of plant products for antibacterial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [14]. There has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years [15].

Enicostemma littorale blume (gentianaceae) is a glabrous perennial herb belonging to the family gentianaceae. It grows up to 1.5 feet height and more frequently near the sea throughout India. It is called as Chota-Kirayat in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu in Tamil [16]. The plant is locally used for its medicinal properties in Tamil Nadu, India, such as anti-inflammatory, anti-ulcer activity [17], hypoglycaemic [18], and anti-malarial activities [19]. The leaf possesses antioxidant property and helps in reducing obesity [20]. Hence, the present study was performed to investigate the phytochemical screening, total phenol, terpenoid, antioxidant activity, high-performance liquid chromatography (HPLC) analysis of swertiamarin from the leaf extract of *E. littorale*.

MATERIALS AND METHODS

Collection of *E. littorale*

The healthy plants of *E. littorale* (Fig. 1) were collected from different places of Tamil Nadu, namely, Tirunelveli, Thoothukudi and Chengalpet. The collected plants were brought to the laboratory and maintained at Poonga Biotech Research Centre, Plant Biotechnology division, Chennai – 600 094, Tamil Nadu, India.

Preparation of the plant extract

Preparation of the extracts was done according to a combination of the methods prescribed by Pizzale *et al.,* 2002 [21] and Lu and Foo, 2001 [22]. The dried leaf powder of *E. littorale* plant materials were extracted with

Fig. 1: Mother plant of *Enicostemma littorale* (a) Mother plant of *E. littorale* collected from Tirunelveli, (b) Shade dried plants of *E. littorale*

acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract for 1 minute using an ultra turax mixer (13,000 rpm) and soaked overnight at room temperature. The extracts were then filtered through what man No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in an airtight container in the refrigerator below 10°C.

Phytochemical screening of *E. littorale* leaf extracts

The phytochemical screening of leaf extracts were assessed by standard methods [23-25]. Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the leaf extracts tested.

Estimation of total phenol content in leaf extracts of *E. littorale*

Total phenolic content in the leaf extracts was determined by the Folin–Ciocalteau colorimetric method [26]. For the analysis, 0.5 ml of aliquot of sample was added to 0.5 ml of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of total terpenoid content in leaf extracts of *E. littorale*

Total terpenoid content in the leaf extracts were assessed by standard method [27]. 1 g of *E. littorale* leaf powder was taken separately and soaked in alcohol for 24 hrs. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoid.

Qualitative analysis of antioxidant activity of *E. littorale*

The antioxidant activity of leaf extracts of *E. littorale* was determined by standard method [28,29]. 50 µl of leaf extracts of *E. littorale* were taken in the microtiter plate. 100 µl of 0.1% methanolic 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered to be strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *E. littorale*

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf extract of 100 µl were mixed with 2.7 ml of methanol and then 200 µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank containing the same amount of methanol and DPPH solution was prepared and measured as a control [30]. Subsequently, at every 5 minutes interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of butylated hydroxy toluene (BHT). The experiment was carried out in triplicates.

The capacity of scavenging free radicals was calculated as scavenging activity (%) =

$$\frac{\text{Absorbance in control} - \text{Absorbance in sample}}{\text{Absorbance in control}} \times 100$$

HPLC analysis of swertiamarin compound

The fine powder of the leaf biomass was extracted with 75% of ethanol and then the extract was evaporated. The residue of extract was mixed with n-butanol and water (2:1) and both the upper layer of n-butanol and lower layer of water were separated and evaporated under vacuum. The residues were washed with petroleum ether to remove fatty components and then extracted with methanol. The concentrated extract in methanol was separated and analyzed using -HPLC as per standard method [31].

The extracts were filtered through sartorius regenerated cellulose-membrane syringe filter (0.2 µ) and 20 µl of filtrate was injected into the HPLC. Chromatography was performed using Shimadzu HPLC (Model SPD-10A UV-VIS Detector) and supelcosil LC-18 column (25 cm × 4.6 mm, 5 m) with mobile phase consisting of acetonitrile, water and acetic acid (50:50:0.1). Flow rate was maintained at 1.0 ml/minute with a back pressure of 250 psi and the compounds were read at 210 nm using a UV detector. The total run time was 20 minutes, but preferably it was extended up to 40 minutes [31]. The results were compared with standard.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaf extracts of *E. littorale* collected from different places namely Tirunelveli, Thoothukudi and Chengalpet has been shown in Tables 1-3, respectively. The study revealed that among various extracts, the acetone leaf extract of *E. littorale* (Tirunelveli accession) were rich in secondary metabolites such as tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenol, steroid, coumarins and alkaloids followed by other accessions.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc., [32]. Thus, the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development [33]. The presence of alkaloids and saponins in the leaf extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities [34]. Saponins have properties of precipitating and coagulating red blood cells, and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity [35] and traditionally saponins have been extensively used as detergents and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [36]. Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics.

Table 4 shows the estimation of total phenol content in the acetone leaf extract of *E. littorale.* The higher content of total phenol (16.32 mg GAE/g) was found in leaf extract of *E. littorale* (Tirunelveli accession), followed by other accessions. Phenolic compounds are important plant antioxidants, which exhibited considerable scavenging activity against radicals. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [37]. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [38-40]. Similarly, Shahidi and Naczk [41] reported that naturally occurring phenolics exhibit antioxidant activity.

The total terpenoid content of *E. littorale* leaf extract (Tirunelveli accession) is shown in Table 5. The result revealed that acetone leaf extracts of *E. littorale* recorded terpenoid content of 71.3 mg/g. Terpenes play an important role as signal compounds and growth regulators of plants [42].

Table 1: Phytochemical screening of *E. littorale* leaf extracts (Tirunelveli accession)

Phytochemicals	*E. littorale* leaf extract				
	Aqueous	Acetone	Ethanol	Chloroform	Petroleum ether
Tannins	+	++	+	+	–
Saponins	+	++	+	–	–
Flavonoids	+	++	+	+	–
Quinones	+	+	+	+	–
Glycosides	–	++	–	–	–
Cardiac glycosides	++	++	+	–	–
Terpenoids	+	+	+	+	+
Phenol	+	+	+	–	–
Coumarins	++	++	++	–	–
Steroids	–	+	+	+	+
Alkaloids	+	+	+	–	–

++: Strong positive,+: Positive, –: Negative, *E. littorale: Enicostemma littorale*

Table 2: Phytochemical screening from leaf extracts of *E. littorale* (Thoothukudi – accession)

Phytochemicals	*E. littorale* leaf extract				
	Aqueous	Acetone	Ethanol	Chloroform	Petroleum ether
Tannins	+	+	+	+	–
Saponins	+	++	+	–	–
Flavonoids	+	+	+	+	–
Quinones	+	+	+	+	–
Glycosides	–	+	–	–	–
Cardiac glycosides	+	+	–	–	–
Terpenoids	+	+	+	+	+
Phenol	+	+	+	+	+
Coumarins	+	++	++	–	–
Steroids	–	+	–	–	–
Alkaloids	–	–	+	–	–

++: Strong positive, +: Positive, –: Negative, *E. littorale: Enicostemma littorale*

Table 3: Phytochemical screening from leaf extracts of *E. littorale* (Chengalpet accession)

Phytochemicals	*E. littorale* leaf extract				
	Aqueous	Acetone	Ethanol	Chloroform	Petroleum ether
Tannins	+	+	+	–	–
Saponins	+	++	+	–	–
Flavonoids	–	–	–	+	–
Quinones	+	+	–	–	–
Glycosides	–	+	–	–	–
Cardiac glycosides	+	+	–	–	–
Terpenoids	+	+	+	+	+
Phenol	+	+	+	–	–
Coumarins	+	+	+	–	–
Steroids	–	+	–	–	–
Alkaloids	–	–	+	–	–

++: Strong positive, +: Positive, –: Negative, *E. littorale: Enicostemma littorale*

The presence of terpenoids has been reported in medicinal plants [43,44]. Terpenoid promotes glutathione-S-transferase and cancer cell apoptosis; hence, terpenoids have been used for anti-cancer properties [45].

E. littorale leaf extracts were further analyzed for the presence of antioxidants. Table 6 shows the qualitative antioxidant analysis in the leaf extracts of *E. littorale* collected from various accessions. The results revealed strong positive response for acetone leaf extract (Tirunelveli accession), followed by others. Scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Free radicals have a broad range of effects in biological systems. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants [46-48].

Table 4: Estimation of total phenol content of acetone leaf extract of *E. littorale*

Serial number	*E. littorale* leaf extract	Total phenol content (mg GAE/g)
1	*E. littorale* - Tirunelveli accession	16.32
2	*E. littorale* - Thoothukudi accession	13.64
3	*E. littorale* - Chengalpet accession	9.11

E. littorale: Enicostemma littorale, GAE: Gallic acid equivalents

The percentage of DPPH radical scavenging activity of leaf extracts of *E. littorale* from various accessions is shown in Fig. 2. The results revealed that among three accessions and five different solvent extracts of *E. littorale*, the acetone leaf extract collected from Tirunelveli had maximum DPPH radical scavenging activity (91.33%), followed by Thoothukudi

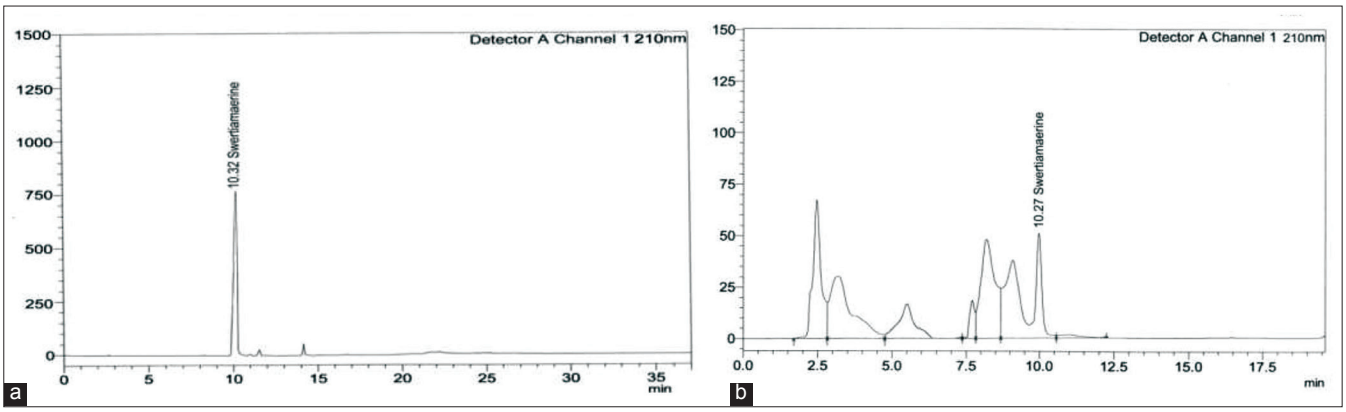

Figure 3: HPLC analysis of swertiamarin compound from *Enicostemma littorale* leaf extract (Tirunelveli accession) (a) Swertiamarin standard (1 mg/1ml) -Sigma aldrich (b) Leaf extract of *Enicostemma littorale*

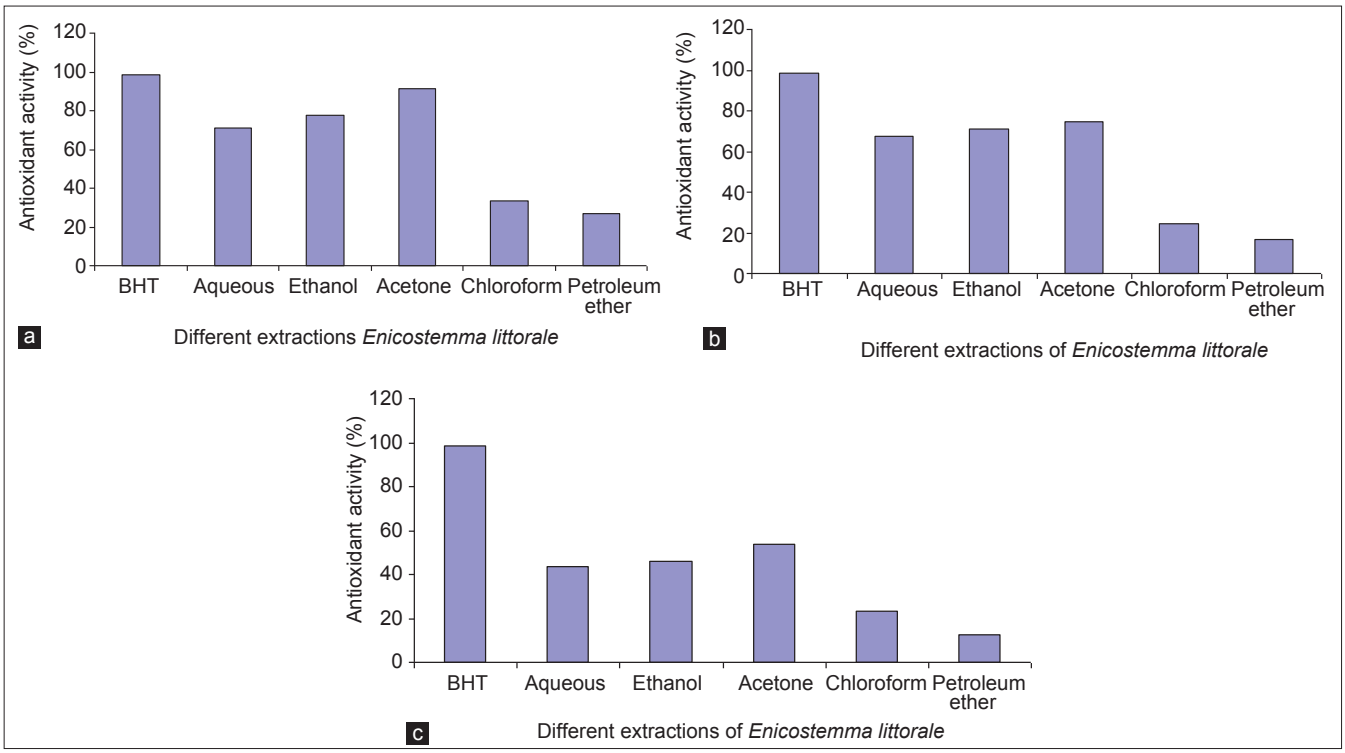

Fig. 2: Quantitative analysis of antioxidant activity of *Enicostemma littorale* leaf extracts (a) Tirunelveli, (b) Thoothukudi and (c) Chengalpet

(74.6%) and Chengalpet accession (56.9%), when compared with that of synthetic antioxidant BHT as a positive control (98.36%). In all accessions, acetone leaf extracts recorded higher percentage of free radical scavenging activity followed by ethanol, aqueous, chloroform and petroleum ether.

The HPLC analysis of swertiamarin compound from *E. littorale* leaf extract along with the standard swertiamarin has been represented in Fig. 3. Swertiamarin compound eluted through HPLC analysis and based on standard retention time (Rt) 10.32 min. The *E. littorale* leaf extract (Tirunelveli accession) used for HPLC analysis recorded a Rt of 10.27 minutes and *standard* swertiamarin compound recorded a Rt *of* 10.32 minutes, thus confirming the presence of swertiamarin compound in leaf extract of *E. littorale*.

CONCLUSION

The present study revealed that acetone leaf extract of *E. littorale* was rich in phytochemical constituents and high levels of total phenolic and terpenoid compounds. The leaf extract of *E. littorale* also possessed strong antioxidant potential and was thus capable of inhibiting, quenching free radicals to terminate the radical chain reaction. The HPLC analysis revealed the identification of active compound namely swertiamarin present in the leaf extract of *E. littorale.* The results indicate that the plant material may become an important source of natural drug compounds with health protective potential and natural antioxidants of significant impact on the status of human health and disease prevention.

REFERENCES

1. Momin RK, Kadam VB. Determination of ash values of some medicinal plants of genus *Sesbania* of Marathwada region in Maharashtra. J Phytol 2011;3(12):52-4.
2. Mary HP, Tina AV, Jeeja KJ, Abiramy MR, Sajina N, Jaya Sree S. Phytochemical analysis and anticancer activity of essential oil from *Myristica fragrans*. Int J Curr Pharml Rev Res 2012;2(4):188-98.
3. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical

Table 5: Estimation of total terpenoid content of acetone leaf extract of *E. littorale*

Serial number	*E. littorale* leaf extract	Total terpenoid content (mg/g)
1	*E. littorale* - Tirunelveli accession	71.3 mg

E. littorale: Enicostemma littorale

Table 6: Qualitative analysis of antioxidant activity of *E. littorale* leaf extracts

Serial number	*E. littorale* leaf extracts	Qualitative response of DPPH assay		
		Tirunelveli accession	Thoothukudi accession	Chengalpet accession
1	Aqueous	+	+	+
2	Ethanol	+	+	+
3	Acetone	+++	++	+
4	Chloroform	–	–	–
5	Petroleum ether	–	–	–

E. littorale: Enicostemma littorale, DPPH: 1,1-diphenyl-2-picrylhydrazyl

screening of some species of Iranian plants. Iran J Pharm Res 2003;2:77-82.
4. Parekh J, Chanda S. Phytochemicals screening of some plants from Western region of India. Plant Arch 2008;8(2):657-62.
5. Hagerman AN, Rield KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, *et al*. High molecular weight plat polyphenolics (tannins) as biological antioxidants. J Agric Food Chem 1998;46:1887-92.
6. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic Res 1995;22(4):375-83.
7. Cespedes CL, El-Hafidi M, Pavon N, Alarcon J. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* [Elaeocarpaceae], *Maqui.* Food Chem 2008;107(2):820-9.
8. Reddy BS, Reddy BP, Raghavulu SV, Ramakrishna S, Venkateswarlu Y, Diwan PV. Evaluation of antioxidant and antimicrobial properties of soymida febrifuga leaf extracts. Phytother Res 2008;22(7):943-7.
9. Chu YH, Chang CL, Hsu HF. Flavonoid content of several vegetables and their antioxidant activity. J Sci Food Agric 2000;80(5):561-6.
10. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28.
11. Halliwell B. Antioxidant characterization. Methodology and mechanism. Biochem Pharmacol 1995;49(10):1341-8.
12. Gülçin I, Oktay M, Küfrevioglu OI, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol 2002;79(3):325-9.
13. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India 2004;52:794-804.
14. Afolayan AJ. Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. Pharm Biol 2003;41(1):22-5.
15. Okunji CO, Wu MW, Duncan AR. New antimicrobials of plant origin. In: Janick J, editor. Perspectives on New Crops and New Uses. Alexandra, V.A: ASHA Press; 1999. p. 457-62.
16. Thirumalai T, Viviyan TS, Elumalai E, David E. Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume. Asian Pac J Trop Biomed 2011;1(5):381-5.
17. Roy S, Niranjan C, Jyothi T, Shankrayya M, Vishawanath K, Prabhu K, *et al.* Antiulcer and anti-inflammatory activity of aerial parts *Enicostemma littorale* Blume. J Young Pharm 2010;2(4):369-73.
18. Vishwakarma SL, Sonawane RD, Rajani M, Goyal RK. Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptozotocin-induced type 1 diabetic rats. Indian J Exp Biol 2010;48:26-30.
19. Ookokon J E, Nwafor PA, Abia GO, Bankhede H K. Antipyretic and antimalarial activities of crude leaf extract and fractions of Enicostema littorale. Asian Pacific Journal of Tropical Disease 2012; 442-447.
20. Gite VN, Pokharkar RD, Chopade VV, Takate SB. Hepatoprotective activity of *Enicostemma axillare* in paracetamol induced hepato-toxicity in albino rats. J Pharmocol 2010;1(2):50-3.
21. Pizzale L, Bortolomeazzi R, Vichi S, Conte LS. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. J Sci Food Agric 2002;82(14):1645-51.
22. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem 2001;75(2):197-202.
23. Finose A, Gopalakrishnan V. Phytochemical screening, HPTLC and GC-MS profiling in the rhizomes of *Zingiber nimmonii* J. Graham Dalzell. Asian J Pharm Clin Res 2014;7(2):54-7.
24. Savithramma N, Linga RM, Bhumi G. Phytochemical screening of *Thespesia populnea* [L.] *Soland* and *Tridax procumbens L.* J Chem Pharm Res 2011;3(5):2834.
25. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies of *Merugan kizhangu*. Bull Med Ethono Bot Res 1981;3(1):84-96.
26. Slinkard K, Singleton VL. Total phenol analysis: Automation and comparison with manual Methods. Am J Enol Vitic 1977;28(1):49-55.
27. Ferguson NM. A Text Book of Pharmacognosy. New Delhi: Mac Milan Company; 1956. p. 191.
28. Hsiao G, Teng CM, Wu CL, Ko FN. Marchantin H as a natural antioxidant and free radical scavenger. Arch Biochem Biophys 1996;334(1):18-26.
29. Abirami MS, Muthuswamy. Antioxidant potential, total phenolic and total flavonoids content of various extracts from whole plant of *Polycarpaea corymbosa* lam. Asian J Pharm Clin Res 2013;6(4):121-4.
30. Lee SE, Hwang HJ, Ha JS, Jeong HS, Kim JH. Screening of medicinal plant extracts for antioxidant activity. Life Sci 2003;73(2):167-79.
31. Shimizu T, Muroi T, Ichi T, Nakamura M, Yoshihira K. Analysis of red cabbage colors in commercial foods using high performance liquid chromatography with photodiode array detection-mass spectrophotometry. J Food Hyg Soc Japan 1997;38(1):34-38.
32. Britto JD, Sebastian SR. Biosynthesis of silver nano particles and its antibacterial activity against human pathogens. Int J Pharm Pharm Sci 2011;5:257-9.
33. Doss A, Mubarak HM, Dhanabalan R. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J Sci Technol 2009;2(2):41-3.
34. Stary F. The Natrural Guide to Medicinal Herbs, and Plants. London: Tiger Books International; 1998. p. 12-6.
35. Sodipo OA, Akiniyi JA, Ogunbamosu JU. Studies on certain characteristics of extracts of bark of *Pansinystalia macruceras* [K schemp] pierre exbeille. Glob J Pure Appl Sci 2000;6:83-7.
36. Shi J, Kakuda Y, Yeung D. Antioxidative properties of lycopene and other carotenoids from tomatoes: Synergistic effects. Biofactors 2004;21(1-4):203-10.
37. Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. Why do we expect carotenoids to be antioxidants *in vivo*? Free Radic Res 1997;26(4):381-98.
38. Zheng W, Wang SY. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J Agric Food Chem 2003;51(2):502-9.
39. Chinnici F, Bendini A, Gaiani A, Riponi C. Radical scavenging activities of peels and pulps from cv. golden delicious apples as related to their phenolic composition. J Agric Food Chem 2004;52(15):4684-9.
40. Huang Z, Wang B, Eaves DH, Shikany JM, Pace RD. Total phenolics and antioxidant capacity of indigenous vegetables in the Southeast United States: Alabama collaboration for cardiovascular equality project. Int J Food Sci Nutr 2009;60(2):100-8.
41. Shahidi F, Naczk M. Method of analysis and quantification of phenolic compounds. Lanchester: Technomic Publishing Company; 1995. p. 287-93.
42. Onyeike EN, Ikewuchi JC, Ikewuchi CC, Uwakwe AA. Quantitative high performance liquid chromatographic analysis of simple terpenes, carotenoids, phytosterols and flavonoids in the leaves of *Acalypha wilkesiana* muell arg. Pac J Sci Technol 2010;11(2):480-7.
43. Gill LS. Ethnommedical uses of plants in Nigeria. Benin City, Nigeria: Uniben Press; 1992. p. 276.
44. Rahila T, Rukhasandra N, Zaidi AA, Shamshila R. Phytochemical screening of medicinal plants belonging to family *Euphorbiacea*e. Pak Vet J 1994;14(3):160-2.
45. Dillard CJ, German JB. Phytochemicals: Nutraceuticals and human health. J Sci Food Agric 2000;80:1744-56.
46. Gálvez M, Martín-Cordero C, Houghton PJ, Ayuso MJ. Antioxidant activity of methanol extracts obtained from Plantago species. J Agric Food Chem 2005;53(6):1927-33.
47. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chem 2006;95(2):200-4.
48. Mammadov R, Ili P, Vaizogullar HE. Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora*. Iran J Chem Chem Eng 2011;30(3):57-62.