Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(8); 1363-1371

ISSN: 0975-4873

Research Article

Screening of Phytoconstituents, FT-IR Analysis and *In vitro* Antioxidant Potential of an Endemic Plant *Crotalaria longipes* Wight & Arn.

Paulpriya K, Tresina P S, Sornalakshmi V, Mohan V R*

Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

Available Online:10th August, 2016

ABSTRACT

The present study is to investigate the phytochemical and *in vitro* antioxidant activity of different solvent extracts of aerial part of *Crotalaria longipes*. Preliminary phytochemical screening was carried out using standard procedures. The total phenolic content of the methanol extract was determined spectrophotometrically according to the Folin-Ciocalteau procedure and total flavonoid content of methanol extract was determined by Aluminium chloride method. The preliminary phytochemical screening revealed that alkaloids, anthraquinones, coumarins, catechin, glycosides, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, sugars and xanthoproteins was found to be present in the methanol and ethanol extracts of aerial part of *C. longipes*. The FT-IR spectrum confirmed the presence of C-I, C-F, C-N, C-O, C=C, C=O, C-H and O-H groups. The total phenolic and total flavonoid content of the methanol extract was found to be 1.08 g 100 g^{-1} dry weight basis and 1.24 g 100 g^{-1} dry weight basis respectively. DPPH, hydroxyl, superoxide radical scavenging activity and ABTS radical cation scavenging activity of methanol extract of *C. longipes* aerial part exhibited the IC_{50} values of 32.41, 29.13, 36.91 and 33.16 µg/mL respectively. The reducing power activity showed increase with increase in concentration of extracts. The antioxidant potential may be directly linked to the phenolic and flavonoid contents present in the aerial part of *C. longipes*. The outcome of the present investigation clearly indicates that *C. longipes* aerial part showed potential phytochemicals and they can be used as antioxidants.

Keywords: Phytochemicals, FT-IR, DPPH, ABTS, flavonoid.

INTRODUCTION

A plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, steroids, tannins, etc. that exert a physiological effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites[1]. These compounds have been extracted with various solvents by different screening techniques[2]. The phytochemical research based on ethno-pharmacological information is generally considered to be an effective approach in the discovery of new anti-infective agents from higher plants[3]. Free radicals have significant role in the causation of several diseases such as diabetes, cirrhosis, cancer and cardiovascular diseases[4,5]. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Thus, compounds or antioxidants that can scavenge free radicals have vital role in the improvement of these diseased conditions[6]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) have also been widely used in food, however, some side effects were registered[7]. Therefore, investigations of new natural sources of antioxidants are on the increase[8]. The efficacy of a plant extract as an antioxidant is best evaluated based on results obtained by commonly accepted assays, taking into account different oxidative conditions, system compositions and antioxidant mechanisms[9,10]. Plants contain a wide variety of free radical scavenging molecules such as phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity[11]. Natural antioxidants tend to be safer and they also possess antiviral, anti-inflammatory, anticancer and hepatoprotective properties[12]. Therefore, the evaluation of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals. The genus *Crotalaria* Linn. (Fabaceae) has 300 species worldwide and about 18 species are reported in India. *Crotalaria* species have been reported to contain alkaloids, saponins, and flavonoids as notable chemical markers with basic N-oxides of the genera Leguminaceae having antileukemic, antitumour, antispasmodic, antineoplastic,

*Author for Correspondence: vrmohanvoc@gmail.com

Table 1: Preliminary phytochemical screening of aerial part of *C. longipes*.

Test	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloid	-	-	+	+	+
Anthraquinone	+	-	+	+	+
Catechin	+	-	+	+	+
Coumarin	-	+	+	+	+
Flavonoid	-	+	+	+	+
Phenol	+	-	+	+	+
Quinone	-	+	-	+	+
Saponin	-	-	+	+	+
Steroid	-	+	+	+	+
Tannin	-	-	+	+	+
Terpenoid	+	+	+	+	+
Sugar	-	-	+	+	+
Glycoside	-	-	+	+	+
Xanthoprotien	-	-	-	+	+
Fixed oil	+	+	+	-	+

+ Presence - Absence

Table 2: FTIR spectroscopic data of *Crotolaria longipes* aerial part.

Stretching Frequency (cm^{-1})	Functional Group	Tentative Assignments
575.29	C-I Stretching	Aliphatic iodo
1021.46	C-F Stretching	Aliphatic fluoro
1157.30	C-N Stretching	Tertiary Amine
1243.46	φ-O-H aryl-O Stretching	Aromatic Ether
1386.16	C-O Bending	Alcohols, Esters, Carboxylic acids and anhydrides
1545.68	C=C Stretching	Aromatic Compounds
1652.14	C=O Stretching	Carboxylic
2927.18	C-H Stretching	Alkyl
3416.16	O-H Stretching	Hydroxyl

cardiodepressant, hypotensive properties[13,14]. The leaves are the excellent remedy for ptyalism, diarrhea, scabies and impetigo. The seeds were powdered and boiled in milk and used for enhancing body strength, life span and also for curing skin diseases, leprosy, flatulence and fever[15]. The genus *Crotalaria* has the largest number of threatened species listed in the Red Data Book. *Crotalaria longipes* is one among the 15 species listed in the Red Data Book. It is a woody shrub growing upto 4 m tall with bright yellow flowers endemic to Nilgiris and Kolli Hills. However, perusal of literature survey reveals that phytochemical screening and *in vitro* antioxidant potential of *C. longipes* is totally lacking. In this view, the present study was designed to investigate the phytochemical screening, total phenolic and total flavonoid contents and to evaluate the *in vitro* antioxidant activities of various solvent extracts of aerial part of *C. longipes.*

MATERIALS AND METHODS

Collection of Plant Material

The aerial part of *Crotalaria longipes* Wight & Arn. was collected from Kothagiri, Nilgiris Biosphere Reserve, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

Preparation of Plant Extract

Freshly collected aerial part of *C. longipes* was dried in shade, and then coarsely powdered separately in a willy mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures[16-18]. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 − 400 cm^{-1}[19].

Estimation of total phenolic content

Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay as previously described by McDonald *et al.*[20] with little modification. To 1 mL of each extract (100 µg/mL) in methanol, 5 mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4 mL (75 g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 mL aliquots of 20, 40, 60, 80, 100

Table 3: IC_{50} values of different extracts of aerial part of *C. longipes*.

Solvent	IC_{50} (µg/mL)			
	DPPH Radical Scavenging Activity	Hydroxyl Radical Scavenging Activity	Superoxide Radical Scavenging Activity	ABTS Radical Cation Scavenging Activity
Petroleum Ether	28.33	22.43	31.16	20.88
Benzene	23.13	23.12	24.16	21.13
Ethyl Acetate	22.14	21.94	21.93	22.13
Methanol	32.41	29.13	36.91	33.16
Ethanol	30.84	24.32	32.62	30.24
Ascorbic Acid	27.34	25.96	24.81	-
Trolox	-	-	-	28.16

µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of flavonoids

The flavonoids content was determined according to Eom *et al.*[21]. An aliquot of 0.5ml of sample (1 mg/mL) was mixed with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1 M). In this mixture, 4.3 ml of 80% methanol was added to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H[22]. The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method[22]. Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (50,100,200,400 & 800 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula. % inhibition = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.*[23]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$**,** 0.1 mL H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of the extract of different concentration (50,100,200,400 & 800 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM , pH 7.9), 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37^0C for 1 hour. 1.0 mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al.*[24]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800 µg/mL), and 0.5 mL Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12 mM) to the mixture, incubated at 25ºC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang *et al.*[25]. ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha[26]. 1.0 mL of solution containing 50,100,200,400 & 800 µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%): The

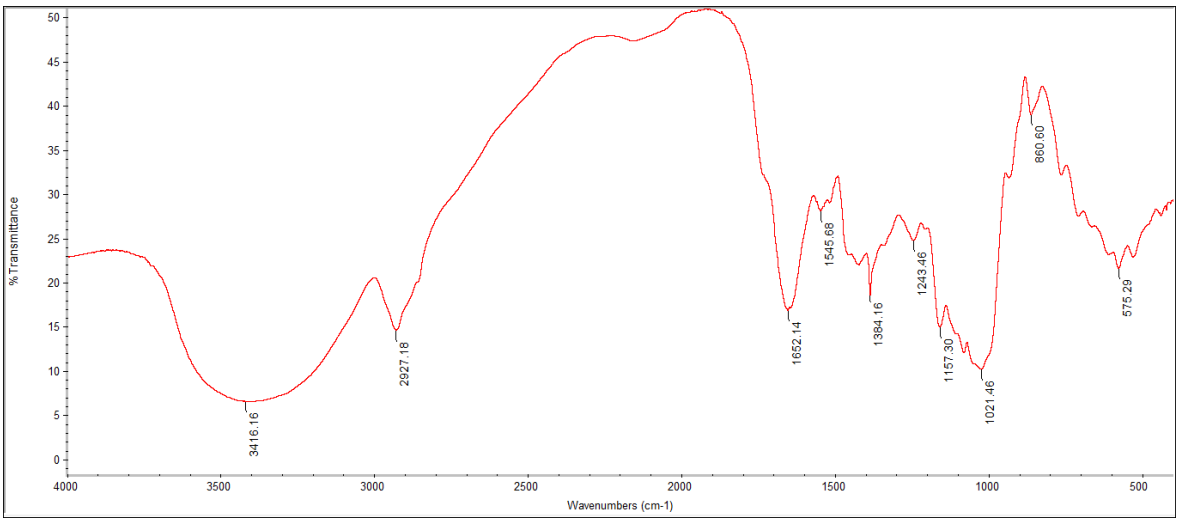

Figure 1: FT-IR Spectrum of aerial part of *C. longipes.*

mixture was incubated at 50ºC for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5ºC) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULT AND DISCUSSION

Preliminary Phytochemical Screening

Preliminary phytochemical investigation was undertaken for the identification of different types of chemical constituents present in the aerial part of *C. longipes*. Results of preliminary phytochemical screening are compiled in Table 1. Screening of methanol and ethanol extracts indicated the presence of alkaloids, anthraquinones, coumarin, catechin, glycosides, flavonoid, phenol, quinone, saponin, tannin, terpenoids, sugar and xanthoprotein. All these compounds are medicinally important. Most of the compounds are detected in the methanol and ethanol extracts when compared to other extracts (petroleum ether, benzene and ethyl acetate), the result may be due to the weak polarity of other solvents because the nature of the solvent, which include polarity that influences the rate of composition and diversity of the extracted compound[27,2]. The phytochemicals found from plants are useful in taxonomic distinction as well as for the detection of pharmacologically important compounds of plants[28].

FT-IR Analysis

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FT-IR spectrum of *C. longipes* aerial part powder was illustrated in Fig. 1. The outcome of FT-IR functional groups and tentative assignments of aerial part of *C. longipes* are represented in Table 2. The functional groups present in *C. longipes* aerial part are hydroxyl, alkyl, carboxylic, aromatic alcohols, esters, carboxylic acid and anhydride, aromatic ether, tertiary amine, aliphatic fluoro and aliphatic iodo compounds. All these compounds belong to secondary plant metabolites as per researcher's explanations[29-31]. Therefore the FT-IR analysis of aerial part of *C. longipes* displayed novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also to identify the medicinally important plants. Further studies are needed with this plant to identify the unknown functional groups, isolate, characterize and elucidate the structure of the bioactive compounds which are responsible for the pharmacological activity. The present study was undertaken with a view to identify the functional groups present in the aerial part of the medicinal plant taken with the help of FT-IR analysis. It helps to identify the chemical constituents, elucidate the chemical structure and also effort was taken to understand the significance of functional groups as bioactive constituents for the treatment of various diseases. The FT-IR analysis of aerial part powder of *C. longipes* gave results that suggest the presence of different functional groups viz. O-H stretching, hydroxyl (3416.16 cm^{-1}), C-H stretching, alkyl (2927.18 cm^{-1}), C=O stretching, carboxylic (1652.14 cm^{-1}), C=C stretching aromatic compounds (1545.68 cm^{-1}), C-O bending, alcohols, esters, carboxylic and carboxyl (1386.16 cm^{-1}), φ-O-H aryl-O stretching, aromatic ether (1243.16 cm^{-1}), C-N stretching, tertiary amine (1157.30 cm^{-1}), C-F stretching, aliphatic fluoro (1021.46 cm^{-1}) and C-I stretching, aliphatic iodo compounds (575.29 cm^{-1}).

Quantitative Estimation of Phytoconstituent (Total Phenolic and Total Flavonoid Contents)

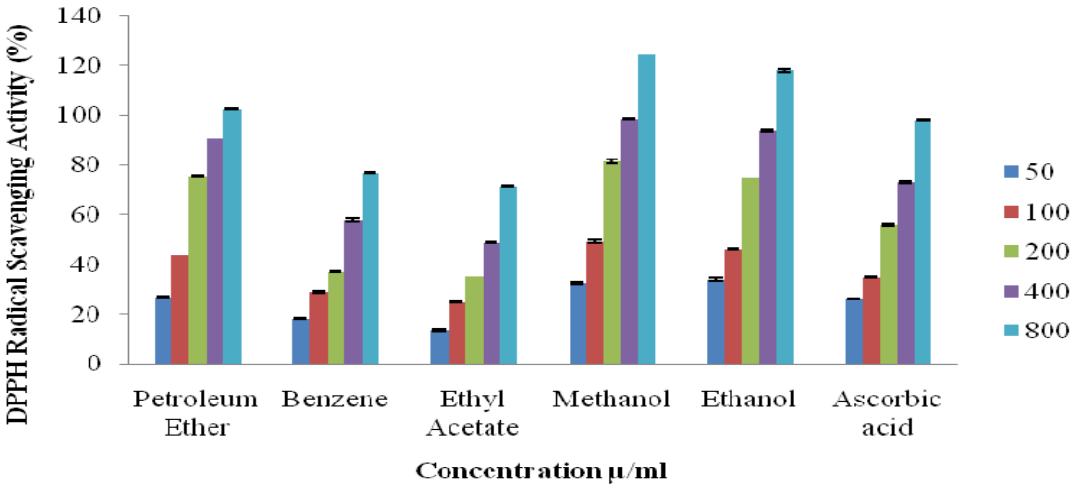

Figure 2: DPPH radical scavenging activity of different extracts of aerial part of *C. longipes.*

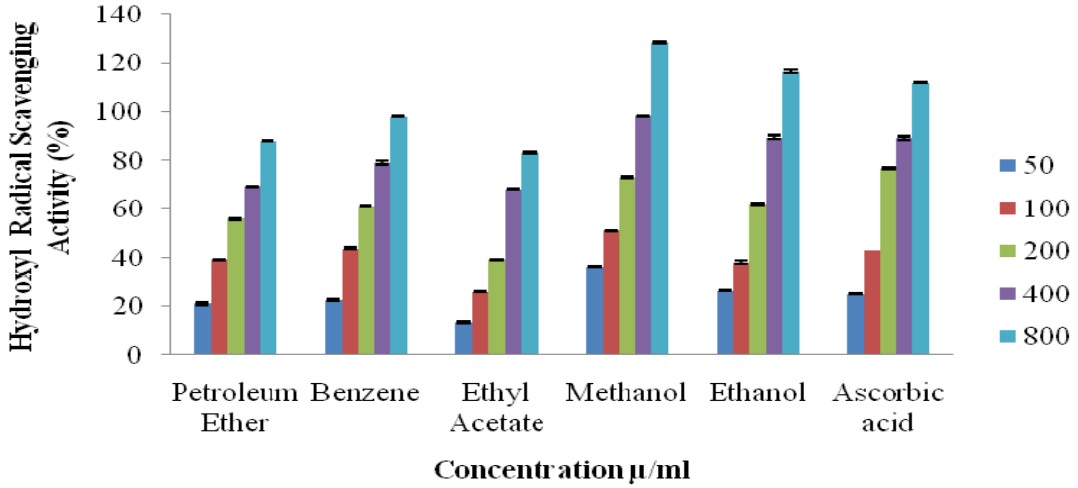

Figure 3: Hydroxyl radical scavenging activity of different extracts of aerial part of *C. longipes.*

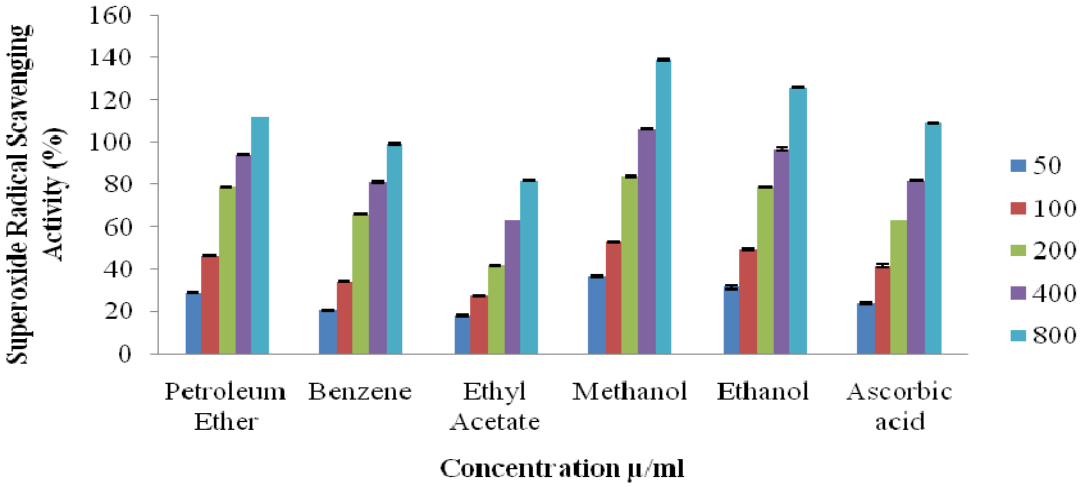

Figure 4: Superoxide radical scavenging activity of different extracts of aerial part of *C. longipes.*

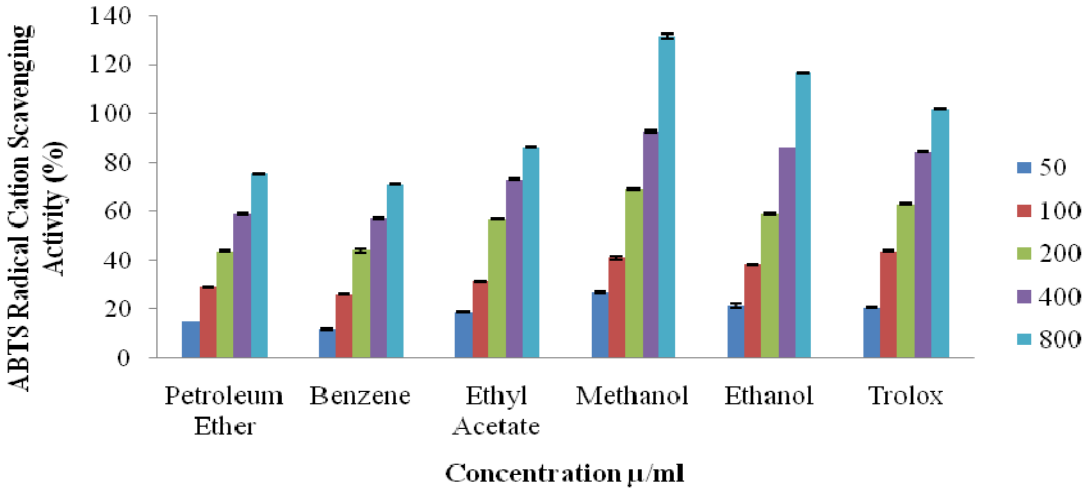

Figure 5: ABTS Radical scavenging activity of different extracts of aerial part of *C. longipes*.

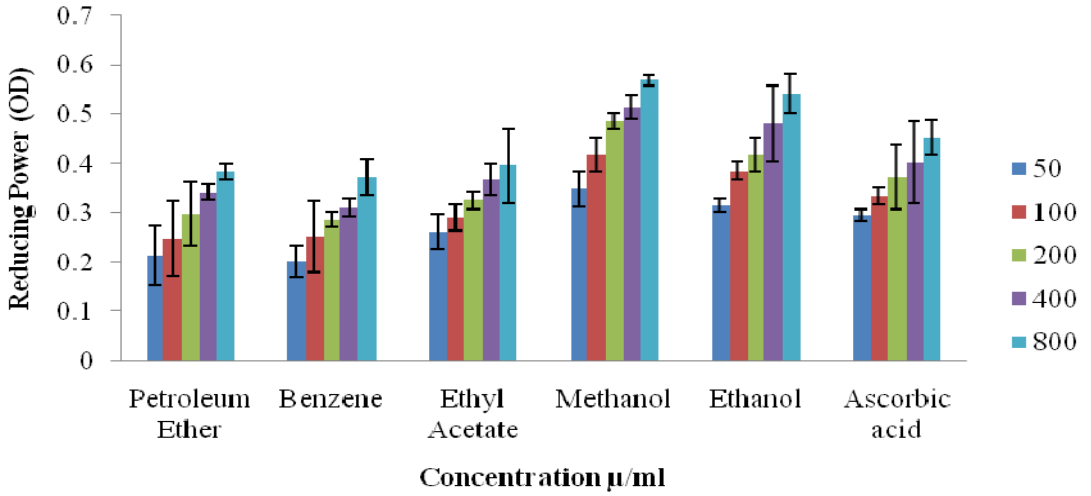

Figure 6: Reducing Power of different extracts of aerial part of *C. longipes*.

The total phenolic and flavonoid content of the methanol extract of *C. longipes* aerial part were found to be 1.08 g 100 g^{-1} and 1.24 g 100 g^{-1} respectively. Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups. It is well known that phenolic compounds are constituents of many plants, and they have attached a great deal of public and scientific interest because of their health promoting effects as antioxidants[32]. The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron donating agents and metal ion chelating properties[33]. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The phenolic compounds in herbs act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators. Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons to free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases[34-37]. Moreover, the highest amount of total phenolic and total flavonoid contents were found in *C. longipes* aerial part extracts. Based on the antioxidant assays, it is thus suggested that phenolics and flavonoids present in *C. longipes* aerial part extracts have strong antioxidant activities. This could be due to the antioxidant mechanisms of phenolics and flavonoids towards free radicals.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *C. longipes* aerial part were depicted in Fig. 2. The scavenging ability increased with the concentration of standard ascorbic acid and plant extracts. Methanol extract showed satisfactory effect in inhibiting DPPH. At a concentration of 800 µg/mL, the scavenging effect of various extracts of aerial part of *C. longipes* on the DPPH radical increased in the following order: ethyl acetate extract < benzene extract < petroleum ether extract <

ethanol extract < methanol extract. The results showed that, among the solvent extracts analyzed for DPPH scavenging activity, methanol extract of aerial part of *C. longipes* showed higher radical inhibition activity. The concentration of methanol extract of *C. longipes* aerial part needed for 50% inhibition (IC_{50}) was found to be 32.41 µg/mL, whereas 27.34 µg/mL (Table 3) was needed for ascorbic acid. The relatively stable organic radical DPPH is widely used in modeling systems to investigate the scavenging activities of several natural compounds, such as phenolics and flavonoids, as well as crude mixtures such as methanol or water extracts from plants. The DPPH radical is scavenged by antioxidants through the donation of electrons forming the reduced DPPH. The colour changes from purple to yellow after reduction and the accompanying decrease in absorbance can be quantified at wavelength 517 nm. The results of this study indicate that all the extracts tested have noticeable effect on DPPH radical scavenging activity. Among the solvent tested, methanol extract of *C. longipes* aerial part exhibited more DPPH radical scavenging activity. It has been reported in literature that the antioxidant activity of many medicinal plants is proportional to their phenolic content and antioxidant activity[38]. The character of phenolics contributing to their electron transfer/hydrogen donating ability is also reported to be associated to the DPPH radical scavenging activity[39]. Radical scavenging activity is very important due to the deleterious role of free radicals biological systems. *C. longipes* has demonstrated good free radical scavenging activity which ensured the ability to reduce the harmful free radicals in the maintenance of health and the management of aging.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of various extracts of aerial part of *C. longipes* were compiled in Fig. 3. The hydroxyl radical scavenging effect of different extracts of aerial part of *C. longipes* increased in the following order: ethyl acetate extract < petroleum ether extract < benzene extract < ethanol extract < methanol extract. The results revealed that, among the solvent extracts tested, methanol extracts of the investigated plant sample exhibited highest radical scavenging activity. The concentration of methanol extract of *C. longipes* aerial part needed for 50% inhibition (IC_{50}) was found to be 29.13 µg/mL, whereas 25.96 µg/mL (Table 3) was needed for ascorbic acid. The hydroxyl radical is the most reactive oxygen species that induces severe damage in biomolecules. The hydroxyl scavenging ability of five different extracts of *C. longipes* aerial part was estimated by generating hydroxyl radicals using deoxyribose method. The hydroxyl radical generated through fenton reaction which degraded deoxyribose using Fe^{2+} as an important catalytic component. The potential of aerial part extracts to inhibit hydroxyl radical mediated deoxyribose damage was determined by means of iron (II) dependent DNA damage assay. In this assay, the test and standard compound colour changes to various shades of pink. Antioxidant efficiency of the aerial part extracts compared to the standard ascorbic acid was determined as the ability to scavenge the free radicals generated. Among the solvents tested, methanol extract possessed more hydroxyl radical scavenging activity when compared with standard ascorbic acid. The ability of the extracts to quench hydroxyl radicals can be related to the prevention of lipid peroxidation. Moreover, it seemed to be a good scavenger of active oxygen species, thus reducing the rate of chain reaction.

Superoxide Radical Scavenging Activity

Different solvent extracts of *C. longipes* aerial part were subjected to superoxide radical scavenging activity and the results were represented in Fig. 4. The superoxide radical scavenging activity of various extracts of aerial part of *C. longipes* decreased in the subsequent order: methanol extract > ethanol extract > petroleum ether extract > benzene extract > ethyl acetate extract. The results exposed that; methanol extract of aerial part of *C. longipes* can act as effective antioxidants by reacting with free radicals. The quantity of methanol extract of aerial part of *C. longipes* required to produce 50% inhibition of superoxide radical was 36.91 µg/mL, whereas 24.81 µg/mL was needed for ascorbic acid. Superoxide radical is known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributing to the tissue damage and various diseases. The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm, *C. longipes* aerial part extract indicated ability to quench superoxide radicals in the reaction mixture. The present study showed potent superoxide radical scavenging activity for *C. longipes* aerial part extracts. Methanol extract exhibited strong superoxide radical scavenging activity among the solvents tested.

ABTS Radical Cation Scavenging Activity

The aerial part of *C. longipes* extracts were analyzed for its ABTS radical cation scavenging activity and the results were exemplified in Fig. 5. The methanol extract of aerial part of *C. longipes* possessed maximum inhibitory effect of about 131.65% at a concentration level of 800 µg/mL. ABTS radical cation scavenging activity of aerial part of *C. longipes* extracts reduced in the consequential order: methanol extract > ethanol extract > ethyl acetate extract > petroleum ether extract > benzene extract. Between the solvents tested, methanol extract exhibited highest ABTS radical cation scavenging action. The IC_{50} values of methanol extract of aerial part of *C. longipes* and standard trolox on ABTS radical cation scavenging activity were found to be 33.16 µg/mL and 28.16 µg/mL respectively (Table 3).

ABT Sradical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The ability in both organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity[25]. In the present study, methanol extract of aerial part of *C. longipes* was fast and effective scavenger of ABTS radical and this activity was higher than that of standard trolox. Proton radical scavenging is

an important attribute of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals[40].

Reducing Power

As represented in Fig. 6, the reducing power of various extracts of aerial part of *C. longipes* augmented with increase in concentration. At a concentration of 800 µg/mL, the reducing power of the investigated plant sample was in the declining order: methanol extract (0.569) > ethanol extract (0.542) > ethyl acetate extract (0.396)> petroleum ether extract (0.384) > benzene extract (0.377). A higher absorbance indicates higher reducing power. Among the solvents tested, methanol extract showed signs of high reducing ability. In reducing power assay, the presence of antioxidants in the samples would result in reducing Fe^{3+} to Fe^{2+} by donating an electron by the extracts. The extracts with reducing power reveal that they are electron donors, reduce the oxidized intermediates and act as primary antioxidant substrates[41]. Increasing absorbance at 700 nm indicated an increase in reductive ability. The higher absorbance of extracts may be due its strong reduction potential. In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of *C. longipes* aerial part extracts in concentration dependent manner when compared to the standard ascorbic acid.

CONCLUSION

The results of the present study showed that the methanol extract of *C. longipes* aerial part, which contains phenolics and flavonoids compound exhibited the great antioxidant activity. This is the first report on the antioxidant property of this plant. The high scavenging property of methanol extract of *C. longipes* aerial part may be due to hydroxyl groups existing in the phenolic compounds, chemical structure that can provide the necessary components as a radical scavenger. Tetradecanoic acid, ethyl ether and squalene were reported in the ethanol extract of *C. longipes* aerial part by GC-MS analysis[42]. These compounds may have the role in antioxidant activity. Further research can also explore the particular antioxidant principle(s) from *C. longipes* extracts which can be one of the potent lead molecule(s) from the arsenal of natural products.

REFERENCES

1. Bhakta T, Deb PK, Bhaumik KN. and Saha J. Pharmacognostic investigation, acute toxicity studies and isolation of steroidal compound from the leaves of *Cassia fistula* Linn. Int. J. Pharm. Sci. Rev. Res. 2014; 25: 210-214.
2. Ncube NS, Afolayan AJ. and Okohs AL. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr. J. Biotechnol. 2008; 7: 1797-1806.
3. Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Comp. Alter. Med. 2006; 6: 35-41.
4. Maeda S. and Omata M. 'Inflammation and cancer': role of nuclear factor – kappa B activation. Cancer Sci. 2008; 99: 836-842.
5. Montecucco F, Pende, A, Quercioli A. and Mach F. Inflammation in the pathophysiology of essential hypertension. J. Nephrol. 2011; 24: 23-24.
6. Burns J, Gardner PT, Matthews D, Duthie GG, Lean ME. and Crozier A. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J. Agric. Food Chem. 2001; 49: 5797-5808.
7. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obutor EM. and Farombi EM. Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol. 2007; 6: 1197-1201.
8. Cai Y, Luo Q, Sun M. and Cork H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004; 74: 2157-2184.
9. Prior RL, Wu XL. and Schaich K. Standardized methods for the determination of antioxidant cap activity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 2005; 53: 4290–4302.
10. Rohman A, Riyanto S, Yuniarti N, Saptura WR. and Utami R. Antioxidant activity, total phenolic and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). Int. Food Res. J. 2010; 17: 97-106.
11. Govindarajan R, Vijayakumar M. and Pushpangadam P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J. Ethnopharmacol. 2005; 99: 165-178.
12. Shariar M, Akhter Md.S. Hussain, Md. I, Haque A. and Bhuiyan MA. Evaluation of *in vitro* antioxidant activity by bark extracts of *Terminalia arjuna*. J. Med. Plants Res. 2012; 6: 5286-5298.
13. Nuhu H, Abdurrahman EM. and Shok M. Comparative analysis of the alkaloids of three *Crotalaria* species. Nig. J. Pharm. Sci. 2009; 8: 54-58.
14. Devendra BN, Srinivas N. and Solmon KS. A comparative pharmacological and phytochemical analysis of *in vivo* and *in vitro* propagated *Crotalaria* species. Asian Pac. J. Trop. Med. 2012; 5:37-41.
15. Warrier PK. 1994. Indian Medicinal Plants: A Compendium of 500 Species. Vol. 3. New Delhi, Sangam Book, Ltd.
16. Saraf A. Phytochemical and antibacterial studies of medicinal plant *Costus specious* Koer. E. J. Chem. 2010; 7: S405-S413.
17. Shajeela PS, Kalpanadevi V. and Mohan VR. Potential antidiabetic, hyperlipidaemic and antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats. J. Appl. Pharmaceut. Sci. 2012; 2: 83-88.
18. Murugan M. and Mohan VR. Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage bengalensis* (L.) Kurz. J. Appl. Pharmaceut. Sci. 2011; 1: 157-160.

19. Kareru PG, Keriko JM, Gachanja AN. and Kenji GM. Direct detection of triterpenoid saponins in medicinal plants. Afr. J. Trad. Complement. Altern. Med. 2008; 5: 56–60.
20. McDonald S, Prenzler PD, Antolovich M. and Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001; 73:73-84.
21. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C. and Cho DH. Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Kor. J. Med. Crop Sci. 2007; 15: 319-323.
22. Blois MS. Antioxidant determination by the use of a stable free radical. Nat. 1958; 181: 1199-1200.
23. Halliwell B, Gutteridge JMC. and Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana. Biochem. 1987; 65: 215-219.
24. Srinivasan R, Chandrasekar MJN, Nanjan MJ. and Suresh B. Antioxidant activity of *Caesalpinia digyna* root. J. Ethnopharmacol. 2007; 113: 284-291.
25. Huang MH, Huang SS, Wang BS, Sheu MJ. and Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo*. J. Ethnopharmacol. 2011; 133: 743-750.
26. Kumar RS. and Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. J. Chem. Pharm. Res. 2011; 3: 259-267.
27. Eloff JN. Which extractant should be used f or the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 1998; 60:1-8.
28. Sugumaran and Vetrichelvan T. "Studies on some pharmacognostic profiles of *Bauhinia purpurea* Linn. (Caesalpiniaceae) leaves". Ethnobot. Leaf. 2008; 12:461 - 468.
29. Paulraj K, Irudayaraj V, Johnson M. and Patric Raja D. Phtochemical and anti-bacterial activity of epidermal glands extract of *Christella parasitica* (L.) H. Lev. Asian Pac. J. Trop. Biomed. 2011; 1: 8-11.
30. Rajan ST, Thirunalasundari and Jeeva S. Anti-enteric bacterial activity and phytochemical analysis of seed kernel extract of *Mangifera indica* Linnaeus against *Shigella dysentriae* (Shiga, corrig.) Castellani and chalmers. Asian Pac. J. Trop. Biomed. 2011; 4: 294-300.
31. Maobe, MAG. and Nyarango RM. Fourier Transformer Infra Red Spectrophotometer Analysis of *Warburgia ugandensis* medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. Global J. Pharmacol. 2013; 7: 61-68.
32. Hollman PCH. and Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food Chem. Toxicol. 1999; 37: 937-942.
33. Rice-Evans CA, Miller NJ. and Paganya G. Structure of antioxidant activity relationship of flavonoids and phenolic acids. Free Rad. Biol. Med. 1996; 20: 933-956.
34. Ferrali M, Signorini C, Caciotti B, Sugherina L, Ciccoli L, Giachetti D. and Comporti M. Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. FEBS Lett. 1997; 416: 123-129.
35. Elliot AJ, Scheiber SA, Thomas C. and Pardini RS. Inhibition of glutathione reductase by flavonoids. A structure activity study. Biochem. Pharmacol. 1992; 44: 1603-1608.
36. Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O. and Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J. Nutr. Sci. Vitaminol. 2001; 47: 357-362.
37. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel BV, Pieters L, Vlietinck AJ. and Berghe DV. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J. Nat. Prod. 1998; 61: 71-76.
38. Rice-Evans C. Flavonoid antioxidants. Curr. Med. Chem. 2001; 8: 797-807.
39. Brand-Williams W, Cuvelier ME. and Barset C. Use of a free radical method to evaluate antioxidant activity. Food Sci. Tech. 1995; 28: 25-30.
40. Adedapo AA, Jimoh FO, Afolayan AJ. and Masika PJ. Antioxidant properties of the leaves and stems of *Celtis africana*. Rec. Nat. Prod. 2009; 3: 23-31.
41. Chanda S. and Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties an overview. Afr. J. Microbiol. Res. 2009; 3: 981-996.
42. Paulpriya K., Tresina PS. and Mohan VR. Assessment of Bioactive Constituents by GC-MS of *Crotalaria longipes* Wight & Arn.: An Endemic Plant. Int. J. Pharmacog. Phytochem. Res. 2014; 6: 1043-1048.