



Evaluation of anti oxidant activities of stem and leaf extracts of *Kedrostis foetidissima (Jacq) Cogn*

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ABSTRACT

The *in vitro* antioxidant activity of four extracts of leaf and stem of the species, *Kedrostis foetidissima (jacq.)cogn.*, belonging to the *Cucurbitaceae* family, were investigated by *in vitro* antioxidant methods -1,1-diphenyl-2-picrazyl(DPPH) scavenging assay and reducing power assay. In each investigation, ethanol extract of leaf showed highest antioxidant activity compared to other extracts. Our results clearly demonstrate the antioxidant capacity of all the extracts. Among the eight extracts, the ethanol leaf extract showed strong scavenging effect on 1,1-diphenyl-2-picrazyl radical(DPPH) and displayed higher reducing activity on ferric chloride than chloroform, ethyl acetate and pet ether extracts. These results suggested that ethanol leaf extract of *Kedrostis foetidissima (jacq.)cogn* may act as a chemopreventive agent, providing antioxidant properties and offering effective protection from free radicals and support that *Kedrostis foetidissima (jacq.)cogn* is a promising source of natural antioxidants.

Key words: *Kedrostis foetidissima (jacq.)cogn.*, antioxidant activity, DPPH radical scavenging assay, reducing power assay.

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources¹. India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species². Many plants have been sources of medicines since ancient times^{3,4,5}. Living cells may generate free radicals and other reactive oxygen species by-products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans⁶. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity^{7,8}.

Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities^{9,10}. The species, *Kedrostis foetidissima(jacq.)cogn.*, belongs to the *Cucurbitaceae* family, traditional medicinal plant, locally named as Appakovai, in Tamilnadu. This species was also found in India, Sri Lanka, Ethiopia and Western Malaysia.. It was reported that, five drops of juice of the leaf of *Kedrostis foetidissima(jacq.)cogn.*, *Cucurbitaceae*, is given orally to treat common cold in children¹¹. The roots of *Kedrostis foetidissima (jacq.)cogn.* crushed, mixed in cold water is taken once a day for the treatment of Measles¹². An ethnobotanical survey on the use of medicinal plants by the Zay people of Ethiopia, revealed that the whole plant of *Kedrostis foetidissima (jacq.)cogn.*, locally named as holobido(Or.) is taken orally for curing chest pain and its leaves are used as a traditional veterinary medicine in the treatment of ALOYE - a cattle disease¹³. The leaf juice of *Kedrostis foetidissima* applied externally on joints cures

diarrhoea in babies of 3-4 months¹⁴. Medicinal plants of Bulamogi in Uganda shows a record that *Kedrostis foetidissima*, wild herb, was used in treatment of diarrhoea and measles. Leaf infusion was taken in treating diarrhoea and leaf decoction was taken orally in the treatment of measles¹⁵. Opportunistic infections are treated with multi-plant extracts of *Mangifera indica*, *Eucla natalensis*, *Carissa edulis*, *Psidium guajava*, *Penisetum purpureum*, *Cymbopogon citratus*, *Punica granatum*, *Musa sp*, *Kedrostis foetidissima*, *Withania somnifera*, *Acacia robusta*, *Eucalyptus sp*, *Ximenia caffra*, *Clerodendrum mrycoides* and *Dichrostachys cinerea*¹⁶. The medicinal herb *Kedrostis foetidissima* (*jacq.*)*cogn.*, and *P.vogelii*. are recommended for further pharmacological test on HIV cases and for domestication to serve them from local extinction¹⁷. Nutritional and antinutritional evaluation on proximate composition of the edible tubers consumed by tribal Valaiyans of Madurai, reveals that the tubers of *Kedrostis foetidissima* and stem of *Caralluma pauciflora* have more crude protein than the other plants. The tubers of *Kedrostis foetidissima* have higher vitamin, niacin content and more starch content. All the investigated wild edible plants appeared to have a higher level of iron content compared to Recommended dietary Allowances (RDA) of NRC/ NAS(1980) for infants and adults, have low *in vitro* protein digestibility, exhibit variations in the levels of total free phenolics, tannins, hydrogen cyanide, total oxalate, amylase and trypsin¹⁸. The chloroform extract of leaf and stem of *Kedrostis foetidissima* showed significant antibacterial activity against bacteria like *Streptococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Serratia marcescens*. The maximum zone of inhibition was found in stem against *Pseudomonas aeruginosa* (12.6mm). Since the plants used in this study have proved to possess antimicrobial properties, and are locally available, they may become alternative sources of antimicrobial drugs that will complement existing antibiotics and are provide novel or lead compounds that may be employed in controlling infection¹⁹. Water extracts of the aerial parts of *Kedrostis foetidissima* have been used for centuries by Kenyans as an effective remedy for complications arising from the measles virus infection in children. The study shows that this extract is encouragingly active against a number of bacterial species: *Klebsiella pneumonia*, *Escherichia coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Vibrio Comma*, *Salmonella typhi*, *Streptococcus pneumonia* and *Enterobacter aerogines*. The extract is also active against the measles virus *Leishmania denovani*, the visceral *Leishmania Parasite*, as well as *Trypanosoma bruce*²⁰. Several of non- protein amino acids were isolated first from members of *Cucurbitaceae*^{21,22}. *Kedrostis foetidissima*, shows mild chromatographic spot for the presence of amino acid, Citrulline and traces of *m-Carboxyphenylalanine*. It also shows spots for the presence of traces of unknown Ninhydrin positive²³. Preliminary studies on this plant in our laboratory revealed the presence of terpenoids, sterols, amino acids. The antioxidant activity of plants owe to the secondary metabolites. Oxidative stress often seems to be the

important factor in many of the diseases and antioxidant activity is hence of recent interest. To the best of our knowledge, there are no such reports concerning the antioxidant activity of *Kedrostis foetidissima* (*jacq.*)*cogn.*, So our present work has been focused to study the antioxidant activity of leaf and stem extracts of *Kedrostis foetidissima* (*jacq.*)*cogn.*

MATERIALS AND METHODS

Identification and collection of plant materials

Leaf and stem of *Kedrostis foetidissima* (*jacq.*)*cogn.*, were collected during October 2010 –February 2011, from Aliyar hills, near Pollachi, Coimbatore District, Tamilnadu. The voucher specimen was submitted to Botanical Survey Of India, Southern Regional Centre, Tamilnadu Agri University, Coimbatore, Tamilnadu and the Specimen was identified as *Kedrostis foetidissima* (*jacq.*) *cogn.* (*Trichosanthes foetidissima* *Jacq.*) CUCURBITACEAE family. Collected plant materials were washed thoroughly to remove mud particles, separated and then shade dried. The leaves were powdered; the stems were crushed and stored in a tightly closed container for further use.

Extraction of plant materials

Crushed leaves and stem(100 g) of *Kedrostis foetidissima* (*jacq.*)*cogn.*, were first defatted with petroleum ether. Ethanol (EtOH), Chloroform(CF), Ethyl Acetate(EA), Petroleum Ether(PE) extracts were obtained by Reflux (6h) using 100g of powdered leaves and crushed stem with 1liter of each solvent mentioned above. Eight extracts, with different polarities, were concentrated to dryness and the residues were refrigerated for performing various assays. The extracts were used at the concentrations of 25,50,100,150,200 µg/ml, in DPPH radical scavenging assay and 100,200,300,400,500,µg/ml, in Reducing power assay. The extracts were labeled as follows: Leaf petroleum extract-KFLPE; Leaf Ethyl acetate Extract-KFLEA; Leaf Chloroform extract- KFLCH; Leaf Ethanol extract – KFLEOH; Stem petroleum extract-KFSPE; Stem Ethyl acetate Extract- KFSEA; Stem Chloroform extract-KFSCH; Stem Ethanol extract – KFSEOH.

IN-VITRO ANTIOXIDANT ACTIVITY

DPPH Free Radical Scavenging Activity:

Preparation of standard solution: Required quantity of Ascorbic acid was dissolved in Methanol to give the concentration of 25, 50,100,150, 200 µg/ml.

Preparation of test sample: Stock solutions of samples were prepared b dissolving 10mg of dried extracts in 10ml of methanol.

Preparation of DPPH solution: 0.3mM of DPPH solution was prepared by dissolving 5.9442µg of DPPH in 30ml of methanol. It was protected from light by covering the container with aluminum foil.

Protocol for estimation of DPPH scavenging activity:

This was assayed as described by Elizabeth and Rao (1990). DPPH (diphenyl 2-picryl hydrazyl radical) (1ml of 0.3mM DPPH in methanol)was added to 100µl of compound with concentrations ranging from 25µg to 200µg. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidants in the sample, it was reduced and the color changed from deep violet to light yellow. This was measured at 517nm²⁴ with the help of UV-Visible spectrophotometer (Shimadzu, UV-1700, Japan) after 30 min. decreasing in the absorbance indicates an increase of the DPPH radical-scavenging activity. DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract.

Reducing Power Assay**Preparation of standard solution**

Ascorbic acid (10 mg) was dissolved in 10 ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 20,40,60,80 and 100µg/ml.

Preparation of test sample

Required quantities of the test samples were dissolved in minimum quantity of methanol and volume were made up to 10ml with phosphate buffer. Separately all the samples were diluted in 10ml volumetric flask with phosphate buffer to give (100,200,300,400 and 500 µg/ml).

Preparation of reagents

Dibasic sodium phosphate (18.75 ml , 0.2M) is mixed with 31.25ml Monobasic sodium phosphate and diluted to 100ml with water (0.2M, pH 6.6). Potassium ferricyanide solution (1%) was prepared by dissolving 2g of potassium ferricyanide in 200ml of distilled water. 10% Trichloro acetic acid solution was prepared by dissolving 4gms of Trichloro acetic acid in 400ml of distilled water. 0.1% Ferric chloride solution was prepared by dissolving 0.1g of Ferric chloride in 100ml of distilled water.

Protocol for reducing power

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu et al., 1986 with a slight modification. Different concentrations (100- 500 µg/ml) of the extract (1ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium hexacyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min. After incubation, 2.5 ml of TCA (10%) was added to terminate the reaction. The upper portion of the solution (2.5ml) was mixed with 2.5 ml distilled water, and 0.5 ml FeCl_3 solution (0.1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture

indicated greater reducing power. Ascorbic acid was used as a positive control²⁵.

Statistical analysis

Each experiment was carried out in triplicate and results are expressed as mean % antiradical activity \pm SD. Data were collected and expressed as the mean \pm standard deviation of three independent experiments and analyzed for statistical significance from control and between each other.

RESULTS AND DISCUSSION**Antioxidant Studies**

In the present study, two commonly used antioxidant evaluation methods such as DPPH radical scavenging activity and reducing power assay were chosen to determine the antioxidant potential of leaf and stem extracts of *Kedrostis foetidissima (jacq.)cogn*.

Free Radical Scavenging Activity

The radical scavenging activity was evaluated by the DPPH assay. DPPH is a molecule containing a stable free radical; in the presence of the antioxidant that can donate an electron to DPPH, the purple colour typical of the free DPPH radical decays, a change that can be followed spectrophotometrically at 517 nm. This simple test can provide information on the ability of a compound to donate an electron, the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. In this study, four leaf extracts and four stem extracts of *Kedrostis foetidissima (jacq.)cogn* were investigated for their radical scavenging activity and the results were shown in Tables 1 & 2 and Figures 1-6. DPPH radical scavenging activities of leaf extracts varied from 24.6 to 88.1% whereas that of stem extracts varied from 47.55 to 86.81%. All of the extracts tested possess radical scavenging activity. This activity was increased by increasing concentration of the sample extract. The highest antioxidant activity was observed in ethanol leaf extract of *Kedrostis foetidissima (jacq.)cogn* than other extracts studied. The lowest activity was shown by petroleum ether leaf extract. It was also found that, all the leaf extracts except petroleum ether extract shows more activity than its corresponding stem extracts. This might be attributed to the secondary metabolites like flavanones, polyphenols etc which are known free-radical scavengers normally present in plant extracts and contributing to anti-oxidant activity. From these results it can be concluded that the antioxidant activity of ethanol leaf and stem extracts of *Kedrostis foetidissima (jacq.)cogn* shows promising activity than other extracts and it can be used as a starting material for the isolation of compounds with antioxidant activity. There is a lack of information available on the chemical composition of *Kedrostis foetidissima (jacq.)cogn* including antioxidant activity. Further investigations on the extracts including fractionations are needed to isolate active constituents and subsequent pharmacological evaluation.

Reducing Power Assay

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides²⁶. For the measurements of the reductive ability, it has been found that the Fe³⁺ - Fe²⁺ transformation occurred in the presence of extract samples which was postulated previously by Oyaizu²⁷. Tanaka et al. have observed a direct correlation between antioxidant activities and reducing power of certain plant extract²⁸. The reducing properties are generally associated with the presence of reductones²⁶, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom²⁹. In this assay, depending on the reducing power of antioxidant compounds, the yellow color of the test solution changes into various shades of green and blue. Therefore, by measuring the formation of Perl's Prussian blue at 700nm, we can monitor the Fe²⁺ concentration. The reducing capacity of a compound may serve as a

significant indicator of its potential antioxidant activity. Reducing power of four leaf extracts and four stem extracts of *Kedrostis foetidissima (jacq.)cogn* and standard Ascorbic acid were investigated and the results were shown in the Tables 3&4 and Figures 7-13. The reducing power of the four leaf extracts and four stem extracts of *Kedrostis foetidissima (jacq.)cogn* shows steady increase with the increase in concentration. The reducing power of ascorbic acid was more pronounced than that of various extracts. The reducing power of *Kedrostis foetidissima (jacq.)cogn* leaf extract ranging from 0.0883 to 0.781 Abs, that of stem extracts ranging from 0.0865 to 0.7117Abs for 100 µg/ml to 500 µg/ml of extracts Figures 7-13. The ethanol leaf extract displayed higher reducing activity compared to other extracts. The lowest reducing activity was shown by leaf pet ether extract. The reducing power of pet ether stem and leaf extracts shows almost similar reducing activity.

Table 1: DPPH radical scavenging activity of various leaf extracts of *Kedrostis foetidissima (jacq.)cogn*.

S.No	CONC (µg/ml)	% INHIBITION (Mean ± SD)									
		STD		KFLPE		KFLEA		KFLCH		KFLEOH	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	25	92.92	0.06	24.62	0.12	56.34	0.34	62.31	0.01	75.11	0.14
2.	50	96.48	0.02	25.31	0.005	61.47	0.06	63.13	0.01	79.86	0.11
3.	100	97.41	0.02	32.05	0	62.19	0.07	66.08	0.09	82.87	0.42
4.	150	98.22	0.007	35.71	0.01	64.20	0.23	67.73	0.10	87.75	0.04
5.	200	99.27	0.007	36.64	0.08	66.52	0.02	68.66	0.11	88.18	0.02

Table 2: DPPH radical scavenging activity of various stem extracts of *Kedrostis foetidissima (jacq.)cogn*

S.No	CONC (µg/ml)	% INHIBITION (Mean ± SD)									
		STD		KFSPE		KFSEA		KFSCH		KFSEOH	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	25	84.926	0.068	47.559	0.021	52.329	0.017	57.319	0.0275	70.725	0.326
2.	50	86.4883	0.0218	47.9736	0	53.750	0.0526	61.4992	0.0052	77.1614	0.05037
3.	100	90.4109	0.0234	50.1256	0.05649	57.4816	0.1269	63.4181	0.0479	81.1943	0.059396
4.	150	94.2264	0.0070	53.9864	0.0374	60.4392	0.0698	65.7757	0.0394	83.7011	0.005312
5.	200	99.2799	0.0071	54.0776	0.05649	62.5074	0.0366	67.3611	0.0754	86.8156	0.212454

Table-3 Ferric Reducing Power of various leaf extracts of *kedrostis foetidissima (jacq.)cogn*

S.No	CONC (µg/ml)	ABSORBANCE 700nm (Mean ± SD)							
		KFLPE		KFLEA		KFLCF		KFLEOH	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	100	0.0883	0.0005	0.1436	0.0006	0.1665	0.0003	0.2014	0.0003
2.	200	0.2023	0.0002	0.3133	5.7735	0.3418	0	0.4114	0
3.	300	0.3241	0.0001	0.4454	0.0020	0.4646	0.0015	0.5223	0.0012
4.	400	0.4061	0.0004	0.5511	0	0.5998	0	0.64283	0.0003
5.	500	0.5113	0.0002	0.6224	0	0.6338	0.0007	0.7841	0.002

Table-4 Ferric Reducing Power of various stem extracts of *kedrostis foetidissima (jacq.)cogn*

S.No	CONC (µg/ml)	ABSORBANCE 700nm (Mean ± SD)							
		KFSPE		KFSEA		KFSCF		KFSEOH	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	100	0.0865	0.0008	0.1148	0.0011	0.1231	0.0004	0.1661	0.0005
2.	200	0.2318	0.0011	0.2555	0.0010	0.2959	0.0008	0.3317	0.0012
3.	300	0.3063	0.0002	0.3885	0.0006	0.413	0.0017	0.4781	0.0007
4.	400	0.3963	0.0005	0.4665	0.0011	0.5221	0	0.6213	0.0004
5.	500	0.5016	0.0003	0.5877	0.0010	0.6031	0.0008	0.7118	0.0002

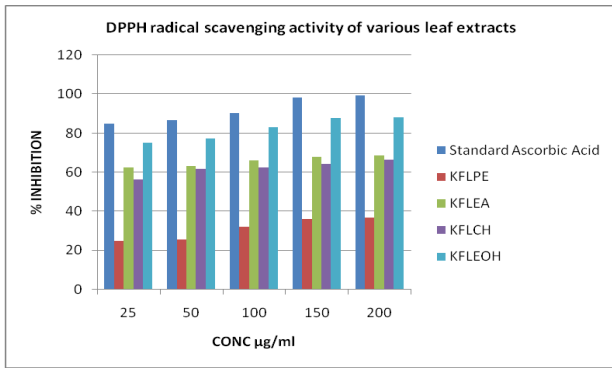


Figure 1: DPPH radical scavenging activity of various leaf extracts

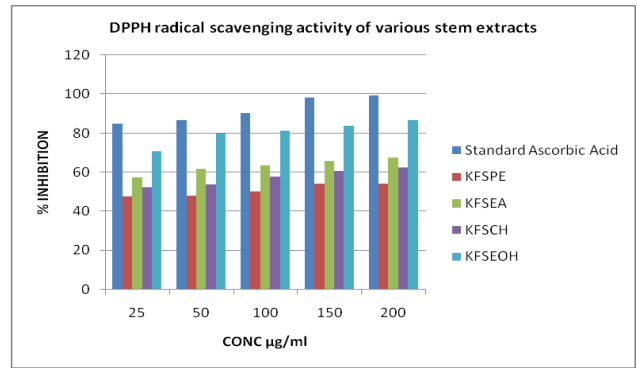


Figure 2 : DPPH radical scavenging activity of various stem extracts

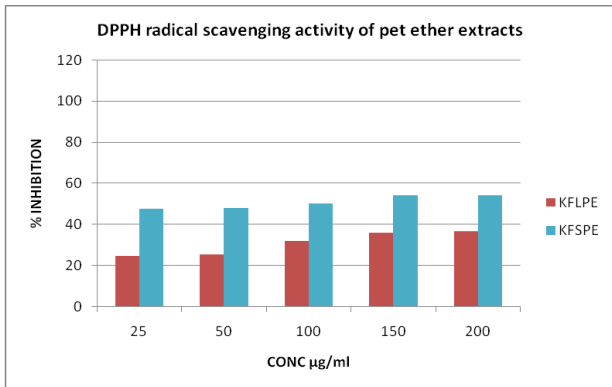


Figure 3 : DPPH radical scavenging activity of Pet ether extracts of leaf and stem

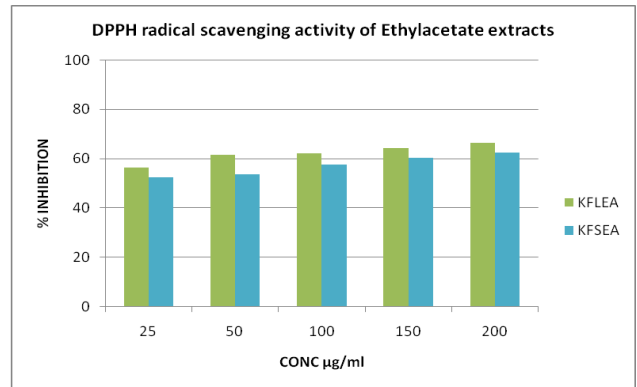


Figure 4 : DPPH radical scavenging activity of Ethylacetate extracts of leaf and stem

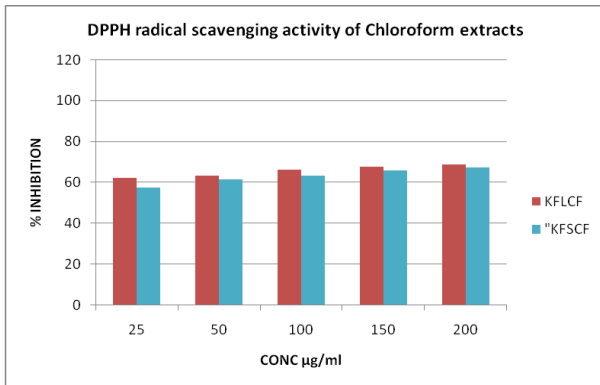


Figure 5 : DPPH radical scavenging activity of Chloroform

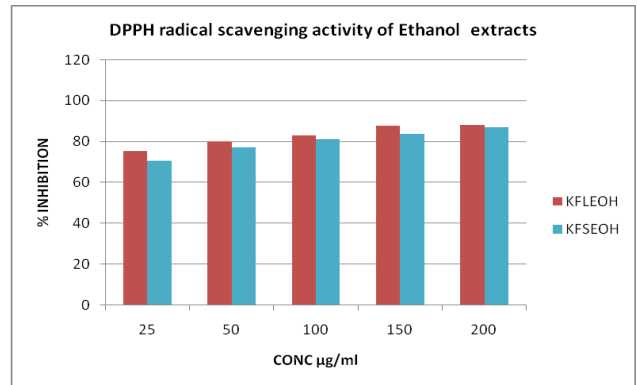


Figure 6 : DPPH radical scavenging activity of Ethanol extracts of leaf and stem

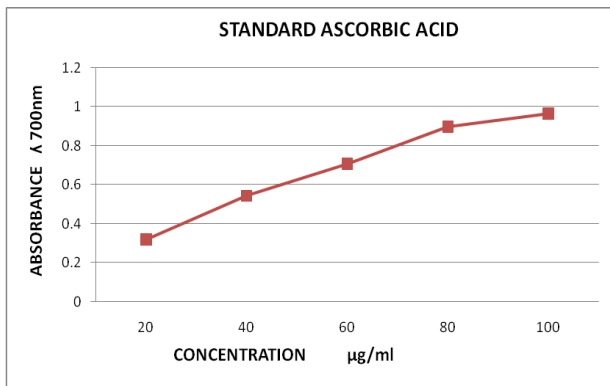


Figure 7 : Reducing Power of Standard Ascorbic Acid

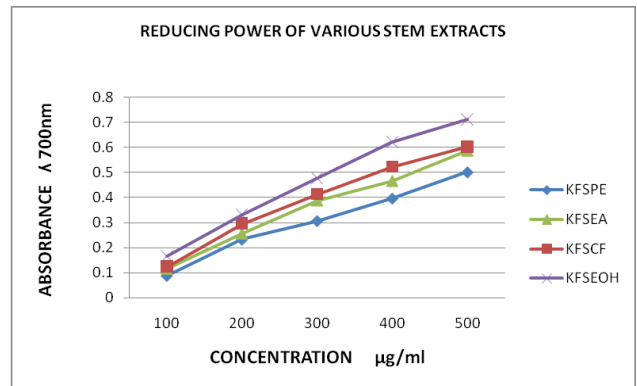


Figure 8 : Reducing Power of Various Stem Extracts

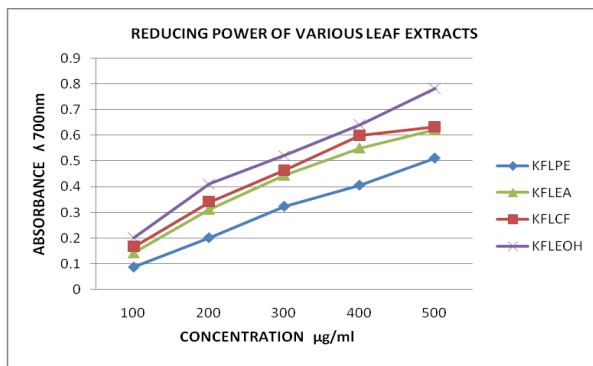


Figure 9 : Reducing Power of Various Leaf Extracts

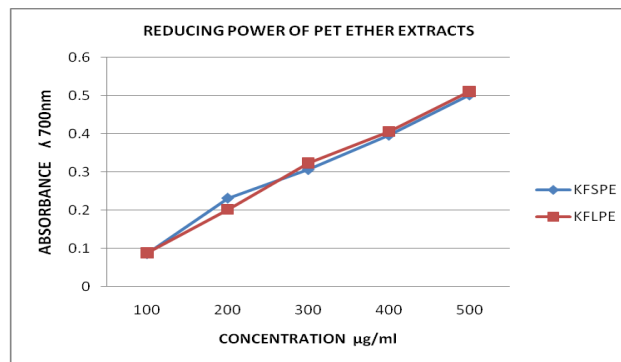


Figure 10: Reducing Power of Pet Ether Extracts Of Leaf and Stem

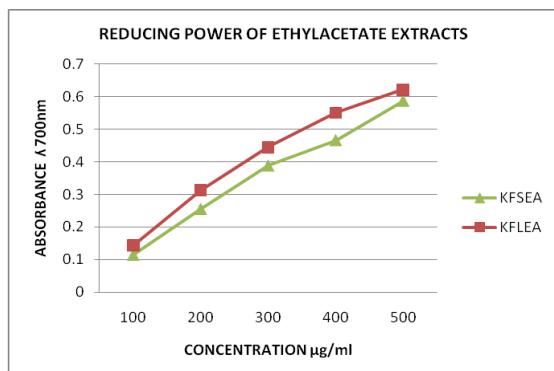


Figure 11: Reducing Power of Ethylacetate Extracts of Leaf and Stem

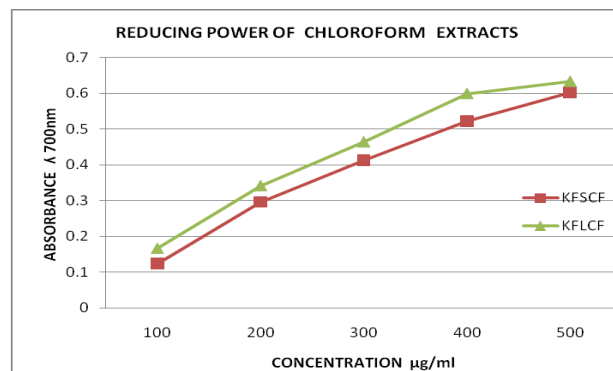


Figure 12: Reducing Power of Chloroform Extracts of Leaf and Stem

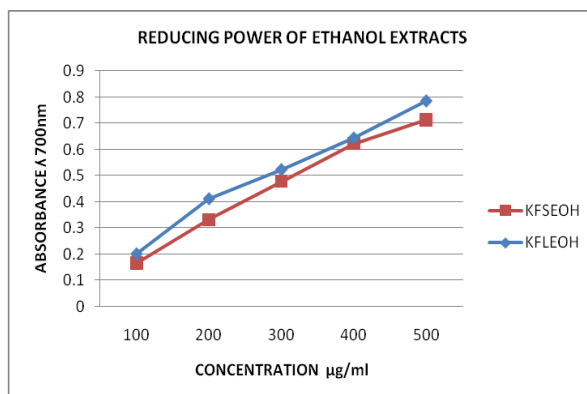


Figure 13: Reducing Power of Pet Ether Extract of Leaf and Stem

CONCLUSION

This paper deals with antioxidant activity of four leaf extracts and four stem extracts of *Kedrostis foetidissima (jacq.)cogn*. The extracts showed significant activities in both antioxidant assays were compared with the standard ascorbic acid. DPPH radical scavenging activity of ethanol leaf extract was 88% at higher concentration. The ethanol leaf extract of *Kedrostis foetidissima (jacq.) cogn* showed potent antioxidant property and contain significant amount of phenolic compounds. Moreover, ethanol leaf extract showed good reducing power. The low antioxidant activity in Pet ether extracts can be attributed to the fact that the compounds are polar in nature and are not completely extracted in Pet ether. These results suggested that ethanol leaf extract of *Kedrostis foetidissima (jacq.)cogn* may act as a chemopreventive agent, providing antioxidant properties and offering effective protection from free

radicals and support that *Kedrostis foetidissima (jacq.)cogn* is a promising source of natural antioxidants.

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