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Original Article

# Therapeutic potentiality of *Kedrostis foetidissima* (Jacq.) Cogn., leaf extracts on free radicals induced oxidative damage in the biological system

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## ABSTRACT

**Objectives:** This study was convened to evaluate the free radical scavenging activity of *Kedrostis foetidissima* leaf extracts. **Methods:** The leaves of *K. foetidissima* (Jacq.) Cogn. were subjected to powder and extracted with various solvents such as water, methanol, acetone, chloroform, and petroleum ether. All the extracts were subjected to nitric oxide radical scavenging assay, hydrogen peroxide scavenging assay, superoxide anion radical scavenging assay, and egg yolk lipid peroxidation (LPO) assay. **Results:** All the extracts of *K. foetidissima* effectively reduced the generation of nitric oxide from sodium nitroprusside over a concentration of 2-10 mg/ml concentration which was significant at $P < 0.05$, showed moderate to potent level of $H_2O_2$ radical scavenging activity over the range of 2-10 mg/ml concentration, effectively reduced the superoxide anion radicals generated from phenazine metho-sulfate/nicotinamide adenine dinucleotide-nitroblue tetrazolium system over a concentration range of 2-10 mg/ml concentration, effectively inhibited the formation of LPO over a concentration range of 2-10 mg/ml. **Conclusion:** The results of this study indicated that among the five solvent extracts, the methanolic leaf extract have beneficial free radical scavenging activity and it also clearly suggested that the leaves may be used as a dietary supplement which can act as a natural antioxidant.

KEY WORDS: Antioxidants, free radicals, *Kedrostis foetidissima*, medicinal plants

## INTRODUCTION

Chemical mobilization of stored fat under various situations (such as lactation, work out, disease and fasting) can occasionally end in increased free radical action and injury [1]. Free radicals may also be produced in various non-enzymatic reactions [2]. At low or moderate concentrations, reactive oxygen species (ROS) exert beneficial effects on cellular responses and immune function, but at high levels, free radicals and oxidants generate oxidative stress, which leads to cell damage [3]. Superoxide anion, hydroxyl, nitric oxide radicals, and other reactive species such as hydrogen peroxide, hypochloric acid, and proxynitrite produced during aerobic metabolism cause oxidative damage to cellular organelles [4].

Free radicals play a significant role in the progression of life and biological evolution by implicating their beneficial effects on the organisms [5]. Most of the free radicals exert significant actions such as transmission of molecular signals, gene transcription, and regulation of soluble guanylate cyclase activity in cells [6]. When free radicals and other reactive species (OH, $HO_2$, $ONOO^-$) extract a hydrogen atom from an unsaturated fatty acyl chain, a carbon-centered lipid radical (L) is produced. This is followed by the addition of oxygen to L to yield a lipid peroxyl radical (LOO) [6]. LOO further propagates the peroxidation chain reaction by abstracting a hydrogen atom from a nearby unsaturated fatty acid. The resulting lipid hydroperoxide easily decompose to form a lipid alkoxy radical [7]. This series of free radicals initiate chain propagation reaction in mammalian cells and results in the formation of malonaldehyde (MDA), an end-product in the oxidation of polyunsaturated fatty acids, is considered as a useful indicator of lipid peroxidation (LPO) [8].

Medicinal plants have been the subject for very intense pharmacological studies and serve as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development [9]. In developing countries, it is estimated that about 80% of the population really depends

on traditional medicine for their primary health care. Medicinal plants are considered to be an important source of antioxidants which attributes to their therapeutic properties [10].

Various herbs and spices were reported to exhibit antioxidant activity. A majority of the antioxidant activity was attributed to derivatives of phenolic acids, flavonoids, and tannins [11]. In the current period, antioxidant-based drug formulations are used for the prevention and treatment of complex diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer [12]. Phytochemicals are non-nutritive chemicals that have protective or disease therapeutic and preventative property [13]. *Kedrostis foetidissima* leaf extracts found the presence of, phytochemicals such as alkaloids, phenols, flavonoids, and steroids in all extracts studied; these compounds of leaves may be used to determine bioactive natural products that may serve as leads in the improvement of new pharmaceuticals that address unmet beneficial use [14]. Thus, this study was aimed to evaluate the free radical ability of the plant leaf extract.

## MATERIALS AND METHODS

### Plant Collection and Preparation of Extracts

Leaves of *K. foetidissima* (Jacq.) Cogn. were collected from near Sivagiri, Erode District, Tamil Nadu, India. Fresh leaves were washed and shade dried. The dried leaves were made to coarse powder and used for preparing extract with water, methanol, acetone, chloroform, and petroleum ether (aqueous extract of *K. foetidissima* [AQKF], methanolic extract of *K. foetidissima* [MKF], acetone extract of *K. foetidissima* [ACKF], chloroform extract of *K. foetidissima* [CKF], and petroleum ether extract of *K. foetidissima* (PEKF)]. All the extracts were subjected to *in vitro* free radical scavenging assays.

### *In vitro* Free Radical Scavenging Assays in Various Extracts of *K. foetidissima*

*Nitric oxide radical scavenging assay*

Nitric oxide radical scavenging activity was measured by Green *et al.*, [15] method. To 3 ml of 10 mM sodium nitroprusside in phosphate buffered saline (PBS) (pH7.4), 1 ml of various concentration of (2-10 mg/ml) extracts were added. The resulting solution was then incubated at 25°C for 60 min. To the incubated sample 5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride in 2% orthophosphoric acid) was added and the absorbance of the chromophore formed was measured at 546 nm against a reagent blank. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standards.

*Hydrogen peroxide ($H_2O_2$) scavenging assay*

$H_2O_2$ scavenging activity was estimated by Ruch *et al.*, [16] method. A solution of hydrogen peroxide (43 mM) was prepared in phosphate buffer (1 M pH 7.4). Various concentrations of samples (2-10 mg/ml) were added to hydrogen peroxide solution and after 10 min incubation the absorbance was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard.

*Superoxide anion radical scavenging assay*

Superoxide anion radical scavenging was found by Fontana *et al.*, [17] method. To 1 ml of various concentrations of the extracts (2-10 mg/ml), 1 ml of phosphate buffer, 1 ml of nicotinamide adenine dinucleotide (NADH), 1 ml of nitroblue tetrazolium (NBT), and 0.1 ml of phenazine metho-sulfate (PMS) were added. After 5 min incubation at room temperature, the absorbance was read at 562 nm against a reagent blank to determine the quantity of formazan generated. BHT and ascorbic acid were used as the standards.

*Egg yolk LPO assay*

Egg yolk LPO was measured by Kuppusamy *et al.*, [18] method. For the induction of LPO, 1 ml of egg yolk was emulsified with PBS (0.1 M, pH 7.4) to get a concentration of 12.50 g/L. 0.1 ml of various concentrations of the extracts (2-10 mg/ml) were added to the buffered egg yolk. Then, 0.2 ml of 3 mM ferrous sulfate was added to the above mixture and incubated at 37°C for 1 h. After incubation, the mixture was treated with 0.5 ml of 15% trichloroacetic acid and 1 ml of 1% thiobarbituric acid (TBA). Then, the reaction tubes were kept in boiling water bath for 10 min. On cooling, the tubes were centrifuged at 3500 g for 10 min to remove precipitated protein and absorbance of the supernatant was measured at 532 nm against blank containing buffered egg and ferrous sulfate. Gallic acid and vitamin E were used as standards.

### Statistical Analysis

Data were expressed as mean ± standard deviation of triplicate measurements. Data were evaluated using Student's *t*-test, one-way ANOVA with DMRT analysis using SPSS 10.0 software.

## RESULTS

### Extraction Yield

The extraction yield of various solvent extracts of *K. foetidissima* leaves varied from 2.6% to 10% and can be represented AQKF >MKF > CKF > ACKF > PEKF. The extractive yield was found to be high in AQKF when compared to other extracts. The variation in the extractive yield may be due to the nature of the solvents and chemical nature of the sample.

### Nitric Oxide Radical Scavenging Activity

Nitric oxide radical scavenging activities of various extracts of *K. foetidissima* were presented in Figure 1. All the extracts of *K. foetidissima* effectively reduced the generation of nitric oxide from sodium nitroprusside over a concentration of 2-10 mg/ml concentration which was significant at $P < 0.05$. The inhibition percentage of AQKF, MKF, ACKF, CKF and

PEKF were 36.33-68.67%, 55.67-87.67%, 30.33-66%, 51.17-79%, 22-61% with the 50% inhibitory concentration ($IC_{50}$) value of 5.2, 1.6, 5.6, 2.0 and 6.2 mg/ml, respectively. MKF exhibited strongest nitric oxide radical scavenging activity with maximum inhibition of 87.67% at 10 mg/ml concentration. The extracts radical scavenging activity were effective in the order MKF > CKF > AQKF > ACKF > PEKF. Ascorbic acid and BHT were used as standards at a concentration 20-100 $\mu$g/ml, and the $IC_{50}$ value was found to be 40 and 42 $\mu$g/ml.

## Hydrogen Peroxide Radical Scavenging Activity

The $H_2O_2$ scavenging activity of various solvent extracts of *K. foetidissima* leaves was reported in Figure 2. All the extracts showed moderate to potent level of $H_2O_2$ radical scavenging activity over the range of 2-10 mg/ml concentration. The inhibition percentage of AQKF, MKF, ACKF, CKF and PEKF were 21-60%, 31.17-81.5%, 21.52-73.53%, 27.2-78%, 14.2-57.67% with the $IC_{50}$ value of 8.0, 3.8, 7.0, 5.0, 8.8 mg/ml, respectively. MKF exhibited strongest hydrogen radical scavenging activity with maximum inhibition of 81.5% at 10 mg/ml concentration. The extracts radical scavenging activity were effective in the order MKF > CKF > ACKF > AQKF > PEKF. Ascorbic acid was used as standard at a concentration of 20-100 $\mu$g/ml and the $IC_{50}$ value was found to be 46 $\mu$g/ml. All the leaf extracts of *K. foetidissima* exhibited moderate scavenging activity when compared to ascorbic acid.

## Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activities of various extracts of *K. foetidissima* were represented in Figure 3. All the extracts of *K. foetidissima* effectively reduced the superoxide anion radicals generated from PMS/NADH-NBT system, over a concentration range of 2-10 mg/ml concentration. The inhibition percentage of AQKF, MKF, ACKF, CKF and PEKF were 25-61%, 31.5-82%, 27.67-65.33%, 37.67-71%, 15-53% with the $IC_{50}$ value of 7.4, 3.2, 6.6, 4.2, 9.6 mg/ml, respectively. MKF exhibited strongest hydroxyl radical scavenging activity with maximum inhibition of 87.67% at 10 mg/ml concentration. The extracts radical scavenging activity was effective in the order MKF > CKF > ACKF > AQKF > PEKF. Gallic acid and BHT were used as standards at a concentration 20-100 $\mu$g/ml and the $IC_{50}$ value was found to be 40 and 70 $\mu$g/ml.

## Egg Yolk Lipid Peroxidation Assay

Egg yolk LPO assay in various extracts of *K. foetidissima* was shown in Figure 4. All the extracts of *K. foetidissima* effectively

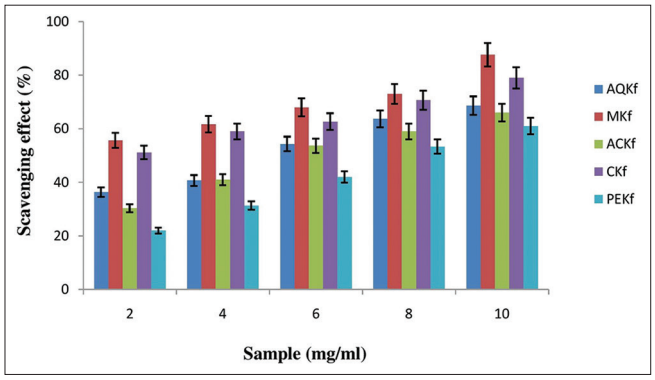


**Figure 1:** Effect of *Kedrostis foetidissima* various extracts on nitric oxide radical scavenging activity. Values mean ± standard deviation; values are statistically significant at $P < 0.05$

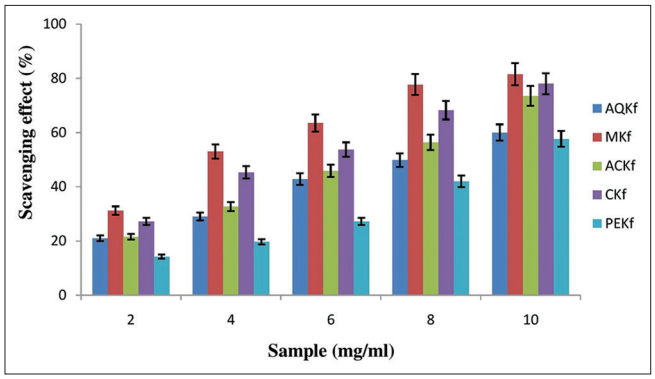


**Figure 2:** Effect of *Kedrostis foetidissima* various extracts on hydrogen peroxide radical scavenging activity. Values mean ± standard deviation; values are statistically significant at $P < 0.05$

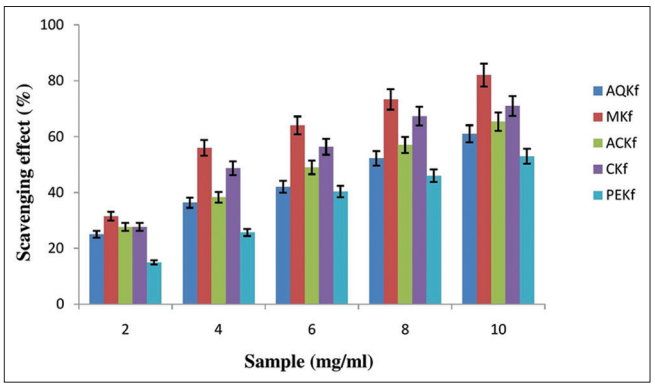


**Figure 3:** Effect of *Kedrostis foetidissima* various extracts on superoxide anion radical scavenging activity. Values mean ± standard deviation; values are statistically significant at $P < 0.05$

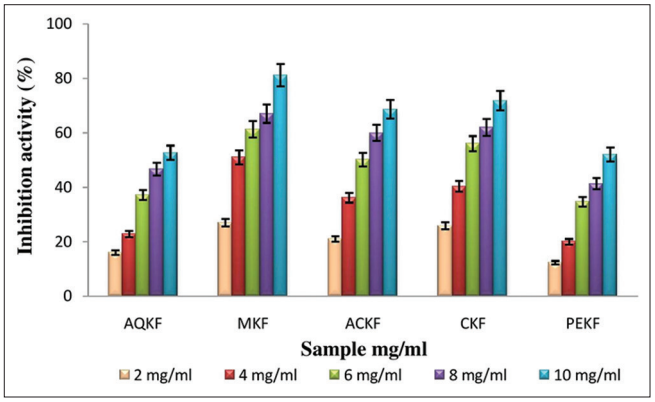


**Figure 4:** Effect of *Kedrostis foetidissima* various extracts on egg yolk lipid peroxidation. Values mean ± standard deviation; values are statistically significant at $P < 0.05$

inhibited the formation of LPO over a concentration range of 2-10 mg/ml. The inhibition percentage of AQKF, MKF, ACKF, CKF, PEKF were 16-52.67%, 27-81.17%, 21-68.67%, 25.89-71.83%, 12.33-52% with the $IC_{50}$ value of 7.4, 3.2, 6.6, 4.2, 9.6 mg/ml, respectively. MKF exhibited the highest inhibition of LPO among the five extracts with maximum inhibition of 81.17% at 10 mg/ml concentration. The effective order of the extracts LPO inhibition were MKF > CKF > ACKF > AQKF > PEKF. Gallic acid and vitamin E were used as standards at a concentration 20-100 $\mu$g/ml, and the $IC_{50}$ value was found to be 38 and 64 $\mu$g/ml. All the extracts possess lesser activity when compared to standards.

## DISCUSSION

Nitric oxide is a diffusible free radical which plays a significant role as an effector molecule in diverse biological process such as regulation of vasodilation immune response, signal transmission in neurons and control of vasodilation and blood pressure [19]. The degree of inhibition of the nitric oxide radicals was found to be increased in increasing concentration of the *K. foetidissima* leaf extracts; this indicates that the extracts may contain compounds capable of inhibiting the generation of nitric oxide and offers scientific evidence for the use of *K. foetidissima* leaves in the treatment of various diseases. The leaf extract of *K. foetidissima* exhibited higher nitric oxide radical scavenging activity when compared to citrus leaf extract ($IC_{50}$ value 2.82 mg/ml) [20] and lesser activity than *Macrotyloma uniflorum* seed extract (62.47% at 250 $\mu$g/ml) [21].

$H_2O_2$ is a normal cellular metabolite that is continuously generated and maintained at low concentration. Excessive production of this oxidant has been associated with cell dysfunction and generation of diseases. Scavenging of $H_2O_2$ radical by extracts may be attributed to their phenolics, which can donate electrons to $H_2O_2$, thus neutralizing it to water [22]. Thus, the results indicate that the antioxidant components present in the extracts are good electron donors, which may accelerate the conversion of $H_2O_2$ to $H_2O$. The leaf extracts of *K. foetidissima* exhibited lesser activity than leaf extract of *Ecballium elaterium* (39.50% at 0.1 mg/ml) [23], whole plant extract of *Solena amplexicaulis* (82.53% at 125 $\mu$g/ml) and seed extract of *Cucumis callosus* ($IC_{50}$ value 94.71 $\mu$g/ml) [24].

Superoxide anion is one of the most representative weak oxidant produced during various biological reactions. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction and reduces NBT. A decrease in absorbance indicates that the antioxidant activity of the extracts may be due to the inactivation or consumption of superoxide anion radicals produced in the reaction mixture. The superoxide anion radicals scavenging ability was increased in increasing concentration of extracts and found to be moderate when compared to standards. The leaf extracts of *K. foetidissima* showed lower activity than wild bitter melon aqueous fruit extracts (36.6-75.8% at 1-100 $\mu$g/ml) [25] and stem extracts of *Coccinia grandis* ($IC_{50}$ value 95-110 $\mu$g/ml) [26].

Peroxidation of lipids has been shown to be cumulative effect of ROS, which disturb the assembly of the membrane causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes [27]. Egg yolk lipids undergo rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulfate results in subsequent formation of MDA and other aldehydes that form pink chromogen with TBA which was measured colorimetrically at 532 nm. All the extracts and standards inhibited LPO induced by ferrous sulfate in egg yolk homogenates in concentration dependent manner. The results suggest that secondary metabolites extracted within various extracts of *K. foetidissima* leaves could play an important role in protecting the physicochemical properties of membrane bi layers of cells from severe free radical induced cellular dysfunction. *K. foetidissima* leaf extracts were found to have lesser LPO activity when compared to L. *parasiticus* extract ($IC_{50}$ value 1.05 mg/ml) [28] and *H. pauciflorus* leaves ($IC_{50}$ value of 64.7 $\mu$g/ml) [29] using egg yolk as media of peroxidation.

## CONCLUSION

In this study, *K. foetidissima* has shown moderate to potent scavenging activity against various free radicals. Among the five extracts, MKF leaves demonstrated prominent radical scavenging activity against $H_2O_2$, $O^-$, NO and egg yolk LPO. The results of the study indicated that methanolic extract of *K. foetidissima* provides a source of biologically active phytochemicals and it was found to have potential antioxidant properties, which can able to protect the cells from free radicals induced oxidative damage in the biological system may because of various biological active compounds present in the crude extract. Further studies have to be carried out to assess the *in vivo* biological activities and to isolate the specific compounds from the methanolic extract which can be responsible for the antioxidant activity.

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