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Research Article

IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF *KEDROSTIS FOETIDISSIMA* (JACQ) COGN LEAF EXTRACTS

M. Jagadeeswari [1]* and Dr. N. Hemashenpagam [2]

*[1] Department of Microbiology, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamil Nadu, INDIA.

[2] Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, INDIA.

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ABSTRACT

Kedrostis foetidissima is well known for antioxidant and related activity like anti- inflammation due to presence of several secondary metabolites in vast quantity. The recent researches contribute to usefulness of the plant in treating variety of ailments and disorders. In the present study, in vitro antioxidant and anti-inflammatory activity of Kedrostis foetidissima leaf extracts were assessed by DPPH free radical scavenging and inhibition of protein denaturation respectively. The results of qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, phenols, steroids, saponins and glycosides. The methanol leaf extract exhibited maximum antioxidant quenching with75.46 % at the concentration of 150µg/ml and IC_{50} value was found to be 90.78µg/ml. Further, the methanol leaf extract was found to be a potent anti-inflammatory agent and shows maximum inhibition of 80.25 % protein denaturation at the concentration of 800µg/ml.

KEYWORDS: *Kedrostis foetidissima, phytoconstituents, antioxidant, DPPH radical scavenging, anti- inflammation, Protein denaturation.*

INTRODUCTION

Plants serve as a powerful source of medicine containing plenty of ingredients that are most useful remedy for infectious and inflammatory diseases. A report by world health organisation says that about 80% of world population depends mainly on herbals for their welfare because of its effectiveness and nil or minimum side effects. *Kedrostis foetidissima* is a creeper belonging to the family Cucurbitaceae known to have plenty of chemical constituents especially phenolic and flavonoid compounds. Antioxidant, anti-inflammatory and other activities of health and medical relevance relies mainly on the presence of secondary metabolites in plants.

Antioxidants are free radical scavengers able to reduce DPPH radical by donating electrons [1]. Super oxide anion, hydrogen peroxide and reactive hydroxyl radicals are the free radicals formed during normal cellular metabolism, the major cause of chronic and degenerative disorders, ageing, cardiovascular diseases, autoimmune disorders, cancer, inflammatory diseases and so on [2-6]. They initiate damage of biological membrane by peroxidation of polyunsaturated fatty acids, DNA damage and oxidation of biocatalysts [7, 8].

Phenolic compounds and flavonoids are the two major phytoconstituents responsible for antioxidant activity. Phenolic compounds contain 1 or more hydroxyl groups in its aromatic rings able to quench free radicals by forming stabilized phenoxyl radicals. Flavonoid structure is an important determinant of free radical scavenging [9, 10] and it's nucleus contain free hydroxyl group in its C3 position. The stability and activity of flavonoid depends on the number of OH groups present.

Inflammation is nonspecific defence mechanism elicited by our body in response to injury caused by entry of pathogens or other irritants. Inflammation may be acute or chronic. Chronic inflammation leads to severe autoimmune disorders like arthritis, cancer, hay fever, atherosclerosis etc., Even though it is a part of healing process, anti inflammatory medications are required to alleviate the pain and other symptoms caused. Excessive inflammatory response leads to impairment in wound healing and physiological activities. The condition worsens if there is any imbalance in inflammatory factors and onset of inflammation [11]. Nonsteroidal anti inflammatory drugs and corticosteroids are normally employed but they are proven to have adverse effects. Several herbal drugs are known to have anti-inflammatory activities without or minimum side effects. The flavonoids inhibit the encoding of isoforms of enzymes like cyclooxygenase, nitric oxide synthase and lipooxygenase required for production of prostanoids, nitric

*** Corresponding author:**

M. Jagadeeswari

Department of Microbiology,

Rathnavel Subramaniam College of Arts and Science,

Coimbatore, Tamil Nadu, INDIA.

** E-Mail: jaga.prith@gmail.com*

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oxide, leukotrienes and mediators of inflammatory reactions like chemokines and cytokines [12]. Some flavonoids inhibit the production of proinflammatory signalling molecules like prostaglandins [13]. The flavonoids act as an anti-inflammatory agent by inducing the circulating cytokines to fix on to the site of injury. There is a positive correlation between antioxidant potential and anti-inflammatory activity of plant extracts. With this background information our study dealt with antioxidant and anti inflammatory activity of various leaves extract of *K. foetidissima*.

MATERIALS AND METHODS

Collection and identification of plant material:

Fresh plant leaves were randomly collected from the garden and villages of Coimbatore district, Tamil Nadu, India and authenticated at Botanical survey of India, Southern circle, Coimbatore. The voucher specimen was deposited in the Department of Microbiology, Rathnavel Subramaniam College of Arts & Science, Coimbatore for further study.

Preparation of Plant extract:

The fresh, clean and disease free leaves were shade dried, chopped and coarsely powdered. The leaf powder was defatted with petroleum ether for 24 hour and dried completely for solvent evaporation. Exactly 200 grams of defatted plant powder was used for extraction in 800 ml of organic solvents like methanol, ethanol and chloroform for organic solvent extract and distilled water for aqueous extract by using cold maceration method [14]. The defatted plant powder in organic solvents and water were kept in a capped container for a week time with frequent agitation to dissolve soluble matter. The contents were filtered with Whatman no.1 filter paper and then the filtrates were allowed for evaporation and concentrated by rotary vacuum evaporator to make the final volume one-fifth of the original volume. The dried extracts were weighed to determine the % yield by following the formula.

% Yield = (Weight of dry extract / weight of extract before drying) x 100

The crude extracts thus obtained were stored in sterile vials at 4^0 C for further study.

Qualitative phytochemical analysis:

The methanol, ethanol, chloroform and aqueous leaf extracts of *Kedrostis foetidissima* were analysed for the presence of phytoconstituents like alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, steroids, glycosides etc., by following the standard method [15].

***In vitro* Anti oxidant activity:**

The DPPH free radical scavenging activity was determined by modified method [16]. In brief 0.135 mM DPPH solution in methanol was prepared and its absorbance value was adjusted to ≤ 1 at 517 nm. About 3 ml of plant extracts in concentrations ranging from 25 – 150 µg/ml was dissolved in 1 ml DPPH solution and incubated for 30 minutes at room temperature for reaction to take place. The absorbance reading was taken at 517 nm in spectrophotometer. Ascorbic acid was used as a standard drug and DPPH without test sample serves as a control. The experiment was carried out in triplicate and the mean value was taken for calculation. The free radical scavenging activity of plant extracts and standard drug was calculated by using the formula,

Percentage inhibition = (OD of control – OD of test sample) x 100/OD of control

The 50% free radical scavenging activity of plant extracts and the standard ascorbic acid were expressed as IC_{50}. The IC_{50} value of plant extracts and standard ascorbic acid was calculated by linear regression method.

Inhibition of Protein denaturation:

Inhibition of protein denaturation was determined by adopting the method with slight changes to check the anti-inflammatory activity at *in vitro* level [17]. The reaction mixture was prepared by mixing 0.9 ml of 5% bovine serum albumin in water with 0.1 ml of different concentrations (50 – 800 µg/ml) of plant extracts in corresponding test tubes. 1N hydrochloric acid was used to adjust the pH of the reaction mixture to 6.3 and incubated at 57^0 C for 3 minutes. Phosphate buffer with volume of 2.5 ml was added to all the test tubes. Optical density reading was taken at 660 nm in spectrophotometer. The aspirin was used as a standard drug. The experiment was carried out in triplicate and the mean value was taken for calculation. The percentage inhibition of albumin denaturation by test sample was calculated from control that does not contain plant extract by using the formula,

Percentage inhibition = (OD of control – OD of test sample) x 100/OD of control.

Statistical analysis:

The results were presented as mean ± standard deviation (SD) (n = 3). Statistical comparisons of data's were done by one way ANOVA in Excel. $P<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The leaf powder of *Kedrostis foetidissima* was extracted with organic solvents and water and the percentage yield of various leaf extracts were determined to be 7.4 in methanol (KFMLE), 6.5 in ethanol (KFELE), 6.0 in chloroform (KFCLE) and 7.0 in aqueous (KFALE) leaf extracts. The variation in extract yield is due to difference in the nature and type of phytoconstituents extracted by different solvent systems.

Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins and triterpenoids in all four tested extracts. Phenols and steroids were found in methanol, ethanol and chloroform extracts. The tests for Saponins and glycosides show negative in chloroform and aqueous leaf extracts whereas carbohydrates, anthroquinones and phlobatannins were found to be absent in all the tested extracts.

DPPH scavenging assay is a common method for determining free radical scavenging activity of plant extracts by oxidation-reduction reaction. In this assay, the antioxidants present in the extracts react with violet coloured 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and reduces it to yellow coloured diphenyl-β-picryl hydrazine by donating electrons and was detected using spectrophotometer. The degree of discolouration indicates the antioxidant potential of the sample. The percentage DPPH radical quenching of *K. foetidissima* leaf extracts was presented in Table 1 and Fig 1. In our study all the tested extracts revealed free radical quenching activity. An increase in DPPH radical quenching was observed with decrease in absorbance if we increase concentration of tested plant extracts and the standard ascorbic acid. Alcoholic leaf extracts

showed notable activity than other extracts studied. Methanol leaf extract (KFMLE) is the leading one with 75.46% antioxidation capacity at 150 µg/ml concentration (IC_{50} = 90.78 ± 0.01µg/ml) and is comparable to standard ascorbic acid (IC_{50} - 62.92 ± 0.02µg/ml). The ethanol extract (KFELE) has antioxidant capacity of 58.64%, followed by chloroform extract (KFCLE) with 176.74% and aqueous leaf extract (KFALE) with 472.45% at 150µg/ml concentration and their IC_{50} values were predicted as 119.82, 176.74 and 472.45 respectively.

Table No. 1: Antioxidant activity of *K. Foetidissima* leaf extracts

Plant Extract	% DPPH radical scavenging/Conc. µg/ml						
	25	50	75	100	125	150	IC_{50}
KFMLE	14.56±0.02	32.89±0.07	45.26±0.03	56.42±0.03	66.46±0.12	75.46±0.01	**90.78**
KFELE	10.01±0.12	25.23±0.02	34.26±0.12	44.26±0.01	53.41±0.04	58.64±0.06	**119.82**
KFCLE	6.51±0.11	14.44±0.03	23.92±0.11	29.42±0.12	35.59±0.07	41.42±0.12	**176.74**
KFALE	1.62±0.01	2.46±0.17	6.09±0.04	9.51±0.03	12.12±0.11	14.52±0.06	**472.45**
Asc.acid	28.56±0.01	44.68±0.01	58.48±0.02	69.87±0.06	80.26±0.04	90.24±0.01	**62.92**

The results were expressed as ± SD (n=3). The values are statistically significant at the level of p<0.05.

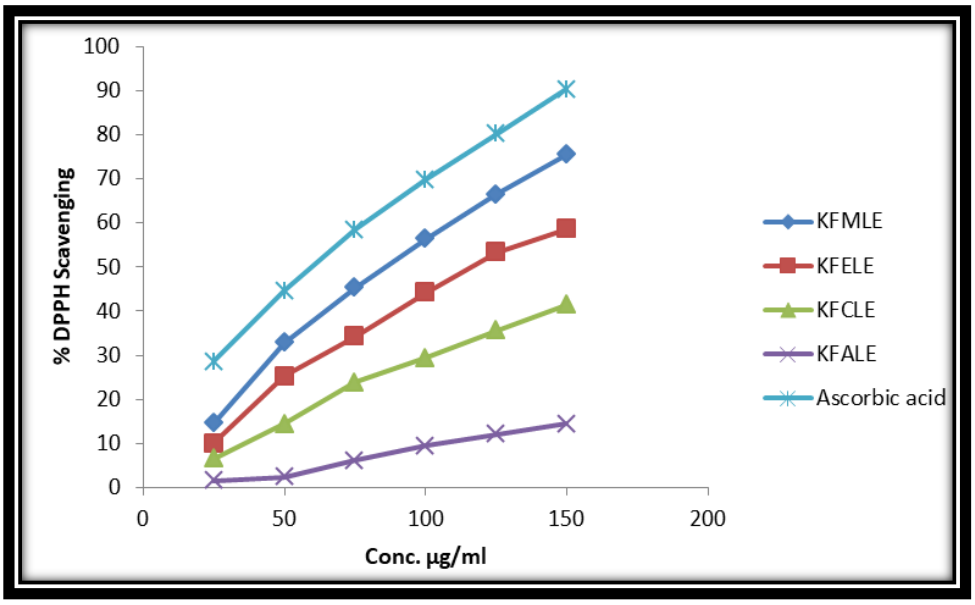

Fig. 1: DPPH Scavenging of *K. Foetidissima* leaf extracts

Our results are in accordance with the results of [18] who reported that the methanol leaf extract of *K foetidissima* revealed strongest antioxidant activity than other extracts tested with EC_{50} value of 1.2. Similarly the methanol extract of C. bonduc elicit utmost antioxidant activity [19, 20] and [21] reported that the ethanol extract of *K foetidissima* displayed highest free radical scavenging activity. The IC_{50} value (1284.93±31) is high with *H. heteroclita* of Cucurbitaceae family when compared to the standard ascorbic acid (31±0.13) [22]. In another study the purified form of flavonoid from *Hyssopus officinalis* showed weak free radical scavenging activity than the extracts with high phenol content [23]. The methanol extract of *Crateva magna* possess a significant DPPH radical scavenging ability than chloroform and petroleum ether extracts and the activity is comparable with standards BHT and rutin [24].

The bioactivity of plant extracts is attributed to the phytoconstituents and have been reported to exhibit multiple biological effects. Primary constituents contain common sugars, amino acids, proteins and chlorophyll, while secondary constituents comprise of alkaloids, flvonoids, saponin, tannin, phenolic compounds and many more [25]. Many studies focused on the role of secondary metabolites like polyphenols and flavonoids in antioxidant potency of plants. The presence of these compounds are usually correlated with their antioxidant activity in the *in vitro* studies like DPPH assay [26]. In our study, the phenolics are the major contributor than flavonoids. Neutralization of free radicals is brought about by phenolic groups present in polyphenols and is present in all parts of the plant and shown to exert protective effect on plants from diseases [27].

The main cause of inflammation is protein denaturation. In our study, all four extracts tested shows inhibitory activity against protein denaturation at different concentrations tested and the results were depicted in Table.2 and Fig.2. Like antioxidant activity, the anti-inflammatory activity also dose dependent and increases with increase in concentration of plant extracts. KFMLE was shown to possess maximum inhibition percentage of 80.25 at 800 µg/ml concentration followed by KFELE (65.75), KFCLE (48.26) and KFALE (24.51). The IC_{50} values were predicted using linear regression equation and was found to be 279.71µg/ml in KFMLE, 486.35 in KFELE, 817.22 in KFCLE and was higher in KFALE. The effectiveness of plant extracts against protein denaturation was in the order KFMLE > KFELE>KFCLE>KFALE. The standard aspirin shows 66.26% and 74.26 % inhibition at 100 and 200µg/ml respectively.

Table No. 2: *In-Vitro* Anti- inflammatory activity of *K.Foetidissima* leaf extracts

Plant Extract	% inhibition of Protein denaturation/Conc. µg/ml						
	50	100	200	400	600	800	IC_{50}
KFMLE	26.12±0.01	35.23±0.02	53.46±0.13	64.45±0.03	72.68±0.16	80.25±0.04	279.71
KFELE	18.17±0.12	24.06±0.04	34.74±0.14	50.94±0.02	58.12±0.03	65.72±0.03	486.35
KFCLE	10.64±0.01	14.12±0.03	21.52±0.12	32.16±0.08	40.21±0.01	48.26±0.14	817.22
KFALE	1.26±0.01	4.62±0.07	11.28±0.01	17.12±0.04	20.78±0.06	24.51±0.02	ND
Aspirin	-	66.26±0.01	74.26±0.01	-	-	-	ND

The results were expressed as ± SD (n=3). The values are statistically significant at the level of p<0.05.ND-Not detected.

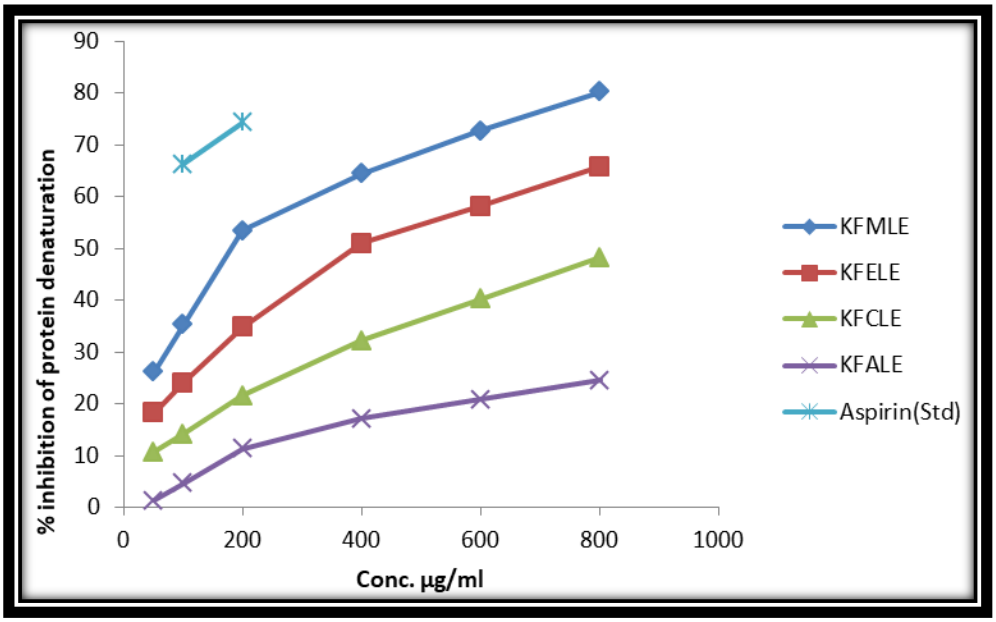

Fig. 2: Anti-inflammatory activity of *K. Foetidissima* leaf extracts

Methanol leaf extract of Oxalis corniculata linn, exhibited 85.92± 1.48 percent inhibition of protein denaturation with IC $_{50}$ value of 288.04±2.78 µg/ml [28] and is analogous to our results. In another study the ethyl acetate extract of *Syzygium zeylanicum* (L) DC leaves possess good anti-inflammatory activity with 64.43 at 100 µg/ml concentration whereas aqueous extract exhibits 76.92% inhibition of protein denaturation at 250 µg/ml concentration [29]. The leaves of Caesalpinia bonducella (Linn) exhibited 48.3% inhibition at the concentration 300 µg/ml with IC_{50} value of 311 µg/ml. Whereas the standard drug aspirin displayed the highest inhibition of 68.15% at 100 µg/ml concentration [30]. A quick wound healing is made possible by polyphenolic biflavonoids like proanthrocyanidines and other tannins synthesized [31]. In our study, an extreme antioxidant correlation was observed with polyphenol in methanol leaf extract of K. *foetidissima* and in turn is correlated with anti-inflammatory activity.

CONCLUSION

The present study concludes that the methanolic leaf extract of *Kedrostis foetidissima* exhibited an efficient DPPH radical quenching and *in vitro* anti-inflammatory activity than other extracts tested. Isolation and identification of potent chemicals in this plant is useful in treating antioxidant dependant inflammatory diseases.

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