Antioxidant capacity and phenolic content of *Elaeagnus kologa* schlecht. an underexploited fruit from India

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ABSTRACT

Introduction: In this study, assessment of total phenolic and flavonoid contents and antioxidant capacity of methanolic extract of fruits *of Elaeagnus kologa* Schldl. were examined for the first time. **Methods:** For the determination of total phenolics (TP) and total flavonoid content (TF) and *in vitro* antioxidative capacity, established assay methods such as 1, 1-diphenyl – 2-picryl hydroxyl (DPPH) radical assay, reducing power, ferric ion chelating assay, superoxide anion, nitric oxide scavenging activity and reduction of lipid peroxidation assays were used with reference to synthetic antioxidant butyl hydroxyl toluene (BHT). One way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were carried out. **Results:** The extract yielded total phenolic content (TP) of 2120 ± 0.012 mg gallic acid equivalents (GAE)/100 g of fresh mass (FM) and total flavonoid content (TF) of 220 ± 0.12 mg quercetin equivalents (QE)/100 g FM. The *E. kologa* fruit exhibited scavenging capacity towards DPPH•, superoxide radical, hydroxyl and nitric oxide. The results also showed that *E. kologa* extract had a strong reductive capacity, strong ferric ion (Fe^{3+}) chelation and remarkable reduction of lipid peroxidation. The antioxidant capacities of the extract were comparable with butyl hydroxy toluene, EDTA and catechin. **Conclusion:** Positive correlations were observed between polyphenolic contents and the antioxidant capacities. The results of the present study revealed that the fruits of *E. kologa* possess potent antioxidant activity.

Keywords: Antioxidant activity, *Elaeagnus kologa,* lipid peroxidation, reductive capacity, total phenolics, total flavonoids.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to damage cellular biomolecules (DNA, proteins, lipids, amines and carbohydrates), resulting in ageing and other degenerative diseases such as certain cancers, diabetes, Alzheimer's disease and Parkinson's disease etc.[1] Considerable attention has already been focused on the isolation, characterization and utilization of natural antioxidants as potential disease preventing agents. Over the past few decades, increasing epidemiological studies and intervention trails have consistently indicated the role of consumption of fruits and vegetables as antioxidants in the prevention of the degenerative and chronic diseases.[2,3] These protective effects of the fruits are mostly related to the antioxidant components i.e. vitamins, flavonoids, and carotenoids.[4,5] Most fruits like blueberries, strawberries, blackberries etc have been proven to combat oxidative stress in *in vitro* and *in vivo* systems.[6,7,8] Phenolics and flavonoids from fruits are best known for their ability to act as antioxidants, but the biological activities exerted by berry phytochemicals *in vivo* extend beyond antioxidation.[9] Therefore, it is of great interest in research concerning the antioxidant ability of underexploited fruits and isolation, characterization and utilization of natural antioxidants as potential disease preventing agents.

Elaeagnus kologa Schldl., belonging to the family Elaeagnaceae, is distributed in the Western Ghats, India. The fruits

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are locally known as '*Kolanga annu*' and are consumed by the local communities of the Nilgiris, India. However, fresh fruits of *E. kologa* are underused.[10] No traditional uses and chemical constituents like flavonoids of *E. kologa* have so far been reported. Earlier, DPPH radical scavenging and insecticidal activities of leaves of *E. kologa* were performed.[11] To our knowledge, no information on the antioxidant properties and health benefits of *E. kologa* fruits is available until now. With this background, we aimed to evaluate the total phenolic content and total flavonoid content, and to examine the potential antioxidant activities using DPPH radical quenching test, ferric reducing power, hydroxyl ion scavenging assay, nitric oxide scavenging and inhibition of superoxide ion assay of methanol extracts of *E. kologa*. The main objectives of the present study encompass (a) to measure the total phenolic and total flavonoid contents, (b) to establish the *in vitro* antioxidant potential of the methanol extract of *E. kologa* underexploited fruits.

MATERIALS AND METHODS

Fruits and preparation of extract

The plant *E. kologa* was taxonomically identified by Dr. R. Gopalan, Taxonomist, Karpagam University and was authenticated in Botanical Survey of India (southern circle), Coimbatore, India (Voucher No. UGC 00). The fully ripened fruits of *E. kologa* were collected from Doddabetta forest range, the Nilgiris (T. N.), India during May 2007 and June 2007. The ripened fruits were manually pooled and were kept in cold (–4° C) dark storage until further analysis. The frozen berries (100 g) were blended, exhaustively extracted with 5 times its volume of methanol (1:5 v/v) and centrifuged (3000 × *g*, Remi, India) for 15 min at 4° C and the supernatant was transferred to an amber bottle. The extraction process was repeated thrice using the same conditions. The supernatants were then combined and filtered over Whatman No. 1 filter paper. The filtered extract was concentrated *in vacuo* at 40 ± 1° C by rotary flash evaporator (Buchi type rotavapor, Switzerland) under reduced pressure to obtain the dry extract. The dry extract was re-dissolved in methanol and the stock solution was kept at –4° C to protect from light until further use. The stock solution was used to determine total phenolics, total flavonoids and antioxidant capacity.

Determination of total phenolics (TP)

The content of total phenolic content (TP) in the *E. kologa* extract was determined colorimetrically using folin-ciocalteu phenol reagent method (Singleton et al.).[12] Briefly, diluted extract (1 mL) was added with diluted folin-ciocalteu reagent (1 N, 1 mL). After 3 min of reaction, Sodium carbonate (Na_2CO_3) (35%, 2 mL) was added and the mixture was incubated for 30 min at room temperature. The absorbance was read at 765 nm using UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). The analyses were performed in triplicates. The TPC was expressed as mg gallic acid equivalents from a gallic acid standard curve (mg GAE/100 g fresh mass, $R^2 = 0.9968$).

Determination of total flavonoids (TF)

The determination of total flavonoid content (TF) in the *E. kologa* extract was based on the method reported previously.[13] The absorbance of TF was measured at 510 nm using UV-Vis spectrophotometer with reference standard prepared with quercetin concentrations. The analyses were performed in triplicate. The TF was estimated from a quercetin standard curve and the results were expressed as mg quercetin equivalents (mg QE/100 g fresh material, $R^2 = 0.9665$).

Scavenging capacity towards DPPH· stable radical

The determination of DPPH· stable radical scavenging activity of the *E. kologa* extract was based on the method as described previously.[14] Briefly, one millilitre of aliquots of the extract and standards (5, 10, 25, 50, 100, 250, 500, 750 and 1000 μg mL^{-1}) was added to MeOH solution of DPPH· (5 mL, 0.1 mM) and vortexed. After 20 min reaction at 25°C, the absorbance was measured at 517 nm against a blank in a UV-Vis spectrophotometer. BHT and ascorbic acid were used for comparison. The percentage quenching of DPPH· was calculated as follows: Inhibition of DPPH· (%) = 1 – $Sample_{517\ nm}/Control_{517\ nm} \times 100$, where, $Sample_{517\ nm}$ was absorbance of the sample and $Control_{517\ nm}$ was absorbance of control. The results were expressed as EC_{50}, which means the concentration at which DPPH· radicals were quenched by 50%.

Measurement of reductive capacity (RC)

The reducing capacity of the *E. kologa* extract was measured using the potassium ferricyanide reduction method.[15] Various concentrations of the extract and standards (50, 100, 250, 500, 750 and 1000 μg mL^{-1}) were added to 2.5 ml of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of freshly prepared potassium ferricyanide [$K_3Fe_3(CN)_6$] (1%) solution and vortexed. After incubation at 50°C for 20 min, 2.5 ml of TCA (10%, w/v) was added to all the tubes and centrifuged (Remi, India) at 3000 × *g* for 10 min. Afterwards, upper layer of the

solution (5 mL) was mixed with deionized water (5 mL). To this, one millilitre of freshly prepared $FeCl_3$ (1%) was added to each test tube and incubated at 35°C for 10 min. The formation of Perl's Prussian colour was measured at 700 nm in a UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicated increasing reducing power. Here, the EC_{50} value is the effective concentration at which absorbance was 0.5% for the reducing capacity. BHT and BHA were used for comparison.

Scavenging capacity towards super oxide anion ($O_2^{\cdot -}$)

Super oxide anion radicals ($O_2^{\cdot -}$) generated in the phenazine methosulfate-reduced form of nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium chloride (NBT) by the extract with some changes (Yu et al. 2006).[16] The $O_2^{\cdot -}$ were generated in 1.25 mL of Tris–HCl (16 mM, pH 8.0), 0.25 mL of NBT (150 µM), 0.25 mL of NADH (468 µM) and different concentrations (50, 100, 250, 500, 750 and 1000 µg mL^{-1}) of *E. kologa* extract and standards. The reaction was initiated by addition of 0.25 mL of phenazine methosulphate (PMS) (60 µM) to the mixture. Following incubation at ambient temperature for 5 min the absorbance was read at 560 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of $O_2^{\cdot -}$ was calculated as follows: Inhibition of $O_2^{\cdot -}$ (%) = 1 – $Sample_{560\ nm}/Control_{560\ nm} \times 100$, where, $Sample_{560\ nm}$ was absorbance of the sample and $Control_{560\ nm}$ was absorbance of control.

Scavenging capacity towards hydroxyl ion ($^{\cdot}OH$) radicals (deoxyribose assay)

Hydroxyl radicals ($^{\cdot}OH$) were generated by a fenton reaction model system, and the scavenging capacity towards the $^{\cdot}OH$ radical was measured using deoxyribose method with minor modifications (Halliwell et al. 1997).[17] To one millilitre of *E. kologa* extract (25, 50, 100, 250, 500, 750 and 1000 µg mL^{-1}), 1 mL of phosphate buffer (50 mM; pH 7), 0.2 mL of EDTA (1.04 mM), 0.2 mL of $FeCl_3$. $6H_2O$ (1.0 mM) and 0.2 mL of 2-deoxy-d-ribose (60 mM) were added. Following incubation in a water bath at 37° C for 60 min, 2 mL of cold TBA (in 50 mM NaOH) and 2 mL of TCA (25% w/v aqueous solution) were added to the reaction mixture. The mixture was then incubated at 100° C for 15 min. After cooling, the absorbance of the pink chromogen developed was recorded at 532 nm in a spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of $^{\cdot}OH$ was calculated as follows: Inhibition of $OH^{\cdot}$ (%) = 1 – $Sample_{532\ nm}/Control_{532\ nm} \times 100$, where, $Sample_{532\ nm}$ was absorbance of the sample and $Control_{532\ nm}$ was absorbance of control.

Scavenging capacity towards nitric oxide (NO)

Nitric oxide (NO) generated from sodium nitroprusside (SNP) in aqueous solution at physiological pH was estimated by the use of Griess reaction with minor changes (Green et al. 1982).[18] The reaction mixture (3 mL) containing freshly prepared SNP (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and the methanol extract of *E. kologa* at different concentrations and standards (25, 50, 100, 250, 500, 750 and 1000 µg mL^{-1}) were incubated at 25° C for 150 min. After incubation, 0.5 mL of the incubated solution containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 mL of N-1-naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25° C. The absorbance of pink coloured chromophore formed during diazotization was immediately measured at 540 nm in a UV-Vis spectrophotometer. BHT and catechin were used for comparison. The percentage scavenging of NO was calculated as follows: Inhibition of NO (%) = 1 – $Sample_{540\ nm}/Control_{540\ nm} \times 100$, where, $Sample_{540\ nm}$ was absorbance of the sample and $Control_{540\ nm}$ was absorbance of control.

Measurement of iron chelating capacity (ICC)

The ICC was investigated using the method of Singh and Rajini (2004).[19] Briefly, different concentrations of *E. kologa* and standards (25, 50, 100, 250, 500, 750 and 1000 µg mL^{-1}) were mixed with 0.1 ml of $FeCl_2$ (2 mM) and 0.2 ml of ferrozine (5 mM). The mixture was made up to 0.8 mL with deionized water. After 10 min incubation at room temperature, the optical density value of ferrous ion-ferrozine complex was measured at 562 nm in UV-Vis spectrophotometer. EDTA and catechin were used as standards for iron chelating assay. The percentage of inhibition of ferrozine-fe^3 complex formation was calculated as follows: Chelating effect (%) = 1 – $Sample_{562\ nm}/Control_{562\ nm} \times 100$, where, $Sample_{562\ nm}$ was absorbance of the sample and $Control_{562\ nm}$ was absorbance of control.

Reduction of lipid peroxidation

Inhibition of lipid peroxidation (LPO) in rat liver homogenate was determined in terms of formation of thiobarbituric acid reactive substances (TBARS) with minor changes.[20] In brief, different concentrations of *E. kologa* extract and standard (25, 50, 100, 250, 500, 750 and 1000 µg mL^{-1})

were individually added to 0.2 mL of liver homogenate (10%) extracted with KCl (15%). To the above mixture, 0.1 mL of $FeSO_4$ (10 mM) solution was added to initiate LPO. The volume of the mixtures was finally made up to 2 mL with phosphate buffer (0.1 mM, pH 7) and incubated at 37° C for 30 min. At the end of the incubation period, reaction mixture (0.3 mL) was added with 1 mL of TBA (0.8%, w/v) and 0.1 mL of TCA (20%) solution. The mixture was then heated on a water bath at 100° C for 60 min. After cooling, *n* – butanol (4 mL) was added in each tube and centrifuged at 3000 × *g* for 10 min. The absorbance of the organic upper layer was read at 532 nm in UV-Vis spectrophotometer (Beuge and Aust 1978). Catechin was used for comparison. The percentage reduction of LPO was calculated as follows: Reduction of TBARS (%) = $1 - \text{Sample}_{532\ nm}/\text{Control}_{532\ nm} \times 100$. Where, $\text{Sample}_{532\ nm}$ was absorbance of the sample and $\text{Control}_{532\ nm}$ was absorbance of control.

Statistical analysis of data

The experimental data were reported as mean ± standard error of three parallel measurements. Linear regression analysis was performed quoting the correlation coefficient. One-way analysis of variance (ANOVA) accompanied with DMRT (SPSS version 10 for Windows 98, SPSS Inc.) was conducted to determine significant difference ($P < 0.05$) between samples.

RESULTS AND DISCUSSION

In this study, in order to determine the antioxidant activity, a series of established *in vitro* protocols were applied. The EC_{50} values were obtained for tested assays and are given in Table 1. The antioxidant capacity was subsequently correlated with TPC and TFC.

Table 1 Antioxidant capacities of methanol extract of *E. kologa* fruit and standards

	EC_{50} (µg mL–1) [a]		
Samples	**Extract**	**BHT**	**Catechin**
DPPH	10.2 ± 1.33 b	26.12 ± 0.04 c[b]	n.d.[c]
RC	110.4 ± 1.02 b	40 ± 0.04 c	n.d.
$O_2^{\cdot -}$	102.4 ± 0.82 a	16.05 ± 0.01 b	30.3 ± 0.05 a
$OH^{\cdot}$	51.1 ± 1.02 a	16.44 ± 0.04 c	19.2 ± 0.01 c
NO	25.2 ± 1.32 a	46.34 ± 0.08 b	62.2 ± 0.04 c
ICC	90.4 ± 1.42 c	n.d.	64.1 ± 0.06 c
LPO	75.6 ± 0.22 c	n.d.	32.2 ± 0.05 c

[a]EC_{50} value: the effective concentration at which the antioxidant capacity was 50%. EC_{50} was obtained by interpolation from linear regression analysis. [b]Data are mean ± standard deviation (n = 3). Values in a column with different letters are significantly different according to Duncan's Multiple Range Test at P < 0.05. [c] Not determined.

Determination of TP and TF

Phenolics are the well known compounds, owing to the potent antioxidant activities and bioactivities, are also known to diffuse the free radicals.[21] The content of TP in *E. kologa* was equal to 2100 mg GAE/100 g of FM. The TP content was found 19–20 times higher than the TF content. It was found in our study that the TP content of the *E. kologa* berry was higher than that of other fruits.[22,23,24] There are strong evidences on the preventive effects of phenolics on age related chronic diseases.[25,26] Hence, the fruits of *E. kologa* can be considered as good source of phenolics. Flavonoids are considered to be the strong scavengers of reactive oxygen species (ROS).[27] Plant flavonoids are important due to their potent pharmacological acticities as free radical scavengers (Cook and Samman, 1996).[28] Many health beneficial properties of flavonoids from edible plants are recognized for their antioxidant and antiproliferative effects that may combat various diseases, such as cancers, cardiovascular disease and inflammation.[29] In this study, TF content was measured by aluminium colorimetric method. The TF content of the *E. kologa* was 220 mg QE/100 g of FM. The TF in fruits of *E. kologa* was higher than the other fruits, pineapple, banana and guava.[30]

DPPH· quenching capacity

The DPPH· assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products (Molyneux 2003).[31] With regard to DPPH· stable radical quenching activity or H-donor activity of *E. kologa* extract, a dose dependent inhibition was observed (Fig. 1). The *E. kologa* exhibited impressive DPPH·

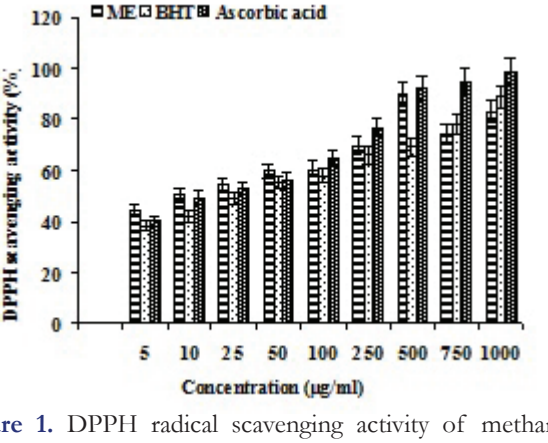

Figure 1. DPPH radical scavenging activity of methanolic extract of *E. kologa*.
Data are mean ± standard deviation (n = 3).

scavenging with 82.9% at 1000 μg mL^{-1} concentration while BHT and ascorbic acid were able to scavenge 88.87% and 98.48% at 1000 μg mL^{-1} respectively. Based upon the measured EC_{50} values, the DPPH· quenching ability (10.2 ± 1.33 μg mL^{-1}) was significantly more efficient than ascorbic acid (11.24 ± 0.02 μg mL^{-1}) ($P < 0.05$) and BHT (26.12 ± 0.04 μg mL^{-1}) ($P < 0.05$). DPPH· activity of the *E. kologa* studied was also significantly higher than its activity against other radical scavenging activities of the extract. The results of this study infer that the *E. kologa* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen ions released from the samples which contain antioxidant.[32]

Reducing capacity

The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is measured by the intensity of the resultant Prussian blue colour complex which absorbs at 700 nm. The higher absorbance at high concentration indicates the strong reducing capacity. From the analysis in Fig. 2, it was found that the *E. kologa* was able to convert the oxidized form of Fe^{2+} into Fe^{3+}. The *E. kologa* caused significant elevation of reducing power with OD value of 0.989, which was significantly more pronounced than that of BHA (0.683) ($P < 0.05$) and comparable to that of BHT (1.022 ± 0.42) at the concentration of 1000 μg mL^{-1}. The EC_{50} value of *E. kologa* was found to be 110.4 ± 1.02 μg mL^{-1}. The RC of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing properties are generally associated with the presence of reductones. The antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom.[33] The result of RC imply that the marked antioxidant activity of the *E. kologa* extract seems to be due to presence of polyphenols which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them into more stable products and terminate free radical chain reaction.

$O_2^{\cdot -}$ scavenging capacity

The $O_2^{\cdot -}$ radical is one of the most dangerous free radicals in humans and also the source of hydroxyl radical (OH·).[34,35] In the PMS/NADH-NBT system, The $O_2^{\cdot -}$ radicals derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. In the present work, the concentration dependent inhibition of $O_2^{\cdot -}$ generation by *E. kologa* fruit extract is illustrated in Fig. 3. The extract exhibited 67.24% of $O_2^{\cdot -}$ scavenging at the concentration of 1000 μg mL^{-1} with an EC_{50} value of 102.4 ± 0.82 μg mL^{-1}. On the other hand, BHT and catechin showed a higher potency than the methanolic fruit extract. As reported in Table 1, the *E. kologa* still exerted noticeable scavenging effect on $O_2^{\cdot -}$ radicals though lower than the BHT and catechin ($P < 0.05$).

·OH scavenging capacity

Hydroxyl radical (·OH) which is the most reactive free radical, has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity.[36] In our present study, the *E. kologa* was evaluated for its ability to scavenge ·OH radicals using 2-deoxyribose degradation assay. As illustrated in Fig. 4, the extract was capable of inhibiting OH· radical formation in concentration dependent manner. Its ·OH scavenging activity was 72.8% at the concentration of 1000 μg mL^{-1}. However, this value was significantly lower than the values of positive controls BHT and catechin (88.1% and 92.3% at 1000 μg mL^{-1}). As it can be seen in Table 1, the EC_{50} value of methanolic fruit extract (51.1 ± 1.02 μg mL^{-1}) was significantly lower when compared to BHT (16.44 ± 0.04 μg mL^{-1}) ($P < 0.05$) and catechin (19.2 ± 0.01 μg mL^{-1}) ($P < 0.05$).

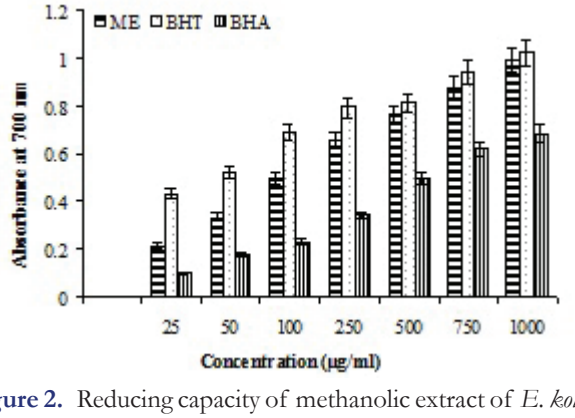

Figure 2. Reducing capacity of methanolic extract of *E. kologa*. Data are mean ± standard deviation (n = 3).

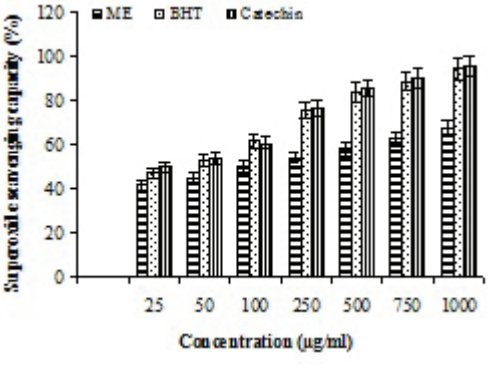

Figure 3. $O_2^{\cdot -}$ Scavenging capacity of methanolic extract of *E. kologa*. Data are mean ± standard deviation (n = 3).

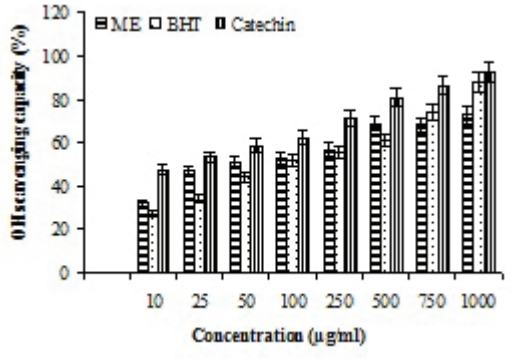

Figure 4. OH radical scavenging capacity of methanolic extract of *E. kologa*.
Data are mean ± standard deviation (n = 3).

No scavenging capacity

In addition to reactive oxygen species, NO is also implicated in chronic inflammation, cancer and other pathological conditions. The NO generated from SNP at physiological pH reacts with oxygen (O_2) to form nitrite ions. The methanolic fruit extract of *E. kologa* competed with O_2 to react with nitrite ions and thus inhibits the NO generation. As shown in Fig. 5. The NO scavenging capacity was depended on concentration of the extract. The *E. kologa* extract was potent in scavenging NO by 82.54% at the concentration of 1000 µg mL^{-1}, while BHT and catechin showed scavenging activity of 94.19% and 93.6% respectively. As compared with the EC_{50} values, the scavenging capacity of *E. kologa* extract (25.2 ± 1.32 µg mL^{-1}) was significantly lower than that of BHT and catechin ($P < 0.05$) (Table 1).

Measurement of ICC

The iron chelating activity is claimed as one of the important mechanisms of antioxidant activity (Wang et al. 2008).[37] As shown in Fig. 6, the formation of this

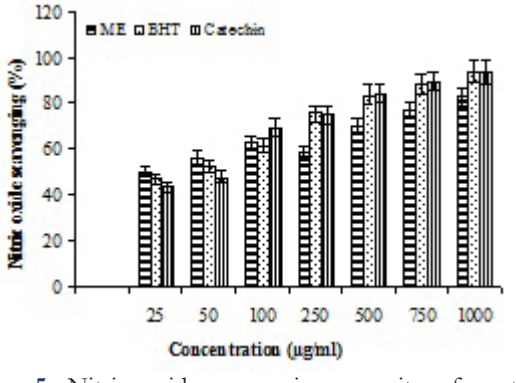

Figure 5. Nitric oxide scavenging capacity of methanolic extract of *E. kologa*.
Data are mean ± standard deviation (n = 3).

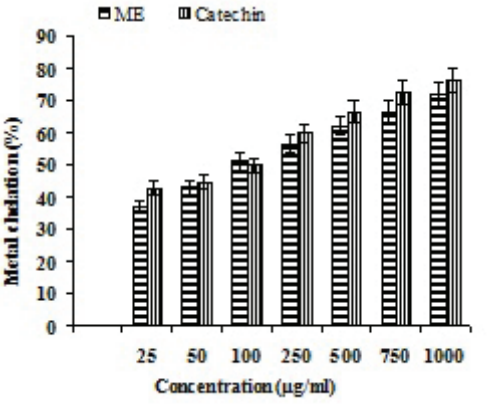

Figure 6. Iron chelating capacity of methanolic extract of *E. kologa*.
Data are mean ± standard deviation (n = 3).

complex was inhibited concentration dependently by the *E. kologa* extract and it strongly chelated fe^{3+} ions at 1000 µg mL^{-1} concentration (71.62%) whilst catechin had considerably lower effect (76.1%). The positive control in this assay EDTA exerted the strongest chelating activity at 1000 µg mL^{-1} with a 96.08 ± 1.64% chelating effect (data not shown), which was significantly higher ($P < 0.05$) than that of fruit extract of *E. kologa*. Iron chelating ability of EDTA was higher than that of phenolic compounds (Andjekovic et al. 2006).[38] The EC_{50} value of the fruit extract (90.4 ± 1.42 µg mL^{-1}) was significantly higher ($P < 0.05$) than that of catechin. The data obtained from this assay reveal that the *E. kologa* extract can act as an effective metal chelator.

Inhibition of lipid peroxidation (LPO)

The damage caused by LPO is highly detrimental to the functioning of the cell (Devasagayam et al. 2003).[39] It plays an important role in causing oxidative damage to biological systems and its carbonyl product, malondialdehyde (MDA) induces cancer and age related ailments. In order to evaluate the effect of the *E. kologa* extract on LPO, we measured the ability of the extract to inhibit the LPO induced by $FeCl_2$ in liver homogenate. From the result, it was found that the *E. kologa* was able to inhibit the concentration of MDA generation in a significant way (Fig. 7). With regard to the EC_{50}, the value of the fruit extract was 75.6 ± 0.22 µg mL^{-1} where as the EC_{50} value of catechin was 32.2 ± 0.052 µg mL^{-1} (Table 1).

This investigation was performed to elucidate the nutraceutical potential and to develop products of added value of underutilized fruits of *E. kologa* which are potentially valuable dietary resource. The results obtained in this study demonstrate that the methanol extract of *E. kologa*

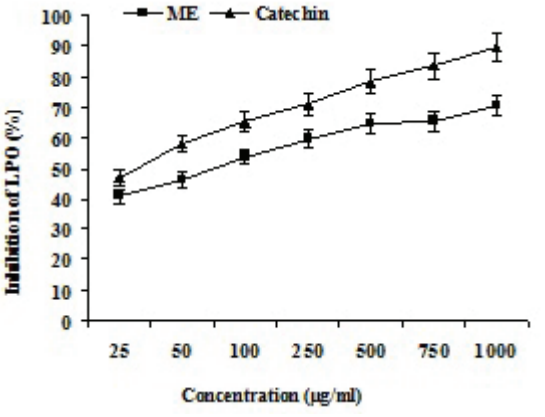

Figure 7. LPO reduction capacity of methanolic extract of *E. kologa*.
Data are mean ± standard deviation (n = 3).

fruits is endowed with very interesting antioxidant capacity in all the assay models. Our findings also indicate that the *E. kologa* fruits are competitive as free radical scavenging agents to BHT and catechin.

Correlations between antioxidant activity and, TP and TF contents

Correlation coefficients between the polyphenolic contents and antioxidant properties of the fruits were performed. An apparent linear relationship between DPPH radical scavenging and TP ($r^2 = 0.845$) and TF ($r^2 = 0.75$) contents was observed. The $O_2^{\cdot -}$ scavenging was in well correspondence with TP ($r^2 = 0.997$) and TF ($r^2 = 0.997$). Significant correlations were observed between antioxidant assays such as RC ($r^2 = 0.994$), $OH^{\cdot}$ scavenging ($r^2 = 0.933$), NO scavenging ($r^2 = 0.931$), ICC ($r^2 = 0.993$), TBARS assay ($r^2 = 0.961$) and TP. The TF was also well correlated with all the tested assays. Many works have shown that there has been a positive correlation between polyphenolic content and antioxidant activity.[40,41] Therefore, it was considered that the high antioxidant capacity of the fruit extract could be attributable to its high amount of polyphenolic content.

CONCLUSIONS

In conclusion, the results of this work indicate that the underexploited *E. kologa* fruit extract efficiently scavenged DPPH, $O_2^{\cdot -}$, NO, $OH^{\cdot}$ free radicals, chelated ferrous ion and inhibited LPO *in vitro*. The outstanding antioxidant capacity of the fruit extract is reported for the first time. These activities of the berries of *E. kologa* were strongly correlated with its phenolic and flavanoid contents. Obviously, this fruit may be used as a potential natural antioxidant and in the development of functional food and raw materials of medicine.

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