European Journal of Medicinal Plants
4(10): 1210-1222, 2014

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Kalanchoe pinnata - a Promising Source of Natural Antioxidants

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Authors' contributions

All the authors have contributed equally in experimental designing, protocol selection and manuscript preparation. Experiments were performed by author SJ. All authors read and approved the final manuscript.

Original Research Article

Received 12th May 2014
Accepted 16th June 2014
Published 3rd July 2014

ABSTRACT

Aims: Evaluation of natural antioxidant potential of *Kalanchoe pinnata* leaves attributable towards its therapeutic properties.
Study Design: *In vitro* experiments to validate antioxidant potential in aqueous and lipid phase.
Methodology: The aqueous-alcoholic whole leaf extract designated as *KPE* (*K. pinnata* extract) was subjected to comprehensive biochemical analysis to reveal its natural strength as an antioxidative agent. In lipid protection ability assay where lipid phase (pre-emulsion) was prepared using linoleic acid with Fe^{2+}, Fe^{3+} and Cu^{2+} as stress-inducers, it's potential to protect against peroxyl radical induced damage in non aqueous environment was tested. Deoxy-D-ribose degradation assay in presence or absence of chelating agent (EDTA) was tested to reveal non site-specific and site-specific hydroxyl radical (OH^{o}) scavenging potential respectively. Sodium nitroprusside based nitric oxide (NO) quenching activity and nitroblue tetrazolium reduction based superoxide radical scavenging potential were also estimated.
Results: Total phenolic content of *KPE* was 28.4±2 µg mg^{-1}. In lipid protection ability assay it exhibited maximally restricted Fe^{2+} induced amplification of peroxyl raical (ROO^{o})

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at 10 mg mL^{-1}. It elicited a significant (P = .05) inhibition of lipid auto-oxidation by directly scavenging peroxyl radicals. In potassium ferrithiocyanate-based reducing power assay, *KPE* exhibited significantly higher potency as compared to the standard synthetic antioxidant butylated hydroxy toluene (BHT), in the range of 100-2000 µg mL^{-1}. The ability of *KPE* to interact at the level of generation of hydroxyl radicals was also tested with deoxy-D-ribose degradation assay that revealed a two-fold higher non site-specific OHº scavenging potential than its site-specific activity. In sodium nitroprusside based NO quenching assay *KPE* showed >50% quenching activity at 0.5 mg mL^{-1}.
Conclusions: *KPE* is a rich source of anti-oxidative properties and has strong protective potential against oxidative stress in both aqueous and lipid phases. Hydroxyl radical scavenging assay showed *KPE*'s ability to scavenge free radicals is more due to its reductive potency than its metal-chelation activity attributable towards its exploration in herbal drug discovery research.

Keywords: Antioxdative activity; OHº scavenging potential; metal chelation activity; nitric oxide quenching; phenolics.

1. INTRODUCTION

Kalanchoe pinnata is naturalized throughout the hot and moist parts of India. It is popularly known as mother of millions, wonder plant or the miracle plant. It has an evolutionary advantage to survive even in the adverse environmental conditions attributed towards its ability to propagate through epiphyllous budding [1]. *K. pinnata* has been traditionally used in Indian medicine for kidney stone treatment popularized it as "pashanchat". It is one of the species used by the tribals in Kerala for treating cancer [2]. Bufadienolides isolated from its leaves have potential chemo-preventive, antibacterial, antitumourous and insecticidal properties [3]. In addition they also show structural similarity with cardiac glycosides - digoxin and digitoxin [4]. Leaf aqueous extract of *K. pinnata*, is used effectively for the treatment of jaundice in folk medicine of Bundelkhand region [5]. It also exhibits antihistamine activity in rodents [6]. The leaves and bark are bitter tonic, used as astringent to the bowels and as analgesic and carminative for curing diarrhoea and vomiting [7]. Its significant wound healing potential (attributed towards presence of steroid glycosides-bufadienolide) and nephroprotection has also been tested in rat model system [8,9]. In addition to its own action, it also enhances the NO-inducing activity of macrophages in a synergistic manner [10].

Phytochemical studies with *K. pinnata* proved it to be rich in alkaloids, triterpenes, glycosides, flavonoids, steroids and lipids [11]. From the dried leaf tissue/aerial parts, epigallocatechin-3-O-syringate, quercetin, kaempferol, rutin, luteolin, ferulic, caffeic and gallic acids along with other compounds have been isolated [12]. These compounds have been reported to possess antibacterial and analgesic properties [13].

Extensive work done in author's laboratory strongly indicated that leaf longevity in *K. pinnata* was somehow linked to its capacity to differentiate epiphyllous buds [14,1]. Subsequently, it has been shown that the progression of leaf senescence is linked with antioxidative status of the foliar tissue during epiphyllous bud differentiation in *K. pinnata* [15]. The present study is an attempt to validate pro-antioxidant activities of leaf extract in both lipid and aqueous phases in the presence of various metal stressors as present in human body. It is considered a necessary pre-requisite to validate its probable use in herbal drug discovery research, attributable towards increasing number of its therapeutic applications.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves of vegetative plants of *Kalanchoe pinnata* Lam. Pers. (Family: Crassulaceae) at 20-24 leaf stage were collected from the experimental garden of the Department of Botany, University of Delhi in August, 2005. Adequate precaution was taken to ensure that the harvested leaves were healthy and devoid of foreign materials like soil, dust, insects and any other extrinsic contamination.

2.2 Preparation of Leaf Extract

The plant material was grinded and extracted thrice in aqueous ethanol (50%) over a period of 24-72 h. The pooled filtrate was evaporated to dryness in a rotary flash evaporator and the yield was determined on w/w basis. The dried extract was designated as *Kalanchoe pinnata* extract (*KPE).* Several aqueous dilutions of *KPE* were made and subjected to assessment of its antioxidative potential by employing specific assays.

2.3 Polyphenol Determination

The polyphenol content of *KPE* was estimated as described [16] using Folin-Ciocalteu reagent. The absorbance of the coloured end product was recorded at 765 nm.

2.4 Ammonium Thiocyanate Assay

In the presence of metal ions: Antioxidative activity of *KPE* was evaluated by ammonium thiocyanate method [17]. *KPE* dilutions were mixed with 5 mL linoleic acid pre-emulsion (3 volumes of linoleic acid mixed with an equal volume of tween-20 prepared in 200 volumes of 30% (v/v) ethanol) containing either 500 μL of copper (10 μM) or ferrous / ferric sulphate (100 μM). Volume was made upto 10 mL with deionized distilled water and reaction samples were incubated at 37ºC for 30 min. The assay mixture contained 2.5 mL of 75% ethanol, 50 μl ferrous chloride ($FeCl_2$:0.1% w/v), 50 μl of 30% ammonium thiocyanate and 50μl of sample. Absorbance was measured at 500 nm.

In the absence of metal ions: A similar analysis was performed (to evaluate the time-course inhibition of linoleic acid degradation by *KPE*) without using the metal ion.

2.5 Antioxidant Activity in Aqueous Phase

The antioxidant activity in aqueous phase of *KPE* was determined as described [18]. *KPE* dilutions (50 µL) were mixed with 200 µL each of 0.2 M phosphate buffer (pH 6.5) and 0.1% potassium ferricyanide and incubated at 50ºC for 20 min. 250 μl of 10% of trichloro acetic acid was added to the above mixture and the supernatant after centrifugation was mixed with 500 µL of demineralized distilled water and 100 µL of 0.1% ferric chloride and further incubated at 37ºC for 10 min. Absorbance was recorded at 700 nm and compared with standard antioxidant butylated hydoxyl toluene (BHT).

2.6 Superoxide-ion ($O_2^{\circ-}$) Scavenging Activity

The $O_2^{\circ-}$ quenching ability of *KPE* was determined by nitroblue tetrazolium reduction assay [19]. Different *KPE* dilutions were mixed with sodium pyrophosphate buffer (0.05 M, pH 8.3) and phenazine methnosulfate (186 µM). Nitroblue tetrazolium (300 µM) was added to the above solution and the final volume adjusted at 3 mL. The reaction was initiated by adding NADH (780 µM) and the contents incubated at 37ºC for 3 min and terminated by adding 1 mL glacial acetic acid, followed by 4 mL of n-butanol. The n-butanol layer was separated by centrifugation (3000 g, 10 min) and its absorbance was measured at 560 nm.

2.7 Hydroxyl Radical (OH°)-scavenging Potential

Non site-specific: OH°-scavenging potential of *KPE* was measured by deoxyribose degradation assay [20]. *KPE* dilutions (500 µL) were mixed with 100 µM each of $FeCl_3$, EDTA, and L-ascorbic acid solution along with H_2O_2 (1mM) and deoxyribose (3.6 mM) in potassium phosphate buffer (pH 7.4). The reaction mixture was incubated for 1 h at room temperature. One mL each of TCA (10 % w/v) and thiobarbituric acid (0.5 % w/v, in 0.025 M NaOH) were added to the mixture and the contents were reincubated at 55ºC for 15 min. Absorbance of the final product was recorded at 532 nm. The percentage inhibition of degradation of deoxyribose was taken as OH° scavenging potential and was quantified by using the formula:

$$\% \text{ Inhibition} = \text{O.D.}_{\text{control}} - \text{O.D.}_{\text{sample}} / \text{O.D.}_{\text{control}} \times 100$$

Site-specific: The procedure for evaluating site-specific OH°-scavenging potential was similar to the one for non site-specific, except for the absence of EDTA from the reaction mixture.

2.8 Nitric Oxide (NO)-scavenging Activity

KPE dilutions were mixed with 5mM sodium nitroprusside (used to generate NO_2^- at physiological pH, that in turn interacts with O_2 to generate nitrite ions), and the volume was made upto 1 mL using PBS and incubated at 25ºC for 150 min. The NO_2^- generated in the system was estimated by adding an equal volume of Griess reagent (6% suphanilamide in 3M HCl + 0.3% napthylethylene diamine dihydrochloride + 7.5% orthophosphoric acid in 1:1:1 ratio). The NO_2^- -scavenging activity was quantified as decrease in percent absorbance of the complex formed by diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine, readable at 546 nm [21].

2.9 Statistical Analysis

The experiment was repeated three times and the values were expressed as Mean ± SE. One-way ANOVA analysis followed by Dunnett's t-test was employed (separately for each parameter) to analyse changes with respect to different treatments at *P* = .05 and .01 level. Computer software *SPSS-10* was used for the analysis.

3. RESULTS

3.1 Antioxidative Potential of Whole Leaf Extract

3.1.1 Total phenolic content

The total phenolic value was calculated from the standard curve obtained by taking quercetin as standard. Stock was serially diluted to obtain concentrations that fall within the range of standard curve. Total phenolic concentration of extract was 28.4±2 µg mg^{-1} *KPE* (as equivalence to quercetin, a standard phenolic).

3.2 Antioxidant Activity in Lipid Phase

3.2.1 Ammonium thiocyanate assay (with Fe^{2+}or Fe^{3+})

Free Fe^{2+} ions, were added to linoleic acid emulsion in order to evaluate the relative effectiveness of *KPE* to metal ion - catalyzed lipid peroxidation. The results are expressed as absorbance at 500 nm vs the ratio of *KPE*/[$metal^{+}$]. *KPE* effectively countered the peroxidation caused by Fe^{2+} maximally at *KPE*/[$metal^{+}$] ratio of 100 with 100µM concentration of Fe^{2+}. Similarly, in case of Fe^{3+}, maximum inhibition was observed at the *KPE*/[$metal^{+}$] ratio of 100. The pattern observed in the first 2 h in both cases was similar, indicating the inherent metal chelation potential of *KPE*. The study was continued up to 4 h and the maximum inhibition of degradation was observed in the range of 30-100 ratios (Fig. 1a).

3.2.2 Ammonium thiocyanate assay (with Cu^{2+})

The antioxidative ability of *KPE* against Cu^{2+}-induced linoleic acid degradation was investigated for 2 and 4 h. 10 µM Cu^{2+} was used to induce lipid oxidation. The significant inhibition was observed in the range of 200-1000 at 2 h while, 100 to 500 was found to be most effective range with maximum inhibition (69.98%) observed at the *KPE* /[$metal^{+}$] ratio of 500 at 4 h (Fig. 1b).

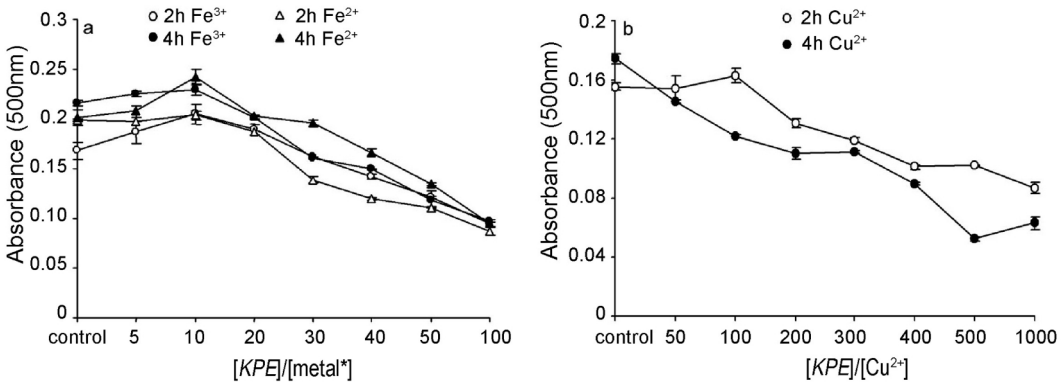


Fig. 1. Effect of *KPE* at different dilutions on metal ion (Fe^{3+}, Fe^{2+})-induced (a)., and Cu^{2+}-induced (b) lipid peroxidation in linoleic acid system

** indicates maximal reduction (P = .05) in absorbance with respect to control*

3.2.3 Ammonium thiocyanate assay (time-course analysis)

The ability of *KPE* to restrict the peroxidation of linoleic acid over an extended period (24 h) of incubation at 37ºC was tested using a metal free emulsion. Time period of 24 h readings was discarded as the control itself gave reduced absorbance indicative of limit of incubation time to be kept for the assay. Significant ROO° scavenging activities were observed for *KPE* at 3-5 mg mL^{-1} for all time periods i.e 2, 4, 24 h (Fig. 2). *KPE* in the range of 2-5 mg mL^{-1} was able to exhibit strong inhibitory activity against lipid peroxidation.

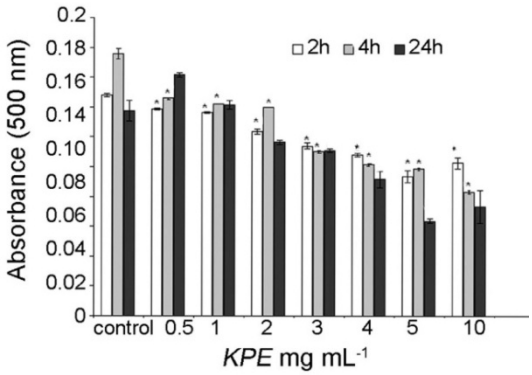


Fig. 2. Effect of *KPE* concentration in preventing auto-oxidation of linoleic acid (lipid phase) over a period of 24 h

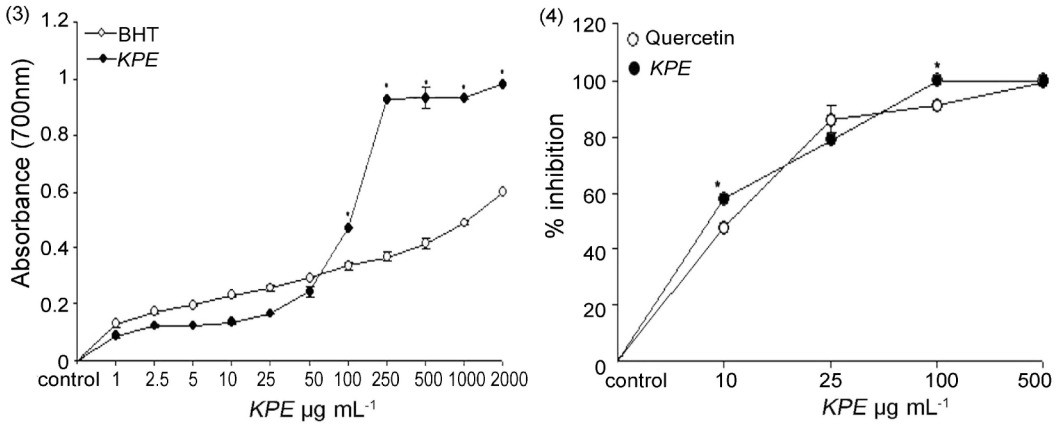
**Significant (P = .05) difference with respect to control at respective time intervals (2h, 4h) evaluated using One Way ANOVA analysis followed by Dunnet's t-test (1 control and 7 test samples) for each time interval*

3.3 Antioxidant Activity in Aqueous Phase

The ability of natural compounds to remove the oxidants from the physiological system is known to have a direct correlation with their potential to act as reducing agents. The antioxidant activity of *KPE* was, therefore, compared with that of a standard antioxidant butylated hydroxyl toluene (BHT). The dosage to attain unit absorbance at 700 nm was >2000 µg mL^{-1} for BHT whereas 250 µg mL^{-1} for *KPE*. Thus, the reducing potential of *KPE* was estimated to be eight times in comparison to BHT (Fig. 3).

3.4 Superoxide-ion ($O_2^{\circ-}$) scavenging Potential

The nitroblue tetrazolium (NBT) was used as a marker substrate whose reduction was indirectly initiated by the involvement of superoxide ion, leading to the formation of blue formazan crystals. The % inhibition of NBT reduction by *KPE* was taken as $O_2^{\circ-}$ scavenging potential of the extract. It was observed that 50% of $O_2^{\circ-}$ was scavenged in the concentration range of 11.5 µg mL^{-1}. The scavenging activity was found to increase in a dose-dependant manner in the range of 10-500 µg mL^{-1} (Fig. 4).



Figs. 3-4. Reducing potential of *KPE* using BHT as standard. *Significant (*P* = .05) difference *KPE* with respect to butylated hydroxyl toluene (BHT), Fig. 4. $O_2^{\circ-}$-scavenging potential of *KPE*. *Significant (*P* = .05) difference with respect to quercetin

3.5 Hydroxyl Radical (OH°) - Scavenging Potential

3.5.1 Non site-specific

It was measured using deoxyribose degradation assay. Maximum hydroxyl radical scavenging potential was evaluated as percentage inhibition of deoxyribose degradation estimated using TBA.

The OH° - scavenging potential was found to increase with rising strength of *KPE* from 10-1000 µg mL^{-1} (Fig. 5). The maximum percent inhibition of *KPE* was 64.20% at 1000 µg mL^{-1}. All the values were found to be significant in comparison to control (0% inhibition).

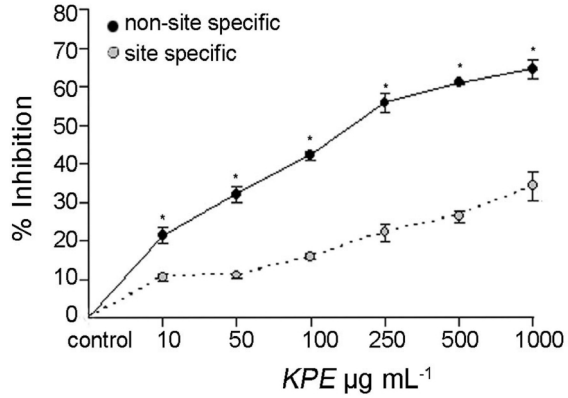


Fig. 5. Site-specific and non site-specific OH°-scavenging potential of *KPE*
**Significantly higher (P = .05) non site-specific activity as compared to site-specific activity at all concentrations*

3.5.2 Site-specific

The procedure for evaluating site-specific OH° scavenging potential was similar to the one for non site specific assay except that it was performed without EDTA.

The OH° scavenging potential was found to increase concomitantly with increase in strength of *KPE* from 10-1000 µg mL^{-1} (Fig. 5). The maximum inhibition was 34.03% at 1000 µg mL^{-1} of *KPE* and found to be significantly higher with respect to control (0% inhibition).

3.6 Nitric-oxide (NO) - Scavenging Activity

It was evaluated as decrease in percent absorbance of the complex formed by diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine readable at 546 nm. The NO scavenging activity of *KPE* was found to increase in a dose-dependant manner in the range of 10-500 µg mL^{-1} (Fig. 6). *KPE* exhibited maximal % inhibition of diazotization complex at 500 µg mL^{-1} (59.44%), which was found to be highly significant as compared to control (0% inhibition).

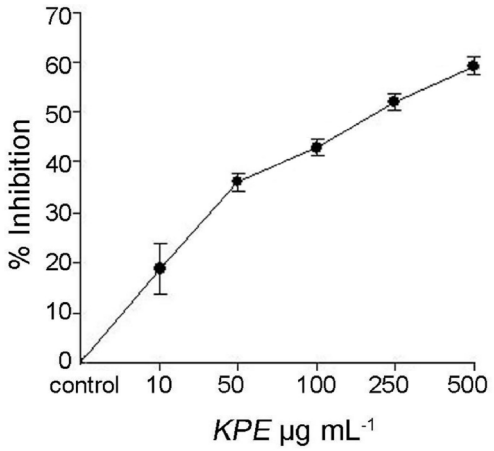


Fig. 6. NO-scavenging activity of *KPE*

4. DISCUSSION

Oxidative damage, a multi-faceted phenomenon, is manifested by increased production of reactive oxygen species (ROS) like superoxide radicals (O_2°), hydroxy radical (OH°), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and amplified by presence of redox active state of free iron and other transition metal ions [22,23]. The exchange of electrons at various levels maintains a physiological balance of antioxidant moieties and pro-oxidant species. Its disruption can lead to the formation of different protein degradation products, DNA-protein cross-linked adducts or other insoluble complexes [24,25]. The external supplementation of antioxidants either prevent the formation of pro-oxidant species (free radicals), or if formed are able to scavenge and remove them [26]. The search for novel antioxidant moieties from natural plant products accounts for a major area of investigation around the world. The present study has evaluated the antioxidant activity of *Kalanchoe pinnata* leaf extract (designated as *KPE*) in both aqueous and lipid phases.

KPE phenolics were estimated to be 28.4±2 µg mg^{-1} of the whole leaf extract. In order to evaluate the lipid protection ability of *KPE*, a lipid phase (pre-emulsion) was prepared using linoleic acid and Fe^{2+}, Fe^{3+} and Cu^{2+} were used as stress-inducers. These transition metals are known to trigger physiological amplification of the oxidative stress [22]. *KPE* exhibited maximally restricted Fe^{2+}-induced amplification of ROOº at 10mg mL^{-1} (Fig. 1). This could be attributed to the metal-chelation ability of the phenolics present in the extract. Due to the presence of aromatic rings and hydroxyl groups on them, the phenolics are able to act at aquo-lipid interface [27]. The number and position of hydroxyl groups in the aromatic moieties of phenols is proportional to its ability to de-localize oxidant radicals and chelate metal ions [28]. Thus, these constituents are able to interact with the transition metals even in lipid phase and are able to chelate it by filling its aqua-coordination sites and generating metal-coordinated insoluble complexes. Such ability of *KPE* can be explored further for therapeutic uses.

Cu^{2+} is an essential constituent of chromatin and its level is known to get elevated during malignancies [29] and cause inter-nucleosomal DNA fragmentation. *KPE* exhibited a dose-dependent decrease in Cu^{2+}- induced ROOº flux (Figs. 1 and 2). Such chelation ability could be explored in inhibiting vascular inflammation and atherosclerotic lesion developed as observed in case of tetrathiomolybedate [30]. In addition, it also exhibited a significant (P = .05) inhibition of lipid auto-oxidation by directly scavenging peroxyl radicals. This could be attributed to the ability of phenolics to stabilize ROOº radicals through generation of stabilized phenoxyl radicals. These results are supported by the presence of quercetin, kaempferol, rutin and luteolin etc in *K. pinnata* reported from dried leaf tissue [12]. Such a stabilization of peroxyl radicals can only be achieved by electron donation. Thus, reducing power of *KPE* was evaluated using potassium ferrithiocyanate-based reduction assay. It exhibited significantly (P = .05) higher reducing power as compared to standard synthetic antioxidant BHT in the range of 100-2000 µg mL^{-1} (Fig. 3). This observation lent further support to the potency of *KPE* in terminating the free radical chain reactions and therefore, its ability to boost the natural antioxidative defence mechanism as reported from other plant systems [31].

$O_2^{\circ-}$ generally acts as pro-oxidant species in the biological systems by converting Fe^{3+} to the redox active Fe^{2+}. *In vitro* generation of this reactive species was achieved by using NADH. *KPE* exhibited significant $O_2^{\circ-}$ scavenging activity in the tested concentration range. These observations indicated that the leaf extract had the ability to inhibit $O_2^{\circ-}$ at very initial phase of the oxidative stress. It can also be attributed to the oxidant stabilizing ability of *KPE* phenolics described above. The biphasic behaviour of polyphenolics i.e. ability to act as chain breaking antioxidants [attributed to di-hydroxyl groups (catechol moieties)] and chain-initiators of radical-induced peroxidation is known as flavanoid paradox [32]. However this phenomenon was not observed in *KPE* in the tested concentration range.

It has been suggested by several workers that the presence of redox active Fe^{2+} and H_2O_2 in the physiological system leads to the generation of hydroxyl radical by Fenton reaction [33]. In view of evaluating the ability of *KPE* to act at the level of generation of hydroxyl radicals, deoxy-D-ribose degradation assay was performed. This assay was used to study the non site-specific (Fe^{2+} + H_2O_2 + EDTA) and site-specific (Fe^{2+}+ H_2O_2) hydroxyl radical scavenging activity in the aqueous system. In the site-specific assay, Fe^{2+} induces hydroxyl generation but maximal attack occurs directly to deoxy-D-ribose prior to hydroxyl generation [34]. On the other hand, in non site-specific assay, the presence of EDTA makes Fe^{2+} non-available for attacking deoxy-D-ribose directly and therefore hydroxyl generation predominates [35]. *KPE's* non site-specific OH° scavenging potential was significantly higher

as compared to the site-specific activity. The results indicated that *KPE* had higher ability to scavenge free radicals by virtue of its potent reductant power as compared to its metal-chelation activity. The metal chelation ability (aqueous phase) was found to be in accord with its ability to reduce metal–induced peroxidative stress (in lipid phase) (Fig. 5). Since in this concentration range antioxidant activity was prominent, the nitric oxide modulatory activity of *KPE* was also evaluated subsequently.

Sodium nitroprusside based generation of NO at physiological pH was used to estimate NO scavenging efficacy of *KPE*. NO is known to act as a pluripotent regulator of diverse functions [36]. They are generated endogenously by the conversion of L-arginine to L-citrulline by NADPH-dependant enzyme NO synthase. It exists in multiple forms (NO^-, NO°, and NO^+) and thus has wide degree of physiological functions and chemical reactivity [36]. NO has been reported to act both as pro-oxidant (being free radical) and antioxidant (electron donating properties) [37]. *KPE* exhibited more than 50% NO scavenging activity at 0.5 mg/mL level (Fig. 6).

The forgoing discussion reveals that *KPE* exhibited an inherent ability to act at initiation level ($O_2^{\circ-}$ scavenging activity), amplification level (metal chelation and ROO° radical scavenging activity) and also in removing harmful end products through its NO, OH° radical scavenging and anti lipid peroxidation potential. In light of these facts several of its medicinal properties can be explained (see Table 1).

Table 1. Analysis of existing therapeutic relevance (reviewed [7]) and correlative potential indicators from present study for overall antioxidative potential spectra of *K. pinnata*

Therapeutic Relevance/ Use in Traditional Medicine	Indicators obtained from present study in line with parallel research
Anti-diabetic activity (hydro-alcoholic extract)	The free radical scavenging activity of *KPE* lies between 60-80% is in line with free-radical induced diabetes by Streptozotocin model [38].
Anti-fungal activity against *Candida* species (ethanolic extract)	Synergism between nitric oxide and azoles is reported against *Candida* species. This can be evaluated for the development of new therapeutic applications [39]. Interactive ability of *KPE* with nitric oxide reported in the study indicates a linkage.
Anti-inflammatory activity (aqueous extract)	Prostaglandins prefer to stay in oil water interface due to its pKa values [40]. Hence anti-inflammatory activity (referred as reduction in prostaglandin levels) requires drugs that can act in both lipid and aqueous phases as described in the our present study with *KPE*.
Anti-convulsant activity (aqueous extract)	Convulsions related to membrane changes (not known locations) is a complex phenomenon and involves free radical formation. This could be in direct consonance with free radical scavenging activity of *KPE*.
Hepato and Nephro protective activity (leaf extracts)	Attributable towards complete spectra of antioxidant activity
Diuretic activity (hydroalcoholic extract)	Mechanism requisite to act at membrane level requiring ability to work at lipid-water interface as possessed by *KPE*

Note - This analysis has considered only those studies which involve aqueous/ aquo-alcoholic extracts

Leaves of *K. pinnata* appear to be a particularly suitable material for further investigations aimed at understanding free radical-mediated disorders and can act as an important candidate for drug discovery research. More work need to be done for targeted standardization of bioactivity-guided fractionation and model formulations through a poly-constituent approach.

5. CONCLUSION

KPE is a rich source of anti-oxidative properties and has strong protective potential against oxidative stress in both aqueous and lipid phases. It has significantly higher reducing potency as compared to the standard synthetic antioxidant butylated hydroxy toluene. Hydroxyl radical scavenging assay showed *KPE*'s ability to scavenge free radicals is more due to its reductive potency than its metal-chelation activity attributable towards its exploration in herbal drug discovery research.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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