



Research Article

Antioxidant activity of methanolic leaf extract of *Etilingera fenzlii* (Kurz) Skronick.& M.Sabu (Zingiberaceae) - The honey bee repellent endemic plant species of the Andaman Nicobar Islands

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Abstract: *Etilingera fenzlii* (Kurz) Skronick.&M.Sabu (Zingiberaceae), is an endemicspecies of the Andaman Nicobar Islands exclusively used by the shompens as a bee repellent source for their honey collection. The favourite food of shompens is the fruit of screw pines and honey from wild bees. By tradition Shompens used leaves of *E.fenzlii*; they smeared their bodies with the leaf sap and also chewed leaves to spat on to the honey comb. The honey bees there upon flew away from the comb and did not sting. It is documented that the volatile constituents of leaves have proved to be effective eco-friendly, possess varying degrees of insect and pest controlling properties. It is also an important folk medicine for malarial fever and gastrointestinal disorders. The present study was performed to investigate the antioxidant activity of methanolic extract of leaves of *E. fenzlii*. The antioxidant potential of leaf extract was evaluated with methods DPPH scavenging assay and reducing power assay. The extract showed antioxidant activity compared to the reference antioxidant ascorbic acid in a dose dependent manner. In DPPH scavenging assay the IC₅₀ value of the extract was found to be 42.50µg/ml while the IC₅₀ value of the reference standard ascorbic acid was 71.31µg/ml. Phenolic content of the leaf extract was also evaluated using Folin-Ciocalteu assay. Total phenolic content of the extract was found to be 53±8.86 µg GAE/mg. The results obtained in the present study indicate that leaves of *E.fenzlii* have potent antioxidant activity and/or free radical scavenging activity.

Keywords: *Etilingera fenzlii*, Antioxidant activity, repellent, DPPH

Introduction

Antioxidant is a substance that has the ability to delay the oxidation of a substrate by inhibiting the initiation or propagation of oxidising chain reactions caused by free radicals. It plays important roles to prevent fats and oils from becoming rancid and protects human body from detrimental effects of free radicals. Nowadays, the synthetic antioxidants are widely used in the food industry and also included in the human diet. The use of natural antioxidant is safer than synthetic antioxidants (Zheng and Wang, 2001). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been widely used around the world for decades. However, they are being scrutinised for possible toxic and carcinogenic effects. As a result, an intense new area of research has been developed concerning the search for identification and characterisation of naturally occurring antioxidants. Natural antioxidants are more ideal as food additives, not only for their free radical scavenging properties, but also on the belief that natural products are healthier and safer than synthetic ones;

thus they are more readily acceptable to the modern consumers (Azlim *et al.*, 2010). Phytochemicals are compound that benefit to health can be found in herbs, fruits and plants.

Free radicals are highly toxic and generate oxidative stress in plants and animals even though; they have an in built wide range of combating mechanism for these free radicals. But, these free radicals mediated toxicity can be effectively eliminated by the compounds from plant origin and many activities have been reported already for a wide range of plants with different compounds (Sulekha *et al.*, 2009). However, these free radicals also play a beneficial role *in vivo* for instance production of ATP molecules, Phagocytosis, regulation of cell growth, intercellular signalling and in the synthesis of biological compounds (Alluri *et al.*, 2009).

Etilingera fenzlii (Zingiberaceae) is an endemic species of Andaman Nicobar Islands (Mathew *et al.*, 2010) exclusively used by the Shompens as a bee repellent source for their honey collection. Shompens are one of the most primitive offshoots of the Mongoloid

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race believed to be migrated from the Malayan Peninsula to this island in the remote past. This semi nomadic tribal group which is presently less than one hundred in population lives in thick tropical evergreen forests of Great Nicobar Island. *E. fenzi* has remarkable significance from ethnobotanical, pharmacological and taxonomical points of view. Chen and Dou (2008) investigated the antioxidant activity and the results showed that *Etilingera* species displayed significantly higher antioxidant properties compared to other genus. Currently, there are no published reports concerning the antioxidant capacity of methanolic leaf extract of *E.fenzi*.

In the present work, the antioxidant ability of the methanolic extract of *E.fenzi* were followed through different methods: 1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical method, reducing power and total phenolic assay. The findings from this work may add to the overall value of the medicinal potential of the species.

Materials and Methods

Methanolic extraction of plant material

The dry leaf (100gm) powder of *E.fenzi* extracted with methanol using a soxhlet apparatus for 4 hours. The vacuum dried extract was stored at 4 °C until further analysis.

DPPH free radical scavenging assay

Radical scavenging activity was determined by spectrophotometric method based on the reduction of the stable free radical 2, 2-diphenyl- 1 – picrylhydrazyl (DPPH) (Saija et al., 1998). Diluted methanol extracts were added at an equal volume to a methanolic solution of DPPH (200µM DPPH). After 15 min at room temperature, the absorbance was recorded at 517nm. The activity was measured as percentage, calculated using the formula:

$$\% \text{ scavenging} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The activity was reported as IC₅₀ value; the concentration of sample (µg/mL) required to scavenge 50% of DPPH radical, relative to the control (DPPH without antioxidant agent added). Ascorbic acid was used as positive control and all determinations were performed in triplicate.

Reducing power assay

Various concentration of the plant extracts were mixed with phosphate buffer (1 ml) and potassium ferricyanide (1ml). This mixture was kept at 50 °C in water bath for 20 minutes. After cooling, 1ml of 10 % trichloroacetic acid was added. The upper layer of solution (1ml) was mixed with distilled water (1ml) and a freshly prepared ferric chloride solution (0.25ml). The absorbance was measured at 700 nm using spectrophotometer. Increased absorbance value of the reaction mixture indicates increased reducing power. Three replicates were

made for each test sample and average data was noted. Here, ascorbic acid at various concentrations was used as standard.

Total phenolic assay

Modified Folin-ciocalteu reagent colorimetric method was used for the determination of total phenolics (Donald et al., 2001). The methanol extract was mixed with 5 ml Folin-ciocalteu reagent. After 5 min, 4ml of 20% Na₂CO₃ solution was added. The mixture was allowed to stand for 30 min and absorbance was measured at 750 nm. The total phenolic content was expressed as mg Galic acid equivalent (GAE) mg/g dry weight of the extract from the calibration curve using standard gallic acid (50, 100 µg) in methanol.

Results and Discussion

In the present study several free radical scavenging activities of *E.fenzi* were evaluated. Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine (Beyer, 1994; Aqil et al., 2006). Antioxidants are molecules that are able to scavenge free radicals or prevent their generation. Isolated compound from rhizome of *Zingiber cassumunins* showed considerably high antioxidant activity (Masuda and Jitoe., 1984). *Zingiber officinale*, *Zingiber zerumbet* and *Zingiber ottensii* have been intensively studied in term of their phytochemicals and bioactivity properties (Murakami et al., 2002; Stoilova et al., 2007). There is an increasing interest in antioxidants, particularly in those intended to prevent the deterioration of fats and other constituents of food stuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999). There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Sa'nchez-Moreno, 2002; Schwarz, et al., 2001). Phenolic compounds, in general, are able to scavenge free radicals or chelate metal ions to prevent generation of free radicals. Free radicals have been implicated in the pathogenesis of more than 50 diseases (Percival, 1996). Currently, there is much interest in herbs and spices as sources of antioxidants. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties. Sources of natural antioxidants are primarily, plant phenolics that may occur in all parts of plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt, 1990, Mathew, 2006). Polyphenols are the main bioactive phytochemicals that have been shown to prevent many types of chronic diseases such as cancer and cardiovascular disease. Previous studies showed that the highest antioxidant activity of different parts of ginger (*Zingiber officinale*) was found in the rhizome (Habsah et al., 2000; Zaeoung et al., 2005).

Oxidation is essential to many living organisms for production of energy for biological processes. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxidation of cell and leads to cell death and tissue damage. Free radicals are responsible for aging and causing various human diseases such as atherosclerosis, diabetes, cancer, hypertension, alzheimer's disease, parkinsonism and cirrhosis (Pal *et al.*, 2010). Antioxidants dealing with an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Sies H, 1997). The antioxidants in the human diet are of great interest as possible protective agents to help human body to reduce oxidative damage. To prevent lipid oxidation food industries have long using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest in antioxidant substances from natural resources (Naveena B.M., *et al.*, 2008).

DPPH free radical scavenging assay

DPPH assay is a valid accurate, easy and economic method to measure the ability of compounds to act as free radical scavengers or hydrogen donors. The assay is based on the measurement of the scavenging capacity of antioxidants towards a stable free radical α , α -diphenyl- β -picrylhydrazyl (DPPH). The method is unique in carrying out the reaction of the sample with DPPH in methanol, which facilitates the extraction of antioxidant compounds from the sample (Kedare and Singh, 2011). DPPH radical scavenging activity assay assessed the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and when it reacts with an antioxidant compound which can donate hydrogen, it is reduced to diphenylpicrylhydrazine. The DPPH method is simple, rapid and sensitive method accepted widely for screening of radical scavenging activity of plant extracts. The extract showed 71.56% radical inhibition at 100 μ l whereas at the same concentration the standard ascorbic acid showed 75.85% inhibitions. Result shows the quantity of extract needed for 50% scavenging of the DPPH free radical (IC_{50}). The changes in colour (from deep-violet to light-yellow) can be measured spectrophotometrically. The IC_{50} of the methanolic extract of *E.fenzlii* was determined as 42.50 μ l/ml where ascorbic acid revealed 71.46 μ l/ml. The present study illustrated that DPPH free radical scavenging activity of *E.fenzlii* increased with the increase of concentration of the extract. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann *et al.*, 1979).

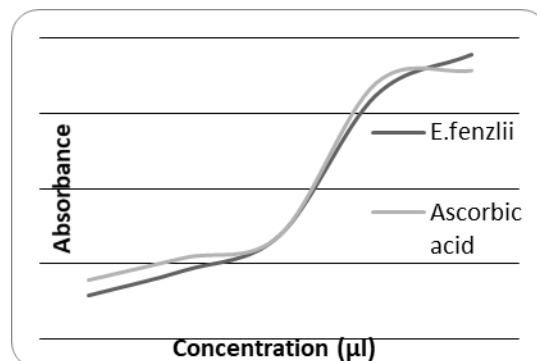


Figure 1. Comparative DPPH radical scavenging activity of methanolic extract of the leaves of *E.fenzlii* and standard ascorbic acid

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. The maximum absorbance for the methanolic extract of *E.fenzlii* found to be 0.28 at 100 μ g/ml while that of the standard ascorbic acid was 1.36 at the same concentration (Fig. 2). The reducing power of the extract increased with increase in concentration. In reducing power assay, with the increase of concentration, the absorbance of the extract and standard increased gradually. Recently, it has been reported that there is a direct correlation between antioxidant capacities and reducing power of certain plant extracts (Tanaka *et al.*, 1988).

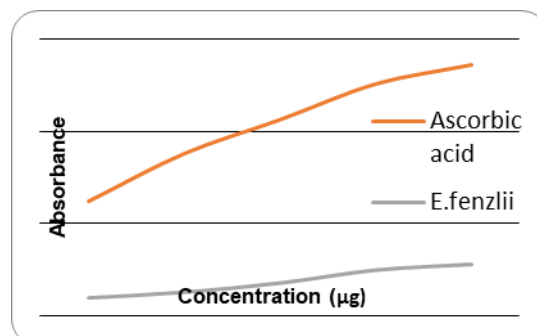


Figure 2. Comparative reducing power assay of methanolic extract of the leaves of *E.fenzlii* and standard ascorbic acid

Total phenolic content

The amount of total phenolic content was quite high in the methanolic crude extract of *E.fenzlii* – $53 \pm 8.86\mu$ g/mg of gallic acid equivalent. The high content of phenolic compounds indicated that these compounds contribute to the antioxidant activity. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts (Cook and Samman, 1996).

The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Rice-Evans *et al.*, 1997). According to our study, the high phenolic content in *E. fenizlii* can explain its high free radical scavenging activity.

Phytochemical compounds especially phenolic compounds such as flavonoids, phenyl propanoids, phenolic acids, tannins are very important components for the free radical scavenging and antioxidant activities of plants. Therefore, the relationship between phenolic content of medicinal plants and antioxidant activity is well documented. The high content of phenolic compounds indicated that these compounds contribute to the antioxidant activity. *E. fenizlii* can be regulated as promising plant species for natural plant sources of antioxidants with high potential value for drug preparation. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds (Okudu, 1994, Tepe *et al.*, 2006). This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001). In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson *et al.*, 2001, Djeridane *et al.*, 2006). The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents. Principle reactions that occur in the determination of total phenolic compounds with Folin Ciocalteu reagent is reduction-oxidation. Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry (Schofield *et al.*, 2001). The reaction forms a blue chromophore constituted by a phosphotungstic-phospho molybdenum complex (Schofield *et al.*, 2001 and Gulcin *et al.*, 2004) where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds (Schofield *et al.*, 2001). Phytochemical compounds especially phenolic compounds flavonoids, phenyl propanoids, phenolic acids, tannins are very important component for the free radical scavenging and antioxidant activities of plants. Therefore, the relationship between phenolic content of medicinal plants and antioxidant activity is well documented (Amin *et al.* 2013; Tanaka *et al.*, 1988). The result of the present study revealed that the presence of high concentration of phenolic components in the extract may effectively eliminate radicals which contribute directly to antioxidant

effect of the system (Duh, 1994). In this context, it can be deduced that the methanolic leaf extract of *E. fenizlii* contains significant antioxidant activities. However, further researches are suggested to find out responsible constituents and possible active principles for these activities.

Conclusion

In general, it is found that the extracting solvents significantly affected the antioxidant activities of *E. fenizlii*. Present studies suggest that *E. fenizlii* have shown potential as sources of natural antioxidants, further studies need to be directed to isolate and characterise antioxidant active compounds from the extracts which could be responsible for the high antioxidant activities. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. *E. fenizlii* can be regarded as promising plant species for natural plant sources of antioxidants with high potential value.

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