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SCREENING AND DETERMINATION OF ANTIOXIDANT SCAVENGING ACTIVITY OF *PIPER LONGUM* AND *EUCALYPTUS CAMALDULENSIS*

Kuppusamy Mohanraj and Naganathan Srinath*
Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore.
*E-mail: sri7486@gmail.com

ABSTRACT

The herbal medicine is used from time immemorial to treat various common ailments like cold, cough and gastric problems. India has various system of treatment like Ayurveda, Siddha, Unani and Homeopathy which are devoid of side effects and treat the person with higher benefits. *Piper longum* fruits and *Eucalyptus camaldulensis* Dehnh., leaves has been used as spices and for medicinal oil preparation for various treatment. We have made an attempt to elevate the importance of these herbal sources by understanding the antioxidant scavenging activity. We have extracted and processed the plants fruit and leaves using different solvents in 1:10 dilution such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water. It was found that ethanol and methanol extract of *Piper longum* and *Eucalyptus camaldulensis* Dehnh., has higher antioxidant activity when compared with the standard Vitamin C and IC_{50} value was observed to be nearly 70µg/ml for all the tests.

Key words: *Piper longum, Eucalyptus camaldulensis* Dehnh., $DPPH^{\bullet}$, $ABTS^{\bullet+}$, FRAP.

1. INTRODUCTION

Cold and cough is always considered as drastic problem which everyone suffers in all parts of world. India a reservoir for various herbs uses plants and their products to treat the common problems time immemorial. The World Health Organization (WHO) has found that out off 3000 plants most of them are available in India and the usages of these plants are nearly by 80 % of the world population (WHO report, 2009). With an increase rate of over exposure towards various antibiotics has created an environment of multidrug resistance microbes such as *Escherichia coli, Klebsiella pneumoniae, Aeromonas* sp., *Mycobacterium tuberculosis, M. leprae*, *Candida* sp., etc. (Waters and Basseler, 2005). This has made the scientists to explore new drugs either using plant sources like medicinal herbs or their parts or chemical sources. Due to the concern related to the side effects by the usages of chemical drugs (Hassan, 2012) made a shift in the path of drug usages and caused the public to use herbal medicinal sources for the treatment like Ayurveda, Siddha, Unani and Homeopathy.

In the current research we have chosen *Piper longum* fruits and *Eucalyptus camaldulensis* Dehnh., leaves as the source with medicinal property for treating the common problem of cold and cough. *Piper longum* belongs to the *Piperaceae* family and is thought to originate from South East Asia and *Eucalyptus* sp. commonly found in the dry and hilly areas are having many medicinal roles in Ayurveda and in modern medicine. Before evaluating any medicinal plant, it is essential to understand its antioxidant potential because there are various free radicals released by our body and by various products intake (Abrahim *et al.,* 1993; Gupta and Ray, 2004; Kumar *et al.,* 2010; Arawwala *et al.,* 2011). It has also been found that these free radicals remain as an adjuvant in causing cancer like breast cancer. These plants are used commonly as spices or for inhalation treatment for curing the common ailment and it is essential to evaluate the antioxidant potential of these drugs as when used and inhaled could sometime reach the blood stream either by absorption from the tissues or from the stomach (Manoj *et al.,* 2004; Sadlon *et al.,* 2010; Chahal *et al.,* 2011). We have aimed to understand the scavenging potential of these plants as they are commonly used in India as a source for treating common cold and cough problems in various parts of India.

2. MATERIALS AND METHODS

2.1. Collection of herbal plants

Piper longam fruits and *E. camaldulensis* Dehnh., leaves were collected from in and around Coimbatore and subjected for plant authentication at Botanical Survey of India, Coimbatore, Tamil Nadu.

2.2. Processing of Plant fruits and leaves using cold percolation method

The plant leaves and stems were processed using cold percolation method as described by Adonizio *et al.* (2008). The cold percolation method helps to retain the medicinal property of any

medicinal plant. The plant leaves and fruits were stored in dark for a period of 2-3 weeks and were powdered. The powders of plant leaves and fruits were sieved and stored in dark bottle. The powdered plant leaves and fruits were weighed and mixed with different solvents in 1:10 dilution in increasing order of polarity such as petroleum ether, chloroform ethanol, hexane, methanol and distilled water.

They were shaken well during the process to avoid fungal contamination and filtered using muslin cloth. The filtrate was kept in watch glass in dark for evaporation and scrapped powders were stored in dark bottle as they are light sensitive. The extracts were subjected to antioxidant scavenging activity and subjected for future research against pathogens.

2.3. Evaluating the various antioxidant properties of extracted Piper longum and Eucalyptus camaldulensis Dehnh. by in vitro free radical scavenging activity

2.3.1. DPPH$^{\bullet}$ scavenging activity

The 2,2-diphenyl-1picryl hydrazyl (DPPH$^{\bullet}$) scavenging activity of *P. longum* and *E. camaldulensis* Dehnh. is performed using the method described by Blois, in 1995. DPPH$^{\bullet}$ is scavenged by antioxidants through the donation of a proton forming the reduced DPPH$^{\bullet}$. Various concentrations of samples were taken along with Vitamin C as standard in different test tubes. The volume was adjusted to 500 µL by adding methanol and 5 mL of 0.1 mM methanolic solution of DPPH was added to these test tubes and vortexed. The tubes were allowed to stand at room temperature for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. The color change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm. The percentage of inhibition radical scavenging activity was measured by the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

The percentage inhibition vs. concentration was plotted and the concentration required for 50 % inhibition of radicals was expressed as IC_{50} value.

2.3.2. ABTS$^{\bullet+}$ radical scavenging activity

The test was based on the relative activity of antioxidants to quench the radical cation ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)$^{+}$ was done using the method described by Re *et al.* (1999). ABTS$^{\bullet+}$ decolorisation assay involves the generation of the ABTS$^{\bullet+}$ chromophore by the oxidation of ABTS$^{\bullet+}$ with ammonium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The reaction was initiated by the addition of 1.0 mL of diluted ABTS to 10 µL of different concentration of extract with high antibiofilm activity of the sample or 10 µL of methanol serve as control. The absorbance was read at 734 nm. Percentage inhibition was calculated by the formula

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.3. Hydrogen Peroxide scavenging activity

The hydrogen peroxide scavenging activity was measured in terms of a decrease in the absorbance as 230 nm in spectrophotometer using the method described by Ruch *et al.* (1989). A solution of H_2O_2 was prepared in phosphate buffer and the H_2O_2 concentration was determined using spectrophotometer at 230 nm wavelength. Various concentrations of plant extracts were added to H_2O_2 and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 and standard compound Vitamin C was calculated using the formula:

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

2.3.4. Hydroxyl radical scavenging activity

Hydroxyl radicals were generated from ferrous ammonium sulphate and EDTA were determined against the scavenging activity of the plant extracts using the method described by Klein *et al.* (1991). Various concentration of plant extracts were added with 1 mL of iron-EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %), and 1 mL of DMSO (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5ml of ascorbic acid (0.22 %) and incubated at 80 to 90 ^{o}C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 mL of ice-cold tri-chloro acetic acid (TCA) (17.5 % w/v). About 3 mL of Nash reagent (75 g of ammonium sulphate, 3 ml of glacial acetic acid and 2 mL of acetyl acetone were mixed and make up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. This was detected by their ability to react with ascorbic acid to produce yellow color complex which was measured at 412 nm against reagent

blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

3.2.5. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of sample was determined using ferric reducing antioxidant power (FRAP) using the method described by Benzie and Strain, 1996. The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mm $FeCl_3$, $6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution and 25 mL acetate buffer. It was freshly prepared and warmed to 37 °C. 900 µL FRAP reagent were mixed with 90 µL water and 30 µL test sample/ethanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 min and the absorbance was recorded at 595nm. An intense blue color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. The calibration curve was plotted with absorbance at 595 nm vs concentration of ferrous sulphate in the range 0.1mM ethanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants.

$$\text{Percentage of radical scavenging activity} = \frac{\text{Control - Sample}}{\text{Control value}} \times 100$$

3. RESULTS AND DISCUSSION

The collected *P. longum* fruit and *E. camaldulensis* Dehnh., leaves were taken to Botanical Survey of India, Coimbatore and authentication no. for the plant is BSI/SRC/5/23/2013-14/Tech./2089. The processing and extraction of the plants fruit and leaves were done accordingly and the ethanolic and methanolic extract of *P. longum* and *E. camaldulensis* Dehnh. were subjected for further scavenging activity analysis based on the work done by Kumar and Laxmidhar (2011).

3.1. Evaluating the various antioxidant properties of extracted Piper longum and Eucalyptus camaldulensis Dehnh. by In vitro free radical scavenging activity

The ethanol and methanol extracts of *Piper longum* and *Eucalyptus camaldulensis* Dehnh., were carried out with antioxidant test and identified that the free radical of the extract were found to have high percentage of inhibition against $DPPH^{\bullet}$, $ABTS^{\bullet+}$, hydrogen peroxide, hydroxyl and FRAP. Vitamin C served as the standard for all the antioxidant assays carried out in the study and when compared the ethanol and methanol extracts of *P. longum* and *E. camaldulensis* Dehnh., found to relatively higher and similar to the standard vitamin C. The results were observed to have higher percentage of inhibition for the extracts and the IC_{50} value was observed as 70 µg mL^{-1} (as shown in Fig. 1, 2, 3, 4 and 5) which was found to be similar to the results of Kumar and Laxmidhar (2011).

This shows that *P. longum* and *E. camaldulensis* Dehnh. has higher scavenging activity as compared with the previous results of Kumar and Laxmidhar (2011). Based on the experimental outcome of various researchers, the antioxidant activities, antimicrobial, antitumour and etc., of the fruit of *P. longum* showed higher activities and proved to be useful in many Ayurvedic preparations for treating various ailments (Manoj *et al.,* 2004; Chahal *et al.,* 2011). Antioxidant activities were measured using FRAP, $DPPH^{\bullet}$, superoxide anion, nitric oxide and hyroxyl radical scavenging assays was also found to higher as the result obtained in the current research (Abrahim *et al.,* 1993).

4. CONCLUSION

This proves that both the plants *P. longum* and *E. camaldulensis* Dehnh., have higher scavenging activity using the ethanolic and methanolic extract of plant fruits and leaves. This shows that these plant extracts can be further studied in future to understand the antibacterial activity as well as can serve as a potent drug in future pharmaceutical research for treating various common diseases and in cancer research.

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Fig. 1. DPPH$^{\bullet}$ radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

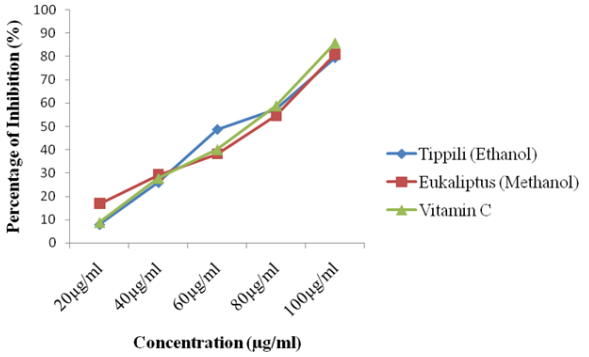

Fig. 2. ABTS$^{\bullet+}$ radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

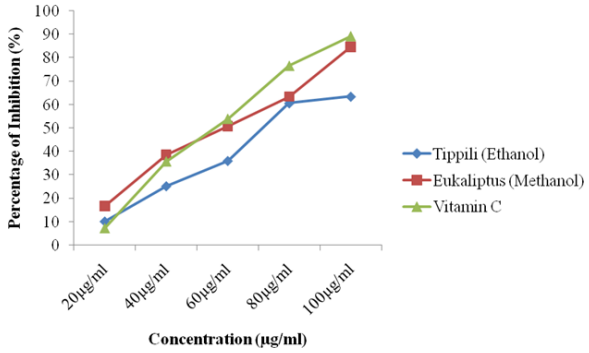

Fig. 3. Hydroxyl radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

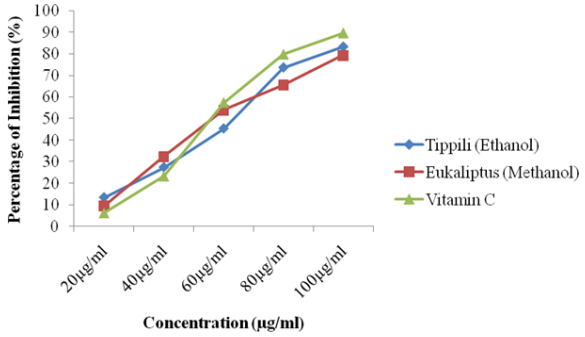

Fig. 4. Hydrogen Peroxide radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.

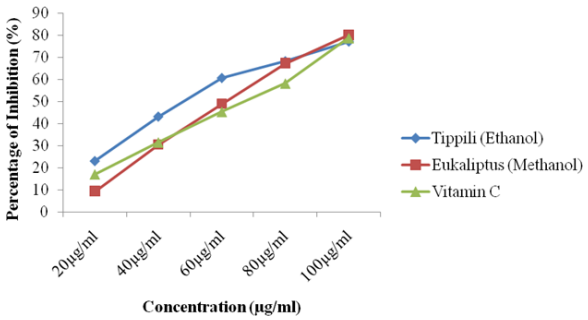

Fig. 5. FRAP radical scavenging activity of *P. longum* and *E. camaldulensis* Dehnh.