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Evaluation of *In vitro* antioxidant and free radical scavenging potential of acetone mace extract (*Myristica Fragrans* Houtt)

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Abstract
Myristica fragrans Houtt is an evergreen tree that belongs to the Myristicaceae family. Mace is the aril part of the nutmeg of *Myristica fragrans* Houtt. Acetone mace extract was tested for its antioxidant and free radical scavenging activities. The extract has shown a significant antioxidant activity as compared to the standard. The extract can be further analysed for its anticancer potential in future.

Keywords: *Myristica fragrans*, antioxidant, free radical scavenging, mace, nutmeg

Introduction

Antioxidant is a active biocompound that protects the living system from the harmful effects of excessive oxidation [1]. Normal metabolic process continuously generate reactive oxygen species and free radicals. When there is a failure in the defence mechanism such as antioxidant compounds and enzymes, it results in oxidative stress. Oxidative stress has been associated with an increased risk of Artherosclerosis, brain dysfunction, cardiovascular disease, cancer and other chronic disease. Natural antioxidant present in the diet prevent oxidative damages and further shield us from oxidative stress and infections [2, 3]. Butylated hydroxytoluene (BTH) and butylated hydroxyanisole (BHA) were the most widely used synthetic antioxidant compounds which have been restricted from usage because of their liver damage and carcinogenic potential. Plants are rich in various secondary metabolites, which can be explored for its antioxidant and antimicribial potential [4, 5, 6, 7].

Plants are important source of natural products with various biological properties. The presence of various phytochemicals especially flavonoids, polyphenols, phenolic acid, tannins, terpenes are known to be responsible for antioxidant and antimicrobial activities of these plant extracts [8, 9].

Nutmeg (*Myristica fragrans* Houtt), is an ever green tree, native of Banda islands of Eastern Indonesia. In India, it is mainly cultivated in South India particularly in Kerala, Tamil Nadu and Karnataka. It belongs to the Myristicaceae family [10]. Mace is the aril part of the seed which constitutes the outermost third integument of the seed, covering its basal part by scarlet yellow ribbon like lobes, and is strongly aromatic in nature [11].

Mace has been used in Indonesian folk medicine as aromatic stomachics, analgesics and a medicine for rheumatism [12]. Mace is traditionally used as a spice and flavouring agent in food. The use of spices in food has been a common practice since ancient times, they impart aroma, mask undesirable odours and can make food more pleasant and tastier. Spices are usually used as flavouring and coloring agents, and also used in food preservation for centuries as they are known to exert antioxidant and antimicrobial activity [13].

Phytochemical analysis of mace acetone extract showed the presence of bioactive compounds such as flavonoids, alkaloids, terpenes, phenols [14]. Plant extracts are used in curing variety of variety of human diseases including microbial infections [15]. Antioxidant and radical scavenging potential of acetone mace extract has been evaluated in this study.

Materials and Methods

Sample Extraction

The dried powder sample of mace (80g) was extracted three times by hot percolation method with 1:5 ratio volume of acetone at room temperature for 72 hrs. The filtrate so were concentrated under reduced pressure at 40 degree Celsius and stored at room temperature for use in subsequent experiments.

DPPH scavenging activity

The antioxidant activity of the extract was estimated on the basis of the radical scavenging effect of the stable DPPHi. Various concentrations of the extract were added to a methanolic 0.4 mM DPPHi solution (0.1 ml) in a 96 well plate. The reaction mixture was shaken vigorously and allowed to stand for 30 min at 37°C. The degree of DPPHi purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. The absorbance of the mixture was determined at 517 nm using UV-Vis microplate reader and ascorbic acid was served as a positive control. The scavenging activity against DPPHi was calculated using the

following equation: Scavenging activity (%) = [1 - (A1 - A2) / A0] x 100% where A0 was the absorbance of control (DPPHi solution without the extract), A1 was the absorbance of DPPHi solution in the presence of the extract and A2 was the absorbance without DPPH· solution.

Superoxide radical scavenging activity

The assay was based on the capacity of the plant extracts to inhibit nitro blue tetrazolium (NBT) upto 50% in the presence of riboflavin-light-NBT system. The reaction medium contains 50 mM phosphate buffer pH 7.6, 20 µg riboflavin, 12 mM EDTA, different concentrations of extract, NBT 0.1 mg/3 ml and BHT was taken in different test tube and the same reagents were added. The reaction was started by illuminating the sample cuvette at regular intervals of 30 seconds and increases in absorbance were measured at 590 nm upto 2.5 min. The superoxide radical scavenging activity was calculated using the formula:

$$\% \text{ inhibition of superoxide radical} = \frac{\text{OD (extract absent)} - \text{OD (extract present)}}{\text{OD (extract absent)}}$$

ABTS radical scavenging activity

ABTS radical cation was produced by the reaction of a 7 mmol/L ABTS solution with 2.45 mmol/L potassium persulphate. The mixture was stored in the dark at room temperature for 12 h before use. The ABTS and the plant extract was diluted with ethanol to an absorbance at 734 nm. After addition of 25 µL of sample or standard to 2 mL of diluted ABTS and the plant extract, absorbance at 734 nm was read after 6 min. A standard curve was prepared by measuring the reduction in absorbance of ABTS at different concentrations of extract. Appropriate blank measurements were carried out and the values recorded. Ascorbic acid was used as positive control.

Results

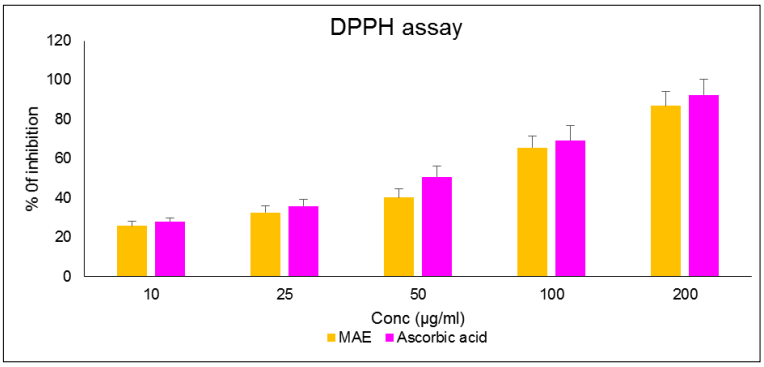

Fig 1

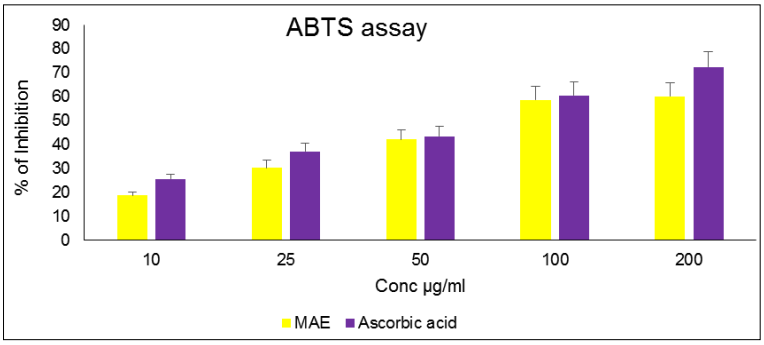

Fig 2

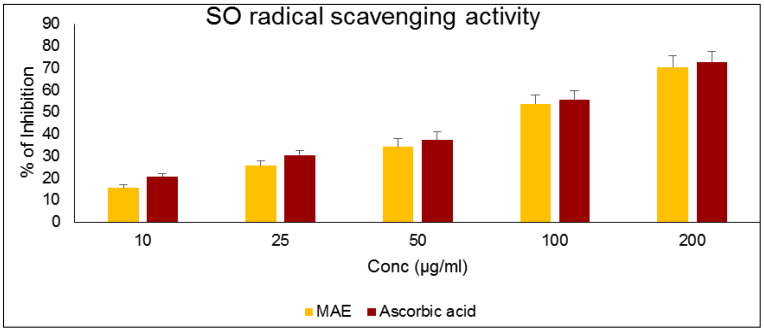

Fig 3

Discussion

The result has revealed that acetone mace extract has significant antioxidant activity.

The strong antioxidant activities of the medicinal plant extracts are attributed to the presence of phenolic and polyphenolic compounds [16]. The phytochemical analysis of acetone mace extract revealed the presence of various phytochemicals such as flavonoids, alkaloids and phenols, contributing to its antioxidant potential [14].

A highly positive relationship between total phenols and antioxidant activity appears to be seen in many plants [17]. It was found that most of the phenolic compounds present in the plants possess antioxidant properties due their redox properties, which make the phenol as reducing agent, hydrogen donator and singlet oxygen quencher [18].

DPPH assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. It is evident that the radical scavenging ability of the acetone mace extract potentially increased with the increasing concentration. IC 50 of the extract was found to be 70 µg/ml (Figure 1) as compared to the ascorbic acid standard.

ABTS radical is stable and soluble in water and organic solvents, enabling the determination of antioxidant capacity of both hydrophilic and lipophilic compounds [19]. The antioxidant potential of the extract as estimated by ABTS method correlated with that of DPPH assay with a IC 50 of 60 µg/ml which is almost equal to the IC 50 of the standard ascorbic acid (Figure)

Superoxide radical scavenging activity is used to estimate the radical scavenging potential of the extract against the superoxide generated by reduction of molecular oxygen in to water in electron transpert chain. Super oxide can be formed by the activated phagocytes such as monocytes, macrophages, Eosinophils and Neutrophils. It is an important factor in killing the bacteria by phagocytosis [20] The IC 50 value of the extract was found to be 80µg/ml which is almost in accordance to the standard ascorbic acid. (Figure 3)

Conclusion

Production and scavenging of free radical by our biological system is inevitable. Our body has a natural defence mechanism to fight against these free radicals. Free radical production need not be always detrimental to life as it is a pathway by which harmful pathogens are eliminated from our system. Owing to the stressful lifestyle, unhealthy eating practices, exposure to pollution and lack of exercises has rendered our body in compatable to fight against the oxidative stress. This may lead to early aging, lack of immunity and in extreme condition may lead to cancer Now it is the responsibility of the scientific community to explore the natural potential antioxidant which can rejuvenate and scavenge the oxidative stress. One such compound is the mace extract, as it is evident that it possess a good antioxidant potential and can be further explored for its other bioactive potentials in future.

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