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Evaluation of Antioxidant and Protease-inhibitory Potential of Ethanolic Extract of *Myristica fragrans* (Nutmeg)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Myristica fragrans* is an important commercial plant used for spices. The plant has been traditionally used as an anticancer, anti inflammatory, antioxidant, sedative hypnotics and antimicrobial agent. Plants have played an important role in maintaining human health & improving the quality of human life for thousands of years and have served humans well as valuable components of medicines.
Methods: Ethanolic extract of *myristica fragrans* was obtained by hot percolation method. Preliminary Phytochemical screening of the extract was done .Antioxidant and anti inflammatory potential of ethanolic extract of *myristica fragrans* was analysed. The data were analysed statistically using two – way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of $p<0.05$.
Results: Ethanolic extract of *Myristica fragrans* (Nutmeg) was rich in the phytoconstituents such as alkaloids, flavonoids, terpenoids and saponins. IC_{50} of antioxidant activity of ethanolic extract of *Myristica fragrans* was found to be 300 µg/ml. IC_{50} of anti-inflammatory potential of the ethanolic extract of *Myristica fragrans* was found to be 360 µg/ml.

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Conclusion: From the study, it was evident that the ethanolic extract of *myristica fragrans* has significant antioxidant and anti-inflammatory potential. In future, the extract can be validated as a drug formulation.

Keywords: Antioxidant; protease inhibitory potential; ethanolic extract; Myristica fragrance; innovative technology; novel method.

1. INTRODUCTION

Myristica fragrans is one of the two species the other being mace derived from the genus myristica, the most important commercial species is *Myristica fragrans*. It is an aromatic evergreen tree indegenious to Bond islands of Indonesia [1]. The plant is native to India and endangered trees are mostly found in western ghat. It is usually cultivated in the asiatic region, caribbean and central American region. The plant has been traditionally used as anticancer, antiInflammatory, anti-Oxidant, Sedative hypnotics, Antimicrobial, Antifertility agent. The plant also possesses Hepatoprotective and cytotoxicity potential [2,3].

Nutmeg or *myristica fragrans* has four parts: The skin, fruit mace and seed. Fruit is a succulent pericarp and the mace is a covering for the endocarp and contains a wrinkled kernek with a ruminant endosperm [3,4]. The seed of nutmeg is firm, fleshy, whitish, transferred by reddish brown veins, and is abundant in oil. The most contrasting feature of this plant is the outer covering of the seed that is the Mace [5]. It is red in colour and is present net covering the seed the mace has its own medicinal properties and has been ingested directly by mouth to treat various being used to treat stomach ulcers, indigestion, liver disorders, and as emmenagogue, nervine, diuretic, diaphoretic, and aphrodisiac [6,7].

This plant enhances the taste and aromatic flavor of food and has been used traditionally as a flavouring agent. Recent scientific studies proved their biological activity according to their traditional claim [3]. It is known to possess Gastroprotective, anticancer, antioxidant, antifungal, anthelmintic properties. Plants have played an important role in maintaining human health and improving the quality of human life for thousands of years & have served humans well as valuable components of medicines [8].

Antioxidants are chemical substances that are protecting the cells of the body from free radicals. Free radicals are unstable molecules that are produced in the body due to oxidation during normal metabolism. Free radicals play an important part in the formation of cancer ,heart disease, stroke and other diseases of aging [2,9]. Protease inhibitors are a class of antiviral drugs that are used on a large scale to treat AIDS /HIV and hepatitis C. Protease inhibitors prevent viral replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors that are necessary for the production of infectious viral particles [10].

Inflammation is the body's immune response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation and acts on the healing process by removing harmful stimuli. hence inflammation is vital to health for its defence mechanism [9,11]. Inflammation if not treated will lead to chronic inflammation and further will lead to chronic inflammatory disease [9]. The level of inflammation relies on the magnitude of the stimuli. A range of anti-inflammatory drugs exist to help control inflammation in the body. However, they often have side effects and in some cases may not be an effective solution [12]. Natural compounds that are present in certain herbal remedies also have the potential to be anti-inflammatory drugs [9,11,13]. Phytochemical constituents that are present in herbs are considered as an effective remedy as an anti-inflammatory agent. Our team has extensive knowledge and research experience that has translate into high quality publications [14-33]. The aim of the study is to evaluate the antioxidant and proteinase inhibitory potential of ethanolic extract of *Myristica fragrans* (Nutmeg).

2. MATERIALS AND METHODS

2.1 Preparation of Ethanolic Extract of *Myristica fragrans* (Nutmeg)

Nutmeg was purchased from a herbal health care centre.Air dried, crushed and made into powder form. Ethanol was added to form 80% ethanolic extract [34,35]. The extract was prepared by a hot percolation method. Later it was dried and used to analyse the antioxidant and anti-inflammatory potential.

2.2 Phytochemical Screening Test

Test for phlobatannin: 1 ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin [36,37].

Test for Carbohydrates: Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids: Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids: 2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids: 2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins: One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins: Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids: One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.Antioxidant activity.

2.3 DPPH Free Radical Scavenging Activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5 mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = (Control OD - Sample OD X 100)/control OD

2.4 Anti-inflammatory Potential of Ethanolic Extract of *Myristica fragrans* (Nutmeg)

Protease inhibitory potential: The test was performed according to the modified method of [38,39]. The reaction mixture (2 ml) was made with 0.06 ml trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4) and 1ml test sample of different concentrations. The reaction mixture was incubated for 10 minutes at 37ºC. Then, 1ml of 0.65% (W/V) casein was added. The mixture was re-incubated for 20 min. After incubation, 2 ml of 2M $HClO_4$ was added to terminate the reaction. The cloudy suspension was centrifuged at 7830 rpm for 15 minutes. The absorbance of the supernatant was measured at 280 nm against. The Tris-HCl buffer was blank. The experiment was performed in triplicate. Anti-inflammatory activity was measured by calculating % inhibition against a range of concentrations.

% inhibition= (1-Ac/At) 100;

Where Ac is absorbance of control; At is absorbance of the test.

2.5 Statistical Analysis

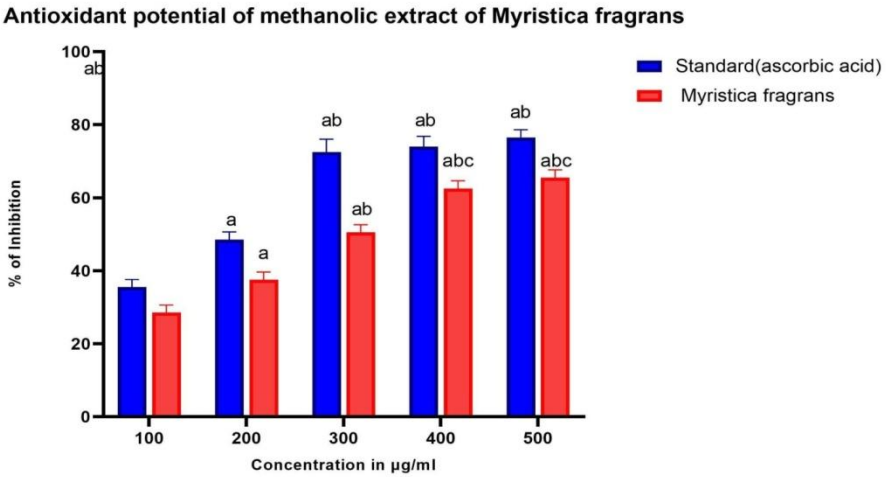
The data were subjected to statistical analysis using two – way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of $p<0.05$.

3. RESULTS AND DISCUSSION

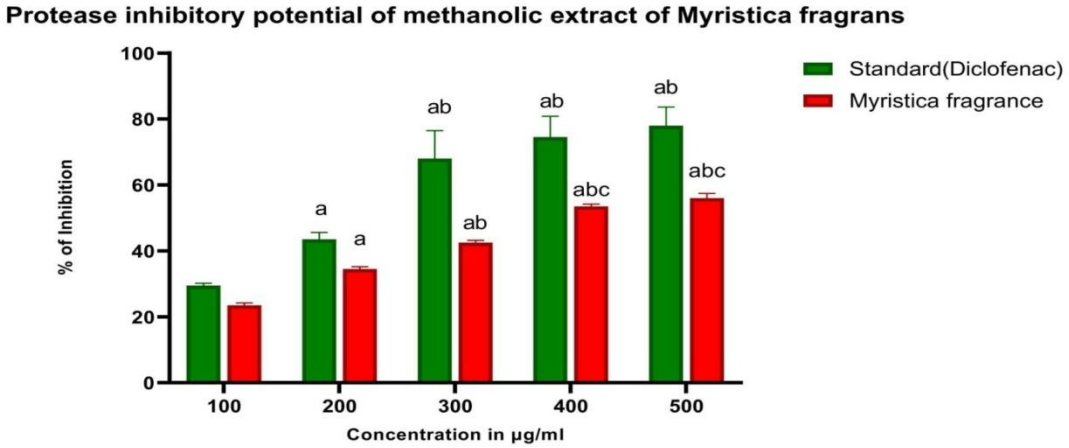
From the above study, it was evident that the ethanolic extract of *Myristica fragrans* (Nutmeg) was rich in the phytoconstituents such as alkaloids, flavonoids, terpenoids and saponins (Table 1). Phytochemicals are secondary metabolites which constitute various medicinal properties of the plant. The unique medicinal and aromatic nature of the plants depends on its phytoconstituents [40].

Table 1. Phytochemical analysis of ethanolic extract of *Myristica fragrans* (Nutmeg)

Phytochemicals	Presence in Ethanolic extract of *Myristica fragrans* (Nutmeg)
AMINO ACIDS	-
PROTEINS	-
TERPENOIDS	-
ALKALOIDS	++
FLAVONOIDS	+
SAPONINS	++
STEROIDS	+
CARBOHYDRATE	+



Graph 1. Represents Antioxidant potential of ethanolic extract of *Myristica fragrans* (Nutmeg)- DPPH Assay as compared with the standard Ascorbic acid. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts.Blue bar represents the standard ascorbic acid, pink bar represents ethanolic extract of *Myristica fragrans* (Nutmeg). Each line represents Mean ± SEM of 3 independent observations . Significance at p <0.05



Graph 2. Represents anti- Inflammatory potential - Protease inhibitory potential of ethanolic extract of *Myristica fragrans* (Nutmeg). X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Green bar represents the standard diclofenac and pink colour represents ethanolic extract of *Myristica fragrans* (Nutmeg). Each line represents Mean ± SEM of 3 independent observations. Significance at p < 0.05

Antioxidant potential of ethanolic extract of *Myristica fragrans* was determined by DPPH Free radical scavenging assay. IC_{50} of antioxidant activity of ethanolic extract of *Myristica fragrans* was found to be 300 µg/ml (Graph 1) and increased in a dose dependent manner as compared to the standard Vitamin C. [41]. Free radicals are molecules possessing an unpaired electron emerging by oxidative stress. Bharadwaj et al 2019 has reported the role of Phenolic compounds in scavenging the free radicals [41,42]. These phenolic compounds are a part of the plant's phytonutrients. The ability of these aromatic compounds in the exchange of electrons makes them potent free radical scavengers. [43].

A dose dependent protease inhibitory potential was observed for the extract and standard drug. In the present study, the standard drug used is Diclofenac. The standard drug showed a greater percentage of inhibition than the extract at the same concentration. The Ic 50 of the ethanolic extract of *Myristica fragrans* was found to be 360µg/ml (Graph 2) [8]. The results revealed that standard drugs are the most potential drugs, but the side effects associated with the long term usage of synthetic drugs needs to be taken care. The potential of any herbal extract can be increased by utilizing downstream processing techniques such to purify the bioactive constituents. J S, Sethi J et al 2018, [9] has analysed that free radicals have an inherent ability to cause oxidative damage to biological macromolecules and thus have been implicated in the aetiology of various diseases such as cellular and metabolic injury, cancer, atherosclerosis, inflammation, aging, immunosuppression, diabetes, ischemic heart disease and neurodegenerative disorder such as Alzheimer's and Parkinson's diseases [44,45]. Reactive oxygen species (ROS) production plays an important role in the modulation of inflammatory reactions Free radicals can cause damage by a variety of different mechanisms which include DNA damage, lipid peroxidation, protein damage, oxidation of enzymes (e.g. anti-protease) and stimulation of pro-inflammatory cytokines release and thus, their neutralization by antioxidants and radical scavengers can attenuate inflammation [46].

4. CONCLUSION

This study can serve as a basis for proper identification, collection and investigation of the plant and their phytochemical constituents. The present study has reported the Phytochemical, antioxidant and anti-inflammatory potential of the ethanolic extract of *Myristica fragrans* (Nutmeg). The plant was screened for phytochemical constituents and found to be an excellent source of medicinally active elements which can be further exploited to isolate and synthesize modern medicines. This work justifies that *myristica fragrans* has effective antioxidant and anti-inflammatory activity and has high potential for development of drugs that can be used in various medical fields through the years of development.

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CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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