© 2020 | International Journal of Scientific Research in Science and Technology
IJSRST | Volume 7 | Issue 3 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
DOI : https://doi.org/10.32628/IJSRST207361

Evaluation of Phytochemicals and Antioxidant potential of aqueous extract of *Myristica fragrans*

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ABSTRACT

Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. The damage caused by antioxidants has been linked to cancer, atherosclerosis, and vision loss. Need of antioxidant with less side effects emerges nowadays. In this present qualitative evaluation shows this plant contains secondary metabolites like terpenoids, flavanoids, saponins, tannins, alkaloid, steroid, glycosides, proteins and coumarins. *Myristica fragrans* have been reported for its versatile antioxidant activity.

Keywords : *Myristica fragrans*, antioxidant activity, radical scavenger, secondary metabolites and free radicals

I. INTRODUCTION

Antioxidants are exogenous or endogenous molecules that mitigate any form of oxidative/nitrosative stress or its consequences. They may act from directly scavenging free radicals to increasing antioxidative defences. Antioxidant deficiencies can develop as a result of decreased antioxidant intake, synthesis of endogenous enzymes or increased antioxidant utilization. Antioxidant supplementation has become an increasingly popular practice to maintain optimal body function. Knight JA et al., 2000 stated that the oxygen-derived free radicals are important in both natural and acquired immunity.Free radical and reactive oxygen species are responsible for lipid oxidation, which is the major chemical change involved in the deterioration of food during processing and storage (Haraguchi H. et al., 1997) Antioxidants have been widely used as food additives to provide protection fromoxidative degradation of foods and oils. The most extensively used synthetic antioxidantsare propylgallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) (Sherwin ER. et al., 1990) but there are some arguments about the safety and adverse effects of these substances when used as food additives. In fact in recent years, researchers have focused on spicy and medicinal plants for extracting natural antioxidants which play an important role in the food industry to combat food deterioration.

Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures (Tapas AR. et al.,

2008). They are sometimes called "free-radical scavengers". The sources of antioxidants can be natural or artificial. Certain plant-based foods are thought to be rich in antioxidants. Plant-based antioxidants are a kind of phytonutrient, or plant-based nutrient.The body also produces some antioxidants, known as endogenous antioxidants. Antioxidants that come from outside the body are called exogenous.Free radicals are waste substances produced by cells as the body processes food and reacts to the environment. If the body cannot process and remove free radicals efficiently, oxidative stress can result. This can harm cells and body function. Free radicals are also known as reactive oxygen species (ROS) (Dillard CJ et al., 2000). Factors that increase the production of free radicals in the body can be internal, such as inflammation, or external, for example, pollution, UV exposure, and cigarette smoke.Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions. This in turn may leads to an excessive release of free iron or copper ions, an activation of phagocytes, a type of white blood cell with a role in fighting infection an increase in enzymes that generate free radicals and a disruption of electron transport chains

Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress. Activities and processes that can lead to oxidative stress includes, Mitochondrial activity, excessive exercise, tissue trauma, due to inflammation and injury, ischemia and reperfusion damage Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to boost overall health. Flavonoids, flavones, catechins, polyphenols, and phytoestrogens are all types of antioxidants and phytonutrients, and they are all found in plant-based foods. Each antioxidant serves a different function and is not interchangeable with another. This is why it is important to have a varied diet like food sources such as pomegranate, plant-based foods, especially fruits and vegetables.

Kuete V et al., 2017 investigated the pharmacological and phytochemical studies on Myristica fragrans have been reviewed. Several compounds were identified in nutmeg and mace of the plant with terpinen-4-ol, β-pinene, and limonene being the dominant constituents common to volatile oil in all species. Several lignans and neolignans have also been isolated in various parts of the plant. Some reported pharmacological properties of M. fragrans include anticancer, antidepressant, antidiabetic, antiobesity, antiinflammatory, analgesic, antimicrobial, antioxidant, hepatoprotective, and memory enhancing. However, the clinical efficacy in long-term trials is still to be investigated.

(Jaiswal P et al., 2009) demonstrated that the biological effects of Myristica fragrans. The chemical constituents of M. fragrans have been investigated for hypolipidaemic and hypocholesterolemic effects. Despite some laboratory studies on the insecti-cidal / molluscicidal activity of M. fragrans, more field studies are recommended for effective control of pests. (Veeru P et al., 2009) represented the scarcity of data regarding the parameters and methods employed for assessing the quality of medicines.

II. Aim and objectives of this study

Extensive scientific research has been carried out all over the world to use the medicinal plants and their extracts from herb as anti-oxidants. Antioxidants can be natural or synthetic. Natural antioxidantsமீ can be taken up through diet as they are present in fruits, vegetables and spices. Versatile phytochemicals which responses for its pharmacological activities. In this study investigate the phytochemical studies for the identification of chemicals for antioxidant analysis in DPPH assay the ascorbic acid as standard.

and antioxidant activity of Myristica fragrans in leaf extract used for the determination process of antioxidant activity in DPPH assay.

III. METHODS AND MATERIAL

Plant collection:

The mature and healthy plant leaves of Myristica fragrans was collected from in and around area of Thanjavur districts of Tamilnadu, and also at shop (Naattu Marunthu Kadai) India.

Extraction

The collected leaves were washed with fresh water and properly dried in a shaddow dry and grounded into a coarse powder with the help of a suitable mixer. As a final point, crushed powder stored in a cool, dark, and dry place in an airtight container, and kept until analysis commenced. By using soxhlet's apparatus, 100 gm of dried plant leaf material were refluxed with Water continuously for 24 hrs. The vapor flows through a coil where they condense back to a liquid which is then collected in the receiving vessel. The whole mixture was successively filtered through a piece of clean, white cotton material and Whatman no.1 filter paper.All the extracted samples were stored in a refrigerator at 4 °C. Mostly we consider the Soxhlet extractor only used for organic solvents. But it heats up a alcohol or water which turns into a gas. The condenser then cools this gas back into liquid form which drips over the herbal material. So in this study this procedure was used.It was followed by the preliminary phytochemical studies.The procedure used for this priliminary studies were given as a very short procedure in the table form.

Qualitative determination of the phytochemicals

S.no	Phytochemical	Reagent mixture	Amount of Aqueous extract (ml)	Confirmation
1	Terpenoids	2 ml of chloroform + conc.H2SO4 (after the addition of *Myristica fragrans*	2	Appearance of reddish brown color.
2	Flavanoids	3 drops of 10% lead acetate and extract	1	Appearance of yellow color
3	Saponins	5 ml of distilled water + 3 drops of of extract (shaked vigorously) drops	5	Formation of foam
	Tannins	2 ml distilled water and plant extract + 3 drops of 0.1% ferric chloride	2	Appearance of green color.
5	Alkaloids	3 drops of Hager's reagent	2	Formation of yellow

6	Steroids	2 ml of chloroform + 5 drops of conc.H2SO4	2	Reddish brown ring was formed.
7	Glycosides	2 ml of chloroform + 2 ml of acetic acid	2	Color change from Violet to blue to green.
8	Phlobatannins	2 ml of1% HCl	2	Formation of red precipitate.
9	Proteins	1 ml of conc.H2SO4	2	Formation of white precipitate.
10	Coumarins	3 ml of 10% NaOH	2	Appearance of yellow color

Preparing DPPH solution

DPPH (7.89 mg) was weighed on a chemical balance with a minimum weighing limit of 10 μg or smaller. Scavenging activity of the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method by Shen et al., 2010. Briefly, an 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to 3 ml of the solution of the *Myristica fragrans* plant extract in methanol at different concentration (100,200,400 & 800μg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Exactly 30 min after the addition of the DPPH solution, the absorbance of the solution at 517 nm was measured. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = {(A0 – A1)/A0)*100}

The absorbance at the addition of the analytical sample was expressed as As, the absorbance at the addition of ethanol instead of the sample as Ac, and the inhibition ratio (%).

In the analytical procedure distributed, the measurements at six points of concentration, including control, were required. The measurement of the DPPH radical scavenging activity for the analytical sample solution at each concentration was repeated three times.

Calculation of IC50

The IC50 of each analytical sample was calculated according to the following procedure:

1) Inhibition ratios (y) were plotted against the sample concentrations (x) at all six points, and the respective regression line (y = ax + b) was drawn.The regression line was not required to pass through the origin. In this step, we verified that all of the measurement points were basically on the regression line. In addition, it was also verified that two points at around the 50% inhibition did not have a

deviation from the regression line. In fact, because the inhibition curve is not completely straight, but slightly curved, we decided to calculate the IC50 value using the interpolation method by joining the two points around the 50% inhibition with a straight line as follows:

2) Two points enclosing a 50% inhibition ratio were selected, and a regression line (Y = AX + B) was drawn. The regression line was not required to pass through the origin.
3) X (sample concentration) was calculated, when Y in the regression equation of (2) was substituted with 50.

IV. RESULTS AND DISCUSSION

Phytochemical screening

Qualitative analysis *Myristica fragrans* leaf extracts

PHYTOCHEMICALS	*Myristica fragrans*
Alkaloids	-
Flavonoids	+
Carbohydrates	+
Protein	+
Phenols	+
Saponins	+
Tannins	-
Phytosterols	+
Terpenoids	+
Phlobatannins	-

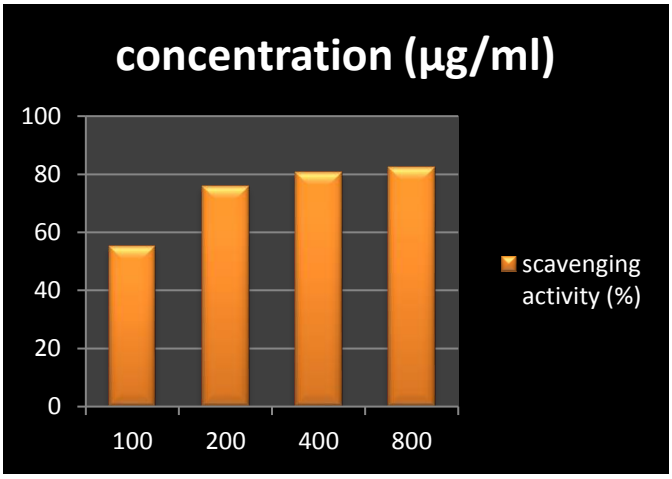
The above qualitative analysis indicates the presence of flavonoids, carbohydrates, protein, phenol, saponin, phytosterol and terepenoids are present *Myristica fragrans* leaf extracts.

Antioxidant activity

Antioxidant activity of Myristica fragrans

Test material	Concentr	Scavenging
Myristica fragrans by DPPH assay		55.20
		75.80
		80.60
		82.40
Ascorbic acid		80.70
		85.40
		90.30
		93.10

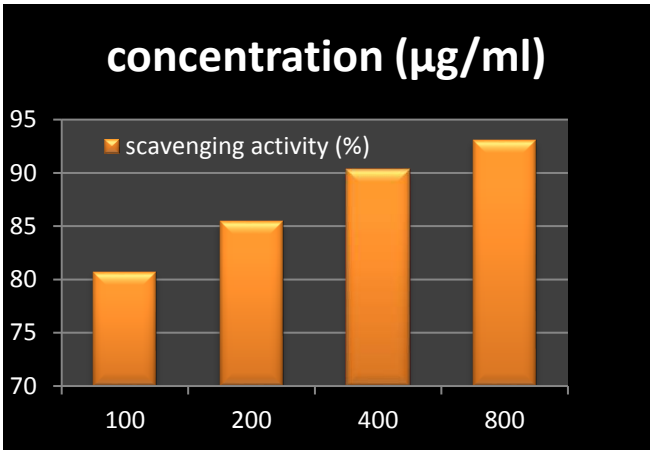
Scavenging activity of *Myristica fragrans* (%)



Aqueous extract of *Myristica fragrans* has significant antioxidant free radical scavenging activity by DPPH assay. It shows 55.20, 75.80, 80.60 and

82.40% for different concentrations such as 100, 200, 400 and 800(µg/ml)

Scavenging activity of Ascorbic acid (%)



Comparing the ascorbic acid activity as standard against the *Myristica fragrans* plant extract. It shows 80.70, 85.40, 90.60 and 92.10% for different concentrations such as 100, 200, 400 and 800(µg/ml).

Discussion:

Antioxidant potential of phenolic compounds from green pepper (*Piper nigrum*L.) and lignans from fresh mace (*Myristica fragrans*) were evaluated for their ability to scavenge 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical, inhibit lipid peroxidation and protect plasmid DNA damage upon exposure to gamma radiation. Nutmeg essential oils were analysed by GC and GC–MS. These oils, together with 16–18 components found to be present, were tested for antioxidant properties at final concentrations of 0.05–2.5×10^4 ppm in an egg yolk-based thiobarbituric acid reactive substances (TBARS) assay and also undiluted in a β-carotene agar diffusion assay. Antioxidant and antimicrobial activities of nutmeg (*Myristica fragrans* Houtt) seed extracts were evaluated. Seeds were extracted with acetone, ethanol, methanol, butanol and water. All the extracts have shown significant antioxidant and antimicrobial activities against the tested microorganisms. Activity guided fractionation to identify major antioxidant compounds were carried out by dot blot tests on TLC.

Essential oil is also demonstrated to possess radioprotective ability as evaluated by plasmid DNA protection assay for first time in this study. Commercially dried powder of nutmeg mace (*Myristica fragrans*) and pimento (*Pimenta dioica*) spices was investigated for their high performance liquid chromatography phenolic profile and their antioxidant and hypoglycaemic properties by α-amylase and α-glucosidase inhibition tests. Silver nanoparticles (AgNPs) from aqueous seed extract of *Myristica fragrans* (nutmeg) were characterized and it shows the antimicrobial potentialz

V. SUMMARY AND CONCLUSION

Medicinal plants frequently used as a raw source for the extraction of active ingredients which need for the novel arrival of disease curing drugs. Edible parts from the plants has many phytochemicals which perform many physiological effects.

Plants have been a source of nutrition, medicines and crop protection agents for centuries. It is believed that over 80% of all medication taken in the developed world is derived from plant origins

(Suchandra Chatterjee[a] *et. al*). Plants are clean, environmentally friendly factories that produce a wealth of novel, biologically active compounds and, with time, we expect to discover an increasing number of active compounds. There is a resurgent interest in traditional plant- based remedies in search of active principles. In recent years significant progress has been made evident in this direction (Thomas RA *et.al* 2015*).* More than 30% of the pharmaceutical preparations are based on plants. An increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from medicinal plants. Searching for new drugs in plants implies the screening the plants for the presence of novel compounds and investigation of their biological activities. The path that leads from an intact plant to its pure constituent is long and the work may last from weeks to years.

Myristica fragrans leaf extract extracted by soxhlet's apparatus was screened for its chemical constituents. It showed the presence of versatile phytochemicals which responses for its pharmacological activities. It also showed a strong antioxidant activity against ROS, through DPPH assay using ascorbic acid as standard. In future, it might be studied in depth by researchers for its enhancement of diseases therapies. Hence *Myristica fragrans* have strong activity in DPPH assay.

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Cite this article as :

Malarvizhi V, Jenni. P, "Evaluation of Phytochemicals and Antioxidant potential of aqueous extract of Myristica fragrans", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 7 Issue 3, pp. 352-356, May-June 2020. Available at doi : https://doi.org/10.32628/IJSRST207361

Journal URL : http://ijsrst.com/IJSRST207361