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MOLECULAR DOCKING OF NOVEL COMPOUNDS FROM MYRISTICA FRAGRANS WITH ONCOGENIC PROTEINS-NEW TARGETS FOR ORAL CANCER PREVENTION

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ABSTRACT

Background: Globally, oral cancer is the sixth most prevalent cancer. Excessive consumption of alcohol and tobacco can cause oral cancer. Initially it grows slowly then, converted to a middle stage and later it will be identified as fully developed malignancy. **Methods:** Molecular docking study was conducted based on the chemical nature and similarities of compounds extracted in the plant with target oncogenic proteins EGFR (PDB ID: L858R) and MAPK (PDB ID: 6K731) using Biovia Discovery Studio Client 2021 and Auto dock Vina softwares. After the protein preparation by Biovia Discovery Studio Visualizer, ligands were imported for virtual screening through PyRx. According to the PyRx result and Lipinski's Rule of Five, 3-O-Methyl-d-glucose, and Squalene demonstrated sub-maximal binding capacity against oncogenic proteins like EGFR, MAPK. **Results:** The results of molecular docking study between 3-O-Methyl-d-glucose, Squalene and Oncogenic proteins EGFR and MAPK indicate that methyl glucose has effective inhibitory constant (10.5Mm) on EGFR and MAPK, the ligand efficacy was 0.21, while the inhibitory constant for Squalene was 44.99µM with ligand efficacy of 0.20. Moreover, both the compounds were shown to suppress the action of oncogenic proteins by binding and inhibiting the LSY721 site. The same molecules were further docked through Biovia Discovery Studio Client 2021 and the interaction was visualized under PyMol. **Conclusion:** In silico studies on 3-O-methyl glucose and Squalene found in

Myristica fragrans revealed that these compounds potentially inhibit the progressive activity of oncogenic-proteins (EGFR, MAPK) in oral cancer.
Keywords: Oral cancer prevention, Myristica fragrans, O-3-Methyl glucose, Squalene, In-silico, EGFR, MAPK.

INTRODUCTION:

Oral cancer is one of the most frequently occurring fatal diseases across the globe. According to Globocan report (2020)[1], nearly 77,757 people die each year while 377, 713 new cancer cases add up globally. Amongst South Asian countries, oral cancer is most frequent malignancy in India with 1/5th of deaths and 1/3rd of new cases being identified every year.

Literature indicates that of all the oral cancers, the most prevalent cancer is oral squamous cell carcinoma (OSCC), which accounts for more than 90% cases[2]. Oral carcinoma is caused due to chronic inflammation, human papillomavirus or candida infections, alcohol and tobacco abuse, ultraviolet radiation, immunosuppression, genetic susceptibility, and dietary habits[3].

Oral inflammation may also have a role in the pathophysiology of oral cancers due to the involvement of several inflammatory pathways, such as Cyclooxygenase (COX)-2, phosphatidylinositol 3-kinase, mitogen-activated protein kinase (MAPK), nuclear factor-κB (NFκB)[4]. Some studies reported the frequent presence of *Candida albicans* genotype A strain in the oral cancer patient than other diseases. Interestingly, this genotype strain is linked with leucoplakia lesions[5].. Research suggests that patients undergoing renal or bone marrow transplantation are more likely to develop oral cancer due to Immuno-suppression[6].

Although new drugs and modern strategies are available for treating oral cancer, the success rate is only below 50%. In this context, many researchers explored various plants for bioactive compounds which can potentially play a key role in the successful treatment of oral cancer. Some studies reported that phytochemicals of certain plants are effective against oral cancer[7-11]. In the light of these findings, herbal products have gained significance in the management of obesity as they are effective, affordable, and also associated with fewer side effects[12-14].

Myristica fragrans commonly known as Nutmeg or mace is used as spices and it belongs to the family of *Myristicaceae*[13]. It is used in culinary preparations and also an alternative medicine for its anti-inflammatory, aphrodisiac, memory enhancer, and anticancer property[13-14]. In Ayurveda medicine, it is used to suppress appetite, hyperlipidaemia, cancer, and atherosclerosis and heart ailments. It is also used to treat rheumatism, sore mouth, diarrhoea and insomnia[15]. This plant was reported to have two bio-active compounds 3-O-Methyl-d-glucose and Squalene with anti-cancer properties. A perusal of the available literature reveals that no previous studies were available on these two compounds for their potential use in the treatment of oral cancer. Therefore, we performed molecular docking to evaluate the molecular interactions of 3-O-Methyl-d-glucose and Squalene with the oncoproteins like EGFR and MAPK and their potential to cure oral cancer.

MATERIALS & METHODS

Collection and Authentication of plant: Fresh seeds (Nutmeg) of *Myristica fragrans* were purchased from Ayurveda medical store in Nandyal town and it was authenticated by the Department of Botany, Rayalaseema University, Andhra Pradesh. The herbarium number of the nutmeg is 201/2016-17. Dried nutmeg was ground to a fine powder in a grinder. This powder was later used for the preparation of extract with three different solvents (petroleum ether, chloroform, and ethanol) by using Soxhlet apparatus. The yield extract was stored and used for further study.

Extraction of plant material: Solvent extraction was done using three solvents petroleum ether, chloroform, and ethanol in a Soxhlet apparatus. Dried seeds of *Myristica fragrans* were finely powdered in a grinder and stored in closed container. From container, 30 g of powder was taken and placed in the Soxhlet basket. The Soxhlet flask was filled with 300 ml of solvent. The Soxhlet connected with condenser and tap water was running through the inlet and outlet of the condenser. The solvent was heated to reflux. The solvent vapour travels up a distillation arm and floods into the Soxhlet basket which contains solid.

The condenser ensures that any solvent vapour cools, and drips back down into the sac which was filled with mace powder in the basket. The Soxhlet basket was filled slowly with solvent. Some of the desired compounds in the powder were dissolved in warm solvent. When the basket was almost full, it was automatically emptied by a side arm, and the solvent running back down to the soxhlet flask. This cycle was allowed to repeat many times in 24 h. After many cycles, the desired compounds were concentrated in the distillation flask. Finally, the extracted solvent was placed on the water bath for evaporation for yielding the extracted compound. The resultant solid extract was measured[16].

Phyto-constituent analysis: Three extracts (petroleum ether, chloroform, and ethanol) were tested to identify the phytochemicals depends on their polarity[17-19]. Preliminary phytochemical analysis (qualitative) was performed to identify the presence of phytoconstituents (alkaloids, phenol, tannins, carbohydrate, glycosides, saponins, steroids, flavonoids, terpenoids, resins, and proteins) in three different extracts[17].

I. Test for Alkaloids (Mayer's test): To 5 ml aqueous extract 2 ml of 1% HCl. was added. Later, Mayerand Wagner's reagent was then added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

II. Test for Saponins (Foam Test): Aqueous extract (5 ml) was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam indicates the presence of saponins.

III. Test for Tannins (Lead acetate test): About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl_3$ Solution were added. Formation of green precipitate indicates the presence of tannins.

IV. Test for quinines: To 1 ml of extract, few drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of red colour indicates presence of quinones.

V. Test for Glycosides: To 1ml of extract, 1ml of Fehling's solution was added and heated. Orange precipitate indicates the presence of glycosides.

VI. Test for Flavonoids (Ferric chloride test): To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

VII. Test for polyphenols: Small amount of various extracts was taken separately in water and tested for the presence of phenolic compounds with dilute ferric chloride solution. Violet colour indicates the presence of phenolic compounds.

VIII. Test for Terpenoids: To 2 ml of chloroform added 3 ml of concentrated sulphuric acid and made upto consecutively to 5 ml of extract. A reddish brown interface in the solution indicates the presence of Terpenoids.

IX. Test for Proteins (Biuret Test): The test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

X. Test for Sterols (Liebermann Burchard Reaction): A small amount of extract of sample and a few crystal of sodium nitrate were taken in a dry test tube and heated gently for a minute. It was cooled and 0.5 ml of concentrated sulphuric acid was added. Appearance of bluish-green colour shows the presence of sterols.

RESULTS & DISCUSSION

The extract was prepared with *Myristica fragrans* (nutmeg) powder by Soxhlet apparatus on continuous hot extraction process for 24 h using three different solvents petroleum ether, chloroform, and ethanol based on their polarity. Table 1 indicates that the percentage of yield was more (16.70%) with ethanol compared to the other solvents. The yield was calculated by the formula given below

$$\textbf{Percentage of yield} = \frac{\textbf{Extract obtained}}{\textbf{Total powder used}} \; \textbf{X 100}$$

Preliminary phytochemical analysis revealed the presence of different constituents in the three different solvents used in the present study and corresponding results are presented (Table. 2). It is observed that the concentration of saponins, flavonoids, and polyphenols was more in ethanolic extraction.

GC-MS analysis: The constituent phytochemicals were isolated by column chromatography and their purity was tested by High Performance Liquid Chromatography (make) equipped with Phenomenex Luna

C18 column (150–4.6 mm, 5 m). The chemical structure of the isolated compound was determined by physicochemical and spectroscopic data analyser. (1D- and 2D –NMR and MS data). The GC-MS study revealed the presence of Squalene and 3-O-Methyl-d-glucose with molecular weight (MW) 410 and 194, and highest percentage of peak area observed at retention time (RT) of 28.87 and 12.62 minutes, respectively, along with other chemical compounds (Table.3)

Molecular docking study (in-silico study): Present study explains about interaction between chemical compounds identified in plant extract and oncogenic proteins, and it was used as primary evolutionary study and confirming the inhibitory effect of phytochemicals. With this confirmation, the anti-cancer property of Squalene and 3-O-Methyl-d-glucose are established. The binding capacity of the plant molecules –oncogenic proteins (receptor) interactions is crucial to describe how well the drug binds to the target molecule. [table 4]

Figure 1 (a-d) illustrates the molecular docking of phytochemical constituents 3-O-Methyl-d-glucose and Squalene with oncogenic proteins EGFR and MAPK. It is clear that the phytochemicals showed sub maximal inhibitory binding capacity from the MolDcok score & H- bond energy[21]

Table 3: shows the molecular interaction and inhibitory property of the photochemical compounds on monogenic proteins (EGRF and MAPK). In the docking study, LYS721, and THR830 of oncogenic protein EGFR were effectively inhibited by **3-O-Methyl-d-glucose**, which was extracted from *Myristica fragrans*. They demonstrated significant affinity by forming hydrogen bonds with different hydrogen and oxygen atoms of the phytochemical Methyl-d-glucose (ASP831 and GLU738) (Figure 1 and Table.3).

The ligand Squalene showed hydrogen bond and hydrophobic interaction with LEU694, ALA719 amino acids of oncogenic proteins which indicate effective bonding between the ligand and oncoproteins. The inhibitory interaction was established with hydrogen bonds in between proteins of EGRF and MAPK[22]. This inhibition mechanism holds the key to control and prevent the oral cancer and regulate other cancer causing pro-active compounds.

In the previous study[23,24] the residues that participated in the formation of hydrogen bonds inside the active binding site of targeted proteins which causes cancer. The resulting hypothesis may be an incredible starting point for the creation of some new pathways as potential EGFR, MAPK inhibitors that improve affinity as well as intrinsic function. The results of this work show that powerful analytical tools are capable of recognizing potential ligands. The use of computational methods in the discovery and creation of drugs could be used to minimize time and minimize the work of a medical chemist.

The molecular docking simulation of the phytochemicals provided by *Myristica fragrans* reveals that perhaps the plant constituents all have comparatively high binding energy. Analysis of these complexes also revealed that all most all the compounds formed the hydrogen with the amino acids LYS-729 &THR-830(Figure 1). So, these amino acids might be responsible for functional of the target protein. Further experimentally analysis is needed to confirm this finding.

CONCLUSION

The ethanolic extract of leaves of *Myristica fragrans* possess various potent bioactive compounds which can be effectively used for the prevention of oral cancer. The results indicate that this plant has phyto-pharmaceutical importance and can be classified as medicinal plant.

Although, the in-silico analysis of the present study is highly promising, further in-vitro and in-vivo studies are required to establish the pharmaceutical potential of O-Methyl-d-glucose & Squalene, since they have no or minimal side-effects. Once such holistic studies are conducted, these phytochemicals can be confidently employed as a candidate drug in the treatment of oral cancer and other oncology fields.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest and are in full agreement with the results of this study.

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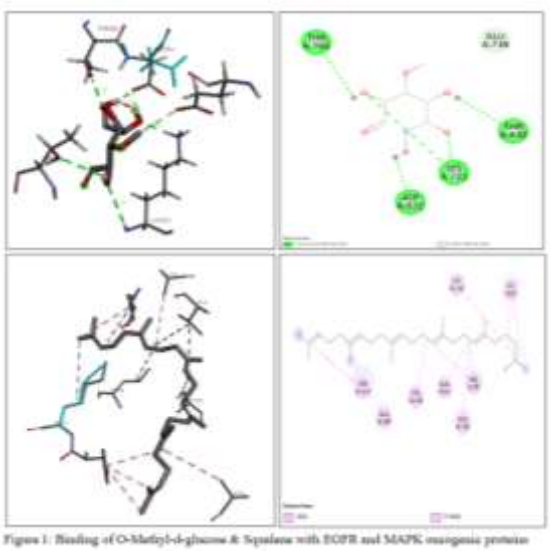
IMAGES



TABLES

Solvent used	(ml)	Mf powder weight (g)	Extract obtained (g)	% of yield (w/w)
Ethanol	300	30	5	16.7
Petroleum ether	300	30	4.5	15
Chloroform	300	30	4.8	16

Table 1: *Myristica fragrans* extract yielded with different solvents

Constituent	Petroleum ether	Chloroform	Ethanol
Alkaloids	+	+	+
Saponins	+	+	++
Tannins	+	+	+
Quinones	+	-	-
Glycoside	-	+	+
Flavonoids	+	+	++
Polyphenols	+	+	++
Terpenoids	+	-	+
Proteins	-	+	+
Steroids	+	-	+

+ (Present), ++ (Highly present), - (Absent)

Table 2: Phytochemical analysis in three extracts of *Myristica fragrans*

Ligand	Protein	Binding Residues	Binding Energy (kcal/mol)	Vdw_hb_ desolv_energy (kcal/mol)	Inhibition Constant	Ligand efficiency
3-O-Methyl-d-glucose	EGFR-1m17	LYS721, THR830, THR766, ASP831, GLU738(Hydrogen bonds)	-6.7	-5.06	10.5(mM)	0.21
Squalene	EGFR-1m17	LEU694, ALA719, LYS721, LEU820 (Hydrophobic bonds)	-5.93	-10.35	44.99 (uM) Old value 328.46(nM)	0.20
3-O-Methyl-d-glucose	MAPK	ALA35, LYS54, LYS151, ASP167, SER153 (Hydrogen bands)	-6.05	-4.47	31.29(mM)	0.16
Squalene	MAPK	ILE31, VAL39, ALA52, LEU156, CYS166, ALA189, LYS114, TYR113(Hydrophobic bonds)	-5.56	-9.97	84.71 (uM)	0.19

Table 3: Interaction and inhibitory property of phytochemical compounds (O-Methyl-d-glucose & Squalene) on oncogenic proteins (EGRF and MAPK)

RT	Name of the compound	Molecular formula	MW	Peak area (%)
3.13	Butane,1,1-diethoxy-3-methyl-	C9H20O2	160	0.69
4.49	Propane,1,1,3-triethoxy-	C9H20O3	176	0.16
5.95	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	C10H18O2	170	0.19
8.89	α-Cubebene	C15H24	204	0.21
10.05	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	C13H18O	190	0.76
10.97	Dodecanoic acid	C12H24O2	200	0.34
11.85	Megastigmatrienone	C13H18O	190	1.16
12.62	3-O-Methyl-d-glucose	C7H14O6	194	51.44
13.36	Tetradecanoic acid	C14H28O2	228	0.88
13.95	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo [4.1.0]heptan-3-ol	C14H22O2	222	0.85
14.13	10-Methyl-8-tetradecen-1-ol acetate	C17H32O2	268	2.80
14.40	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	1.38
16.14	n-Hexadecanoic acid	C16H32O2	256	6.17
16.44	Hexadecanoic acid, ethyl ester	C18H36O2	284	0.64
18.39	Phytol	C20H40O	296	4.47
18.71	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	0.87
18.80	9,12-Octadecadienoyl chloride (Z,Z)-	C18H31ClO	298	4.31
19.11	Octadecanoic acid	C18H36O2	284	1.15
22.13	Eicosanoic acid	C20H40O2	312	1.39
28.87	Squalene	C30H50	410	16.52
35.84	Vitamin E acetate	C31H52O3	472	3.13

Table 4: Phytochemicals identified in the Myristica fragrans