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In silico Analysis of Phytochemicals from Clove against Bronchitis

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Authors' contributions

This work was carried out in collaboration among all authors. Author S. Sahu designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AKS and S. Swain managed the analysis of the study. Author S. Swain managed the literature searches. Author DB read and approved the final manuscript supervised and guided the whole study. All authors read and approved the final manuscript.

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ABSTRACT

Bronchitis is an airborne disease, mostly caused by bacteria like *Mycoplasma pneumonia* and also by influenza virus. Due to hyper secretion and over production of mucus by goblet cells which leads to obstractle in airflow. This disease can be cured using (*Syzygium aromaticum*) clove extracts. Phytochemicals are secondary metabolites which are basically non-nutritive compounds but can protect from various diseases caused by microorganism. Bioactive compounds like Acetyl eugenol, Beta-caryophyllene and vanillin, crategolic acid, eugenin tannins, gallotannic acid, mrthyl salicylate (painkiller), flavonoids, kaempferol, rhamnetin, eugenitin, triterpenoid like oleanolic acid are found in clove extract. The enzyme and the phytochemicals were run using Biovia Discovery Studio and molecular docking was performed. By the help of -CDocker energy and -CDocker interaction energy the strength of the interaction was evaluated. The key enzyme involved in the biochemical pathway of mycoplasma pneumonia is Thymidine phosphorylase. High positive values for both the parameters indicated that out of different phytochemicals, Myricetin can interrupt the life cycle of Mycoplasma pneumonia spp. Effectively by deactivating the enzyme Thymidine phosphorylase (Pdb no 4LHM). The present study is to evaluate the action of phytochemicals present in clove (against brochitis diseases).

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1. INTRODUCTION

Bronchitis is an airborne disease caused by bacteria and virus. It is transmitted by saliva, skin to skin contact. Two types of bronchitis are present namely acute bronchitis and chronic bronchitis. Acute bronchitis is the most common bronchitis and it last for few week and it is does not cause much problem. Chronic bronchitis is the most serious bronchitis as it comes back again and again. In chronic obstructive pulmonary disease (COPD) chronic bronchitis is common. Due to hyper secretion and overproduction of mucus by goblet cells which leads to obstractle in airflow chronic bronchitis is caused. The major symptoms of these disease are shortness in breathing, decline in lung function, in smoker development of airflow obstruction. Chronic bronchitis is commonly found in smokers. Chronic bronchitis is usually caused due to mucociliary dysfunction, mucusproduction and hypersecretion [1].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source [2]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phyto chemicals, which can be used for therapeutic purpose. Plant based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds. Various medicinal plants and their phyto extracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Cloves (*Syzygium aromaticum*) is an aromatic herb and gives a pleasant smell. It is used to enhance the taste of certain dish like cakes. Clove is an English name that has been derived from the latin word 'nail' due to its external appearance. Cloves are used as a medicine and species since hundreds of year. Naturally this plant is grown in India, West Indies, Tanzania, Srilanka, Brazil and Madagascar. Both clove flower and clove oil are used since 2000 years by both Indian and Chinese as traditional medicine. It has the ability of storage of food without harming the food product when eaten [3]. Cloves can be categorized into three different forms i.e. oil, ground and whole. Oil has the highest potency than any other as it can be used by mixing with other oils like almonds. Whole cloves have the medium potency and ground cloves have the least potency. Oil is present in all the three different types of cloves but in different concentration. Cloves have antibacterial, antiviral, antifungal and antiseptic properties in them [4,5,6] and [7]. Due to the presence of eugenol is has the natural anaesthetic effect which are helpful in tooth ache.

Cloves have antimicrobial, chemo preventive, hepato protective activity, hepato- protective activity, antioxidant activity, antidiabetic activity, anti-inflammatory activity, anti-pyretic effect, anaesthetic activity, aphrodisiac, mosquito repellent, insecticidal activity [8]. Cloves are used for treating various health problems like cholera, digestive disease, cough, teeth robles, head ace, earache, nausea, hypertension and pains and burns caused due to wounds [9,10,11]. Clove can also reduce the sugar level of blood. They are helpful in problems caused due to respiration they are used as an air freshener, repellent for mosquitoes, killing of ants and as fly deterrents [12]. Manganese can be used as an important co-factor for antioxidant property for enzyme super oxide dismutase. The oil of clove is used in making toothpaste, Laxative pills and solution of clovacaine which can be used as a local anaesthetic oral ulceration and anti-inflammatory. For temporary filling clove oil is mixed with zinc oxide. In clove manganese can be found in highest concentration. It is used for metabolism, enzyme contribution, strengthening of bone and the value of ORAC antioxidant is high in clove.

In the modern world the pharmacologist have adapted modern chemical chemistry methods in the field of pharmaceutical research. Some of the modern medical chemistry methods includes molecular modelling which can be used for the

study of the structure activity relationship (SAR) [13]. The most frequently used structure based drug design (SSDB) [14] is used for analyzing the molecular structures according to their molecular interaction binding energies and conformational changes. Molecular docking method has been used to identify the phytochemical from the plant extract, which act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The present study aims to evaluate the action of phytochemicals present in clove (against bronchitis diseases.

2. MATERIALS AND METHODS

2.1 List of Phytochemicals Used for Docking

It has been established that clove belongs to the family Myrtaceae. It has the potential to cure the bronchitis disease. Eugenol is present in 72-90% of essential clove oil. Besides, Eugenol and other active constituents are present in the oil like Acetyl eugenol, Beta-caryophyllene and vanillin, crategolic acid, eugenin tannins, gallotannic acid, mrthyl salicylate (painkiller), Flavonoids, Kaempferol, Rhamnetin and eugenitin, triterpenoid like oleanolic acid. Other minerals are also present like manganese, iron, potassium, selenium, and magnesium [15] percent of essential oil is present in the buds of dried clove.

2.2 Enzyme Found in *Salmonella*

It has been reported that bronchitis can be caused by the microorganism like *Mycoplasma pneumonia, Streptococcus pneumonia, Haemophilus influenza, Moraxella catarrhalis* and *Haemophilu influenza* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *the above species*. It has been found that enzyme thymidine phosphorylase mycoplasma data base code 4HLM is very crucial for survival for particular microbe. Mycoplasma pneumonia spp is responsible for causing bronchitis and the enzyme thymidine phosphorylase mycoplasma is responsible for it. The phytochemical myrecetin cures the disease bronchitis by inhibiting the purine metabolism pathway belongs to Nucleotide and cofactor pathway categories in mycoplasma pneumonia.

2.3 Molecular Docking

The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first, the sdf files for the phytochemicals found in the *clove* plant were downloaded from the website (KEGG). The protein database code of the enzyme thymidine phosphorylase mycoplasma pneumonia i.e. 4HLM was identified from the website (BRENDA). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

By following literature review we had a conclusion that the phytochemicals present in clove were Acetyl eugenol, Beta-caryophyllene and vanillin, crategolic acid, eugenin tannins, gallotannic acid, mrthyl salicylate (painkiller), Flavonoids, Kaempferol, Rhamnetin and eugenitin, triterpenoid like oleanolic acid. The presence of these phytochemicals were studied using different phytochemical test including T.L.C.

Scientist Umesh kumar in the year 2010 studied about preliminary phytochemical screening for colour of clove oil Bromine test, Permanganate test, Phenol test was performed. For carbohydrate Molisch test and Fehling’s test was performed, For protein Biuret test and Million’s test was performed, For terpenoid test Liebermann Burchard test was performed,For Steroid and Sterol Salkowsky test and Libermann Burchard test was performed, For Glycosides test Killer-Kilain test Borntrager’s test and Legal test was performed for saponin test Alkanoids Dragendroff’s test and Wagner’s test was performed ,For flavonoid test Ammonia test was performed [16].

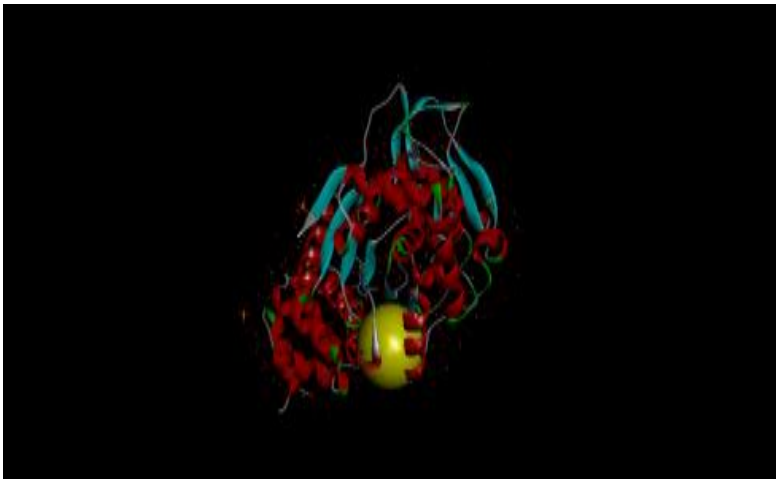

Fig. 1. Active site of Thymidine phosphorylase mycoplasma enzyme

Table 1. Results of CDocking of phytochemicals with Thymidine phosphorylase (receptor)

Sl No	Ligand	-C Docker energy	- C Docker interaction energy	Difference between - C Docker interaction energy and - C Docker energy
1	Beta-caryophyllene	-28.6615	14.5235	43.185
2	Compesterol 3-bet-d-glucoside	FAILED	FAILED	NA
3	Eugenol	8.98281	18.641	9.65819
4	Gallic acid	20.4152	16.4943	-3.9209
5	Kaempferol	16.0252	21.2015	5.1763
6	Myricetin	-22.6653	24.1964	46.8617
7	Oleanoid acid	FAILED	FAILED	NA
8	Rhamnetin	FAILED	FAILED	NA
9	Stigmasterol	FAILED	FAILED	NA
10	vanillin	15.2531	19.041	3.7879

Fig. 1 shows the active site of the thyamidine phosphorylase mycoplasma enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy [17,18].

3.2 Molecular Docking

Through molecular docking using biovia discovery studio we have found that thymidine phosphorylase interaction has the highest positive value of -CDOCKER energy 20.4152 and minimum value of the difference -3.9209 between - C DOCKER interaction energy and - C DOCKER energy followed by phytochemical Gallic acid. Thus the results indicated that phytochemical Gallic acid can effectively deactivate the thymidine phosphorylase mycoplasma enzyme thereby interrupting the biological cycle of Mycoplasma pneumonia spp. The higher values for Gallic acid phytochemical indicated that it was the most active ingredient against *thymidine phosphorylase mycoplasma pneumonia enzyme.* Kaempferol, Eugenol and vanillin also help in the de activation of the enzyme. On the other hand, Beta-caryophyllene, Myricetin, can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Compesterol 3-beta-d-glucoside, oleanoid acid, Rhamnetin, Stigmasterol cannot interact with thymidine phosphorylase enzyme. Thus, the key

phytochemicals preventing bronchitis caused by *thymidine phosphorylase* are Gallic acid, Kaempferol, Eugenol and vanillin.

4. CONCLUSION

It was previously known that *Clove* plant has medicinal action against bronchitis. *Mycoplasma pneumonia spp* Cause the bronchitis disease. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Beta-carophyllene, compesterol 3-beta-d-glucoside, eugenol, gallic acid, kaempferol, myricetin, oleanoid acid, Rhamnetin, stigmasterol, vanillin), which can have a significant interaction with the vital enzyme thyamidine phosphorylase of the microbe. It was found that Gallic acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Compesterol 3-beta-d-glucoside,oleanoid acid, Rhamnetin, Stigmasterol were found to be not interacting with the microbes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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