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Phytochemical screening and antimicrobial activity of Syzygium aromaticum

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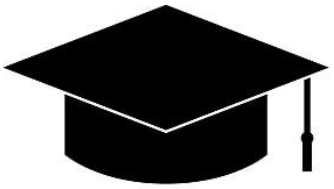

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

عظُم المراد َفهان الطريق

فجاءت لذة الوصول .. لُتمحي مشقةُ السنين

الحمد لله الذي ما تيقنُت به خيراً وأملاً

الا وأغرقني سروراً.

الاهداء

الى من يستحق الاهداء

الى اول من انتظر هذه اللحظات .. الى ذلك الرجل العظيم الذي اقف من اجله هنا ليفتخر ابي .. الى من أرى الدنيا بعينيه ، الى من اعطاني ولم يزل يعطيني بلا حدود ، الضوء الذي ينير حياتي

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الى ملاكي في الحياة .. الى التي كان دعاؤها سر نجاحي ، الى معنى الحب والحنان .. الى من رافقتني في كل اوقاتي .. بسمة الحياة وسر الوجود .. الى (أمي)

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ABSTRACT

Clove (Syzygium aromaticum) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. Clove is native of Indonesia but nowadays is cultured in several parts of the world including Brazil in the state of Bahia. This study talke about Phytochemical analysis of aqueous (water) and alcohol extract of fruit of S. aromaticum and show the presence of carbohydrates, glycosides, alkaloids tannin and resins and give (+) results ,saponin was absent give (-) results. Antibacterial activities of S. aromaticum showed significant bactericidal potential against both gram-positive and gram-negative . General the alcoholic extract was more activity than the aqueous extract, and the activity was increase with increasing concentration ,activity of aqueous extract on Streptococcus (29 mm) at concentration 29% , activity of alcoholic extract found on Streptococcus (40)at concentration 30%

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INTRODUCTION

Clove , *Syzygium aromaticum* , is one of the most valuable ancient and premium essential oil, belonging to family Myrtaceae.

."Clove" because of its resemblance with “nails”, derives its name from Latin and French terms clavus and clou respectively, both of which means "nail”

Cloves are dried and closed flower buds of an evergreen medium-sized tree about 10-20m tall with life span upto 100 years [1] . Syzygium aromaticum (S. aromaticum) (synonym: Eugenia cariophylata) commonly known as clove, is an median size tree from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region[2].

The clove tree is frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after 4 years of plantation. Flower buds are collected in the maturation phase before flowering. [3]

Nowadays, the larger producer countries of clove are Indonesia, India, Malaysia, Sri Lanka, Madagascar and Tanzania specially the Zanzibar island . In Brazil, clove is cultured in the northeast region, in the stat of Bahia in the regions of Valença, Ituberá, Taperoá, Camamu and Nilo Peçanha, where approximately 8000 hectares are cultivated, producing near 2500 tons per year[4-5].

Chemical compounds isolated from clove

Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9381.70 to 14650.00 mg per 100 g of fresh plant material[6].

With regard to the phenolic acids, gallic acid is the compound found in higher concentration (783.50 mg/100 g fresh weight). However, other gallic acid derivates as hidrolizable tannins are present in higher concentrations (2375.8 mg/100 g)[1].

Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations. Concentrations up to 18% of essential oil can be found in the clove flower buds. Roughly, 89% of the clove essential oil is eugenol and 5% to 15% is eugenol acetate [7]. Another important compound found in the essential oil of clove in concentrations up to 2.1% is a-humulen. Other volatile compounds present in lower concentrations in clove essential oil are ß-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate

PHARMACOLOGICAL ACTIVITY OF CLOVE

Antibacterial activity-

Several studies have demonstrated potent antibacterial effects of clove [8,9]. The inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate, β- caryophyllene, 2-heptanone, [6] acetyl-eugenol, a-humulene, methyl salicylate, iso-eugenol, methyl-eugenol, [9] phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnetin, myricetin, gallic acid, ellagic acid and oleanolic acid [10].

These compounds can denature proteins and react with cell membrane phospholipids, changing their permeability. Burst and Reinders 2003, found clove oil effective against non-toxigenic strains of E. coli 0157:H7 [11] . Similarly in another study clove oil was found to be active against food borne gram positive bacteria (S. aureus, B. cereus, E. faecalis, L. monocytogenes) and gram negative bacteria (E. coli, Y. enterocolitica, S. choleraesuis, P. aerugenosa) [8].

Demirpek et al, 2009 showed that aqueous and ethanolic extracts of clove buds inhibit growth of methicillin resistant clinical isolates at 1000 and 500mg/ml concentration [12]. The isolates were multi drug resistant, mostly against beta-lactams, aminoglycosides, tetracyclines, floroquinolones and macrolide antibiotics.

In another study eugenol at 2µg/mL inhibited growth of 31 strains of Helicobacter pylori, after 9 hours of incubation, which is being more potent than amoxicillin and doesn't devlelop resistance [13] .

Antifungal activity

Many studies have reported antifungal activity for clove oil and against yeasts and filamentous fungi, such as several food-borne fungal species [14] and human pathogenic fungi [15] Clove oil and eugenol have also been tested as antifungal agents in animal models [16]. The phenolic components of clove, carvacrol and eugenol, are known to possess fungicidal characteristics [17] including activity against fungi isolated from onychomycosis.In chromatographic analysis eugenol was found to be the main compound responsible for the antifungal activity, due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was also reported by Devi et al [18]. The large spectrum of fungicidal activity of clove oil and eugenol was reported on Candida, Aspergillus and dermatophytes and the mechanism of action was attributed to the lesions of the cytoplasmic membrane [14]. Burt proposed that different modes of action can be involved in the antifungal activity of essential oils.

Analgesic activity

Eugenol is a routine analgesic agent widely used in dental clinics due to its ability to alleviate tooth pain. Its anesthetic effects in dental pain as well as analgesic and anti-inflammatory effects in animal models have been well documented [19]. The effects have been attributed to its capability to suppress prostaglandins and other inflammatory mediators such as leukotriene. It is also believed to depress the sensory receptors involved in pain perception, [20] inhibits the conduction of action potential in sciatic nerves [21] and N-methyl-D-aspartate (NMDA) receptors but potentiates ionotropic y-aminobutyric acid (GABAA) receptors, which are both involved in pain sensitivity [22].

Anti-inflammatory activity

Clove oil clear respiratory passages, acting as an expectorant for treating many upper-respiratory conditions including colds, eye sties, bronchitis, sinus conditions, cough and asthma. One of the studies showed that the essential oil possess significant anti- inflammatory effect at doses of 0.05 ml/kg (90.15% inhibition) and 0.200 ml/kg (82.78% inhibition) [23].

Clove has been used in traditional public medicine to relieve nasal obstruction and musculoskeletal pain which implies its anti-inflammatory activity and the activity is due to COX-2 inhibition [24].

The aromatic oil, when inhaled, can help relieve certain respiratory conditions like coughs, colds, asthma, bronchitis and sinusitis. Clove also contains a variety of flavonoids including kaempferol, rhamnetin and ß- caryophyllene which also contributed to its anti-inflammatory and antioxidant properties [25]. Eugenol (200 and 400 mg/kg) was also found to reduce the volume of pleural exudates without changing the total blood leukocyte count indicating its anti- inflammatory potential [26].

Pain in muscle cramps and some nerve conditions can also be relieved by using clove oil [27].

An active and major component of clove oil is Eugenol, a phenylpropanoid. It is characterized as a pale-yellow liquid having solubility in water but more soluble in organic solvents such as methanol and ethanol It has specific gravity of 1.531-1.054 [28].

Oil extracted from different parts of clove plant possesses different quantities of eugenol. 60-90% eugenol is present in bud oil, 82- 88% in leaf oil whereas 90-95% eugenol is present in stem oil [29].

Eugenol possess a wide range of properties such as antimicrobial, antifungal, anti-carcinogenic, anti-oxidant, antibacterial, antiviral as well as anti- mutagenic and anti-inflammatory [29] .

Clove oil is colorless or pale yellow with a distinct clove flavor and taste. The differences in CEO content and composition depend mainly on sever- al factors like pre-treatments, variety, agro-eco- logical conditions, and extraction processes [30].

MATERIALS and METHODS

1- Preparation of extracts

1- A- Aqueous plant extract

A conical flask containing (10 g) of the plant's powder that dried in the air was used, added 250 ml of distilled water and the mixture was boiled for 2 hours, then filtered with muslin cloth 8 layers of muslin cloth ,after that centrifuged at 5000 RPM for ten minutes. Collected the filtrate] and concentrated in the oven (45oc) until it was wholly dry. The dry extract was preserved at (4oc) in order to be used later [31].

1- B- Alcoholic plant extract

10 grams of the powdered plant was added to 200 ml of ethanol alcohol (70%) in a flask of 250 ml., cover the flask by cotton plug and shaken about (140–220) RPM for (24) hours, Filtered this mixture by muslin cloth (8 layers) and put in a centrifuge at 5000 RPM for about 10 min. Then collected the supernatants. Concentrate the extract at oven 45^{o}c) until it was completely dry. The dry extract was preserved at (4oc) in order to be used later[31] .

2- Phytochemicals Qualitative tests:-

2- A [Test of alkaloids]

The reagent of tannic acid

2 ml from 10% Tannic acid solution was added to 5 ml from extract, Alkaloids give orange color precipitate [32]

2-B [Test for Phenolic Compounds (Iodine Test)]

To 1ml extract, added few drops of dil. Iodinesolution. Presence of transient red colourindicates phenolic compound presence [33].

2-C [Test of flavonoids]

Mix (10) gm of the dried plant in approximately 50 ml of (95%)ethanol solution, later nominatedthe mixture via A. 10 ml of (50 %) ethanol solution and mix with about (10) ml of Potassium Hydroxide (50%) and nominated B. An equal parts of solutions A & B should be combined; the emergence of yellow is a sign of Flavonoids[34]

2-D [Test of coumarine]

An amount of 2 mg of the extracts was dissolved in 2 mL of methanol, and 10% NaOH was added dropwise. The test
was considered positive if a yellow coloration was present and if it disappeared when the solution was acidified. [35]

2-E [Saponin Test] We add 5 ml of aqueous extracts and alcoholic extracts in tube and then we make rapid shaking.. Formation of 2cm thick layer of foam. [36].

2- F [Resin test]

1mL plant extract + Acetic anhydride solution + 1mL conc.H2SO4. considered positive if Orange to yellow was present . [37]

Antimicrobial activity assay

Syzygium water and alcoholic extracts was investigated separately on Brain- Heart infusion Agar (BHIA) medium using well-agar diffusion method. The study was performed using 18- 20hrs bacterial culture of*Streptococcus* , *E. coli* and the fungi *C. albicans*.

Agar well diffusion method

The aqueous and alcoholic extracts of were tested at various concentrations (10%,20%, and 30%).

A nutrient broth was inoculated with loop full growth from each isolate and incubated at 37 °C for 18 hours. Diluted bacterial suspensions in normal saline equal to the McFarland number 0.5, to obtain a uniform suspension with cell density of1.5×108 CFU / ml were used. Petri dishes prepared with Brain-Heart infusion Agar (BHA), the microorganisms isolates were cultured on the media by streaking with sterile swab (for all tested microbes), then created five wells of 6 mm diameters in agar medium utilizing sterile borer and filled with 100µl of every concentration for each extract using sterile micropipette, while the well made in the center contained the control.

Then, the plates were incubated at 37°C for 24 hrs. Gentamicin (5µg) was utilized as a positive control for detection the antibacterial activity,

whereas *fluconazole* (25µg) was considered as a positive control for antifungal activity

Distilled water was considered as a negative control for antimicrobial activities. After incubation, the antimicrobial activity was estimated by measuring the inhibition zone diameter that expressed in(mm) around the well. The assessment was replicated three times and the mean diameter was considered [38] .

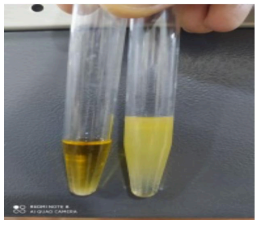
Results and Discussion

Phytochemical analysis

Phytochemical analysis of aqueous (water) and alcohol extract of fruit of *S. aromaticum* displayed the presence of carbohydrates, glycosides, alkaloids tannin and resins ("+" for the presence) while saponin was absent ("-" for the absence) among all the extracts. Similar findings have also been reported by other research workers (39, 40,). The presence/ absence of various phytochemicals indifferent extracts are enlisted in table-1.

The phytochemicals classes reported in the aqueous extracts of Streptococcus have been found to be associated with the antidiarrheal, anti inflammatory, antimicrobial, insecticidal and antioxidant activity (41).

Table 1 (Phytochemical screening of extracts)

picture	Test color	Alcoholic Extracts	Aqueous Extracts	Test Name
	turbidity	+	+	Alkaloids
	Blue- black	+	+	phenols
	turbidity	+	+	Resin Test
	Dark yallow	+	+	Flavonoid Test
	No foam	_	_	Saponin Test
	Bluish green	+	+	coumarin Test

Antibacterial activities

Antibacterial activities of fruit extract in different solvents (aqueous and alcohol) were tested against selected pathogenic bacteria and their zones of inhibition were recorded (table-2). All thefruit extracts of S. aromaticum showed significant bactericidal potential against both gram-positive (Streptococcus) and gram-negative(Escherichia coli) in addition to (Candida). A dose dependent bactericidal activity was noticed in all the fruit extracts. From the results obtained, aqueous (water) extract of fruit displayed the most activity on Streptococcus (29 mm) at concentration 29%, also the most activity of alcoholic extract found on Streptococcus (40 mm) at concentration 30%, in general the alcoholic extrat was more activity than the aqueous extract, and the activity was increase with increasing concentration. Similar findings have also been reported by other research workers (39,40).

S. cumini is a species with widespread use in folk medicine to treat skin diseases and dysentery and improve healing processes [42].

Previous studies documented antimicrobial activity of the seed extract and fruit pulp [43].

The antibacterial effect observed against *E. coli* can be attributed to a synergistic action of the constituents.

Other researches have registered inhibitory and bactericidal activity of the EOSC against *S. aureus* [44], the species was also susceptible to the extract of the seeds of *S. cumini* [45].

According to established techniques, the dried clove was examined for phytochemical and antibacterial properties against pathogenic bacteria and fungi in this study. The qualitative chemical exams and tests for the detection of primary and secondary metabolites were all part of the phytochemical screening process.

These metabolites are claimed to be beneficial to the plant itself, but they can be harmful to other creatures, including humans, according to some sources. The existence of these chemical components in this plant is a signal that, ifadequately screened, the plant has the potential to produce medicines of medicinal value.

A stronger argument for this is provided by the fact that members of the plant's family have been identified as being associated with ethnomedicine in the treatment of a variety of illnesses. Because of the wide range of active phytochemical components in this plant, it has been shown to have therapeutic qualities [46,47,48]

Table-2 (The inhibitory antibacterial effect of aqueousand alcohol against different t microorganism)

flu (mcg)25	GN (mcg) 10		Alcohol			Aqueous		Microorganisms
		30%	20%	10%	30%	20%	10%	
-	13	40	35	30	29	23	20	streptococcus
-	19	19	18	16	0	0	0	E.coli
11	-	30	28	27	0	0	0	C.albicans

Conclusion

Based on the information presented, it could be concluded that clove represents a very interesting and can used as Antibacterial , Antifungal , Analgesic , Anti-inflammatory . It's proved biological activities suggest the development of medicinal products for human and animals uses and confirm why this plant has been employed for centuries

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