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Chemical Composition and Anti-bacterial Effects of Clove (*Syzygium aromaticum*) Flowers

Khalid Abdul Kreem Mohammed^{1*}, H. Mustafa Abdulkadhim² and Sawsan I. Noori¹

¹Chemistry Department, College of Sciences, University of Baghdad, Baghdad, Iraq

²Chemistry Department, College of Sciences, University of Kufa, Iraq

*Corresponding author

ABSTRACT

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The flower of *Syzygium aromaticum* (clove), a common food flavor, has been used as indigenous medicine for the treatment of several diseases in Asian countries. Safety assessment of *S. aromaticum* flower bud (clove) extract with respect to antimicrobial activity against two bacterial isolates. Moreover, the main work was characterizing the chemical components if alcoholic extract of *S. aromaticum* flower bud (clove) in terms of detection of Nitrogen, Sulfur, Halogens, Phenol, aldehydes, ketones and anthraquinones detection. Also Fourier Transform Infrared Spectroscopy (FTIR) and Ultraviolet (UV) spectrum analysis were used for attributing the extract. The result showed that the main composition of the extract was ketones and anthraquinones, the last one was responsible for biological activity of the extract. Furthermore, FTIR results showed morethan eight peaks for represent the main fine composition of the extract. UV spectrum showed that the main absorption at 224, 268 and 348 nm due to the electronic transition from the type $n-\pi$ for group of alcohol that bind with aromatic ring, $n-\pi$ of group carbonyl bind with aromatic ring of quinone and $\pi-\pi$ of the group of ketone that bind with aromatic ring. The present study also showed that the extract has antibacterial activity against two bacterial isolates *Bacillus* and *Serratia marcescens*.

Introduction

Syzygium aromaticum (*S. aromaticum*) (synonym: *Eugenia caryophyllata*) commonly known as clove, is a median size tree. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region and agricultures in Asiatic region and it is used in different field of common life that is why playing a vital role in common life of different populations of different countries (1).

Clove is one of the major vegetal sources of phenolic compounds such as flavonoids, *hidroxibenzoic* acids, *hidroxicinamic* acids and *hidroxiphenyl* propens. The main bioactive compound of clove Eugenol is, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material (2). The phenolic acids and gallic acid are the compounds found in higher concentration. However, other gallic

acid derivatives as hydrolyzable tannins are present in higher concentrations as compared with other compounds (3). Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its group (glycosylated) are also found in clove but 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 and β -cariofileno (4). Another important compound found in the essential oil of clove in concentrations up to 2.1% is α -humulene. Other volatile compounds present in lower concentrations in clove essential oil are β -pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate.

This plant has been used for centuries as food preservatives and as medicinal plants mainly as antioxidant agents and has antimicrobial activities. Recently, many reports confirmed the antibacterial, antifungal, antiviral and anticarcinogenic properties of this plant. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (3). In current study, we focused on analysis of the chemical composition of the extract of flower of plant by Fourier Transform Infrared Spectroscopy (FTIR) analysis and UV spectrophotometer

Materials and Methods

Preparation of Clove Extraction

The standard method of Barre et al. was followed to prepare the Clove extraction. In this method 5 gm of clove leaves was put in thumble flask and put in Soxhlet the extracted was done by ethanol for 25 h. after that the extracted was separated from oil and stored for further experiments (5).

Isolation of Clove (*Syzygium aromaticum*) Compounds

Thin layer chromatography was used to isolate and separate the Clove (*Syzygium aromaticum*) compounds. In this technology silica gel (200*200*2 mm) plates (Merk) was used. Butand–Ethanal-Acetic acid (5:4:1) was used as a solvent (6).

Identification of Active Group of Clove Extract

The active groups in clove extract were detected by TLC technology. The spots was collected from gel and desolved in ethanol. The chemical composition was identified in inorganic chemistry in department of chemistry, college of education, University of Basarh, Iraq (7).

Sodium Fusible

Drops of clove extract were added to small piece of sodium in test tube. The mixture was heated to be red. Many drops of ethanol were added to cooled mixture. The mixture was heated again to deplete sodium and 4-5 drops of distilled water was added to cooled mixture. The mixture was boiled again and filtrated. The filtrated solution was used for the following experiment:

Nitrogen Detection

Drops of extract were put in test tube contained 0.2 gm of ferric sulfate. The mixture was boiled to convert the Fe ++ to Fe +++ after that diluted H₂SO₄ was added to produce the blue complex.

Sulfur Detection

Acidification of extract with acetic acid and mixed with few drops of lead acetate to produce black precipitate of Lead sulfide.

Halogens Detection

Acidification of the extract with few drops of HNO₃ and mixed with silver nitrate to produce yellow precipitate.

Phenol Detection

In test tube, 2 ml of the extract was mixed with few drops of Ferric chloride solution to produce purple solution.

Detection of Aldehydes and Ketones

The carbonyl group was detected by Brady's reagent (2,4-Dinitrophenylhydrazine) and alpha and aroma aldehydes group were detected by Tollen's reagent. In test tube, 2 ml of the extract was mixed with few drops of Brady's reagent after mixing the the appearing of black precipitate indication of positive results of presence of carbonyl group. Similarly, 2 ml of the extract was mixed with few drops of Fehling's reagent and boiled the mixture for 2 minutes, red precipitate indication of aroma aldehydes. The detection of ketones was done by mixing 1 ml of plant extract with few drops of sodium nitroprussid to produce red solution (6).

Anthraquinones Detection

Drops of the extract were mixed with drops of diluted sodium hydroxide with contentious agitation. The color of solution should be changed from brown to black because the oxidation effect in case of positive result (8).

Fourier Transform Infrared Spectroscopy (FTIR) and Ultraviolet (UV) Spectrum Analysis

FTIR (FT-IR-Prestige spectrophotometer) and US shimadzu spectrum

(shimadzu visible UV) analysis were done in the laboratories of College of Science, University of Kufa, Najaf, Iraq.

Antibacterial Activity of Supernatant of Extract

The antibacterial activity of the extract was done on two Isolates of bacteria, *Bacillus* and *Serratia marcescens*. Both of isolates were cultured into Muller Hinton broth prior to experiment (9). The disc diffusion method was used to assessment the antibacterial activity of the extract against *Bacillus* and *Serratia marcescens*. Briefly, filter paper of Whatman no1 was cut to circles with 0.5 cm in diameter and then sterile by autoclave. The piece of papers were dried and submerged in different dilution of the extract (1, 1/2, 1/3; V/V). after overnight the paper s were dried by oven at 37 °C for 20 min. Each bacterial isolate was spread onto Muller Hinton agar and left upright for 2 h. The prepared papers were put onto plates and incubated at 37 °C for overnight. The results were observed by clear zone around the discs (8).

Results and Discussion

Extraction, Separation and Purification of Chemical Compounds from Clove (*Syzygium aromaticum*) Flowers

The extraction was done by Soxhlet with ethanol. By this method the oil components were depleted. In extraction it was observed white pellet, after extraction the yielded extract was oil free. The final extract was run by TLC. By the last technology, many spots were observed and the spots were scratched and the chemical components were extracted by ethanol. Finally, it was gained the alcoholic extraction of clove that used in further experiments (9). Several previous studies focused on the extraction of

different compounds from clove (*Syzygium aromaticum*) flowers and used in different fields of medicine. They found that the extract of clove (*Syzygium aromaticum*)

composed from wide spectrum of chemical components and most of them were safe (10).

Table.1 Inhibition Zone that Produced by Alcoholic Extract of Clove (*Syzygium aromaticum*) Flower against *Bacillus* and *Serratia marcescens* Isolates

Bacteria	Dilution of the extract			
	1	1/2	1/3	Control
<i>Serratia marcescens</i>	18.3 mm	12.4 mm	9 mm	0 mm
<i>Bacillus</i>	14.9 mm	11.6 mm	8 mm	0 mm

Fig.1 Ftir Analysis of Alcoholic Extract of Clove (*Syzygium aromaticum*) Flower. Ftir Analysis of the Extract Showed more than 8 Peaks of Bands

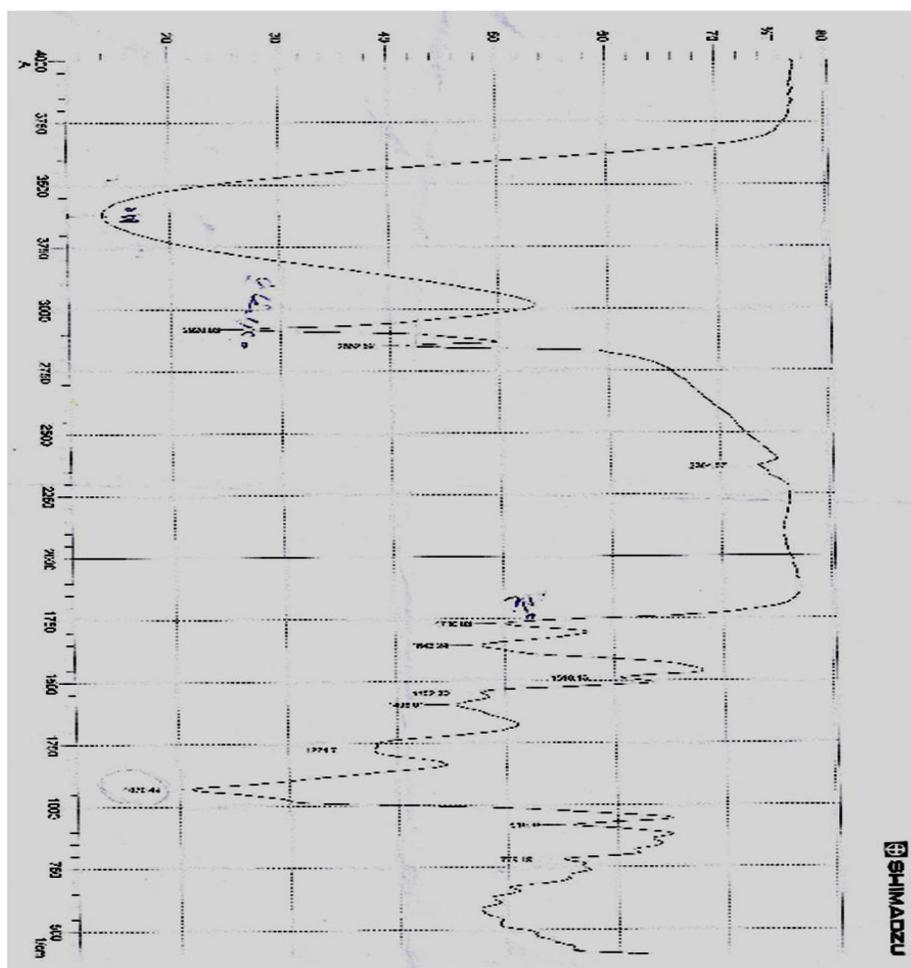
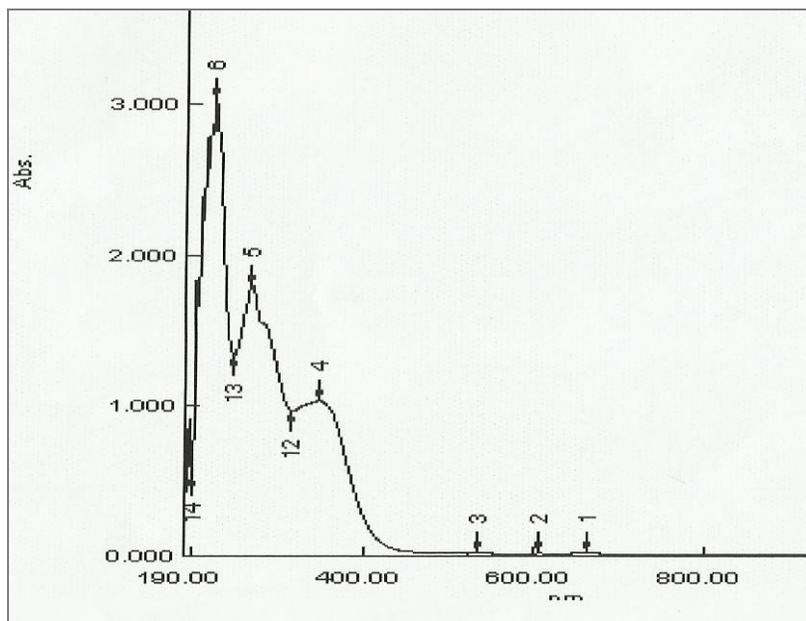


Fig.2 UV Spectrum Analysis of Alcoholic Extract of Clove (*Syzygium aromaticum*) Flower



Identification of Alcoholic Extract of Clove (*Syzygium aromaticum*) Flower

The identification tests of the extract gave good and clear results. Identification tests for Sulfur, Halogens, Phenolic compounds and aldehydes gave negative results. These are going on with results of previous study (11). However, ketones detection test gave positive result by giving red solution in the end of reaction. The anthraquinones detection test is the highly valuable test because we did not find any researcher used this test in spite of the role of anthraquinones in biological activity (antibacterial activity) in alcoholic extract of clove (*Syzygium aromaticum*) flower. In present study this test gave positive results because the oxidation process (8,12).

FTIR Analysis

The results of FTIR analysis showed that there is a clear and high intensive band (3375 /cm) which represent OH groups of alcoholic extract. The moderate band (2920-2852 /cm) that represents frequency

asymmetrical patterns of group CH₂ and CH₃ of alcoholic compound. Another band (1730 /cm) represented frequency patterns of ester group C-O or aromatic ketone group C=O which combine with more than ring. The band 1643/cm which is also the patterns match the frequency of the aromatic carbonyl group belonging to quinine (13) which nearby and similar to aromatic group (C=C). The bands at 1452/cm and 1402 /cm represents the frequency of pattern of group CH₂- and other strong band (1070 /cm) which represents frequency of pattern of group C-O. Another moderate bands at 779 /cm and 919 /cm that represents the frequency of pattern of groups CH₂ and C=C, respectively (14).

UV Spectrum Analysis

Fig 2 showed results of UV spectrum analysis for alcoholic extract of clove (*Syzygium aromaticum*) flower. The absorption at 224 nm due to the electronic transition from the type *n-π for group of alcohol that bind with aromatic ring and absorption at 268 nm is due to electronic

move of type $n-\pi$ of group carbonyl bind with aromatic ring of quinone which reach up to 350 nm and give the yellow color of these compounds. Absorption at 348 nm is belonging to the electronic moving of the type $\pi-\pi$ of the group of ketone that bind with aromatic ring (15).

Antibacterial Activity of Alcoholic Extract of Clove (*Syzygium aromaticum*) Flower

Table 1 showed the antibacterial activity of alcoholic extract of clove (*Syzygium aromaticum*) flower against two bacterial isolates. The results showed that the extract reduced the bacterial growth and the diameter of clear zone related positively with the concentration of the extract. That is why the lowest diameter was observed in case of the highest dilution of extract (1/3). The extract showed highly effect on bacterial growth that because the presence of active groups of quinone in the extract. That play important role in the inhibition process to the content of the carbonyl group of atoms oxygen that the presence of the duples of electronic that react or link with enzymes of active groups in the body of the host, which lead to the inhibition of some important enzymes, as well to what the duty of ketones group (16).

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