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# CYTOTOXIC, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF CRUDE EXTRACT OF *SYZYGIUM AROMATICUM* PLANT

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## Abstract

Antimicrobial, cytotoxic and antioxidant effects of alcoholic extract from the clove plant were studied. The antimicrobial activity was investigated on the growth of three types of pathogenic microbes; two types of Gram –ve bacteria, namely *E. coli* and *P. aeruginosa,* and one Gram +ve yeast, namely *C.albicans*. The results showed no effect for the plants extract on the growth of the yeast, while different effects on bacteria were observed. The highest effects were recorded on the growth of *E. coli* with the diameter of zone of inhibition reached to 22 mm, followed by *P. aeruginosa* with the diameter reached to 18mm. The results also demonstrated that treatment with *S. aromaticum* extract significantly inhibited the growth of cells (MCF-7 cell line) in a concentration dépendent manner. The results indicate that *S.aromaticum* can be considered as a particularly valuable source of effective antiproliferative and cytotoxic agents, also the extract have higher antioxidant activity.
***Key word*:** Antimicrobial activity; Antioxidant activities; Crud extract; Cytotoxicity; *Syzygium aromaticum*.

## Introduction

Herbs have been used since ancient times to improve the flavor and aroma of foods as well as maintain their nutritional value. The majority of spices were initially indigenous to the tropics e.g. cinnamon, pepper, clove and nutmeg (Viuda-Martos, 2007). *S. aromaticum* (Clove bud) is one of the most ancient and valuable spices of the orient (Chaieb, 2007) (Figure 1). In addition, traditional herbal medicines are of great importance in developing countries and 80 percent of the population depends on them for their primary health care needs (Garg, 2011). These compounds are known as secondary metabolites and have biological activities such as modulating detoxification enzymes, promptthe immune system, reducing the aggregation of platelet, modulating the metabolism of hormone, as well as antioxidant, antimicrobial, and anti-cancer activities (Saidulu, 2014). Phytochemicals contain terpenoids, phenolics, and alkaloids (Dillard, 2000).

Cancer is a malignant disease characterized by abnormal division and differentiation of cells, which leads to a large increase in the number of dividing cells that later collect to produce the tumor, or spread to the rest of the body tissue by blood or lymph. The cancer cell feeds itself through a method called the angiogenesis process in which the growth of a network of blood vessels will raise creation of angiogenesis activators and reduce production of angiogenesis inhibitors (Deshmukh, 2011). This research aimed is to detected the antimicrobial, antioxidant, and cytotoxicity activities for the crude extractof *S. aromaticum*.

**Fig. 1 :** *Syzygium aromaticum* plant.

## Materials and Methods

### Plant Materials

### Preparation of extract

Dried *S.aromaticum* were extracted by the soxhlet with ethanol 70%. The extracts were completely removed by using a rotary evaporator to obtain a semi-solid mass and then transferred to an oven to produce the crude extract. Fractions were stored at 4 ºC until assayed.

### Phytochemical Screening of *S. aromaticum* Extract

Phytochemical constituents such as tannins, saponins, phlobatanins, phenolics, reducing sugar, trepenoid,

steroid, glycosides, alkanoids and flavonoids of the crude extracts were analyzed. *S. aromaticum* extracts (2 ml each) were utilized separately for each analysis, in a way that formation of precipitate, color change, or frothing indicates presences of the phytochemicals mentioned above (Sofowora,1993).

**FTIR assay**

Fourier transmission infra-red (FTIR), and UV Spectrum (Shimadzu) analyses were performed in the Lab of IbenSena center/ University of Baghdad, Iraq.

**Antioxidant assay:DPPH assay**

This activity for crud extracts of *S.aromaticum* was measured using stablediphenyl-1- picrylhydrazyl DPPH radicals with minor adjustments as previously described(Tailor,2014). Ethanol extracts of *S.aromaticum* were used to investigate the scavenging activity. Every samples was treated with diphenyl-1-picrylhydrazyl DPPH and the solution was brought up to 1mL by ethanol. The decline in absorbance was measured at 517 nm. Scavenging activity computed according to the equation formula:

$$\text{DPPH Scavenging Activity}\,(\%) = (\text{AC} - \text{AS} / \text{AC}) \times 100$$

Ac, and As that refers to the absorbance values of diphenyl-1-picrylhydrazyl and test sample solvent, respectively.

**Bacterial preparation**

The *E. coli, P. aeuroginosa*, and *C. albicans* isolates were taken from isolated specimens. Isolates were cultured overnight at 18–24 h and 37 ºC on Nutrient broth for the preparation of cell suspensions that were adjusted to 0.5 McFarland standards $5 \times 10^5$ CFU/mL.

**Antimicrobial susceptibility assays**

Antimicrobial activity of *S. aromaticum* extract was investigated by the well diffusion method against two types from Gram –ve *E.coli*, and *P. aeruginosa* and yeast *C. albicans* Gram +ve (MHA and SDA agar, Oxide respectively. Crud extract were dissolved with 10% DMSO, Then a hole with a diameter of 6 mm full with 100, 80, 60 and 20 µg $mL^{-1}$ of crud ,a blank well was carried by adding solvent alone (10% DMSO) to act as -ve control. After incubation period under 37 °C for 24 hr. zones of growth inhibition were measured. All of the experiments were conducted in triplicate. The results are reported as the average of three experiments (Bauer, 1966).

**Radical Scavenging Activity**

**Detection of the Toxicity: Maintenance of Cell Cultures**

Cell line of Lung cancer MCF-7 cell line, from the unit of Cell Bank of Iraq biotech ,Saved RPMI-1640 Completed by 10% from fetal bovine, 100 units/mL of both penicillin, and streptomycin antibiotic's . Cells were passaged by Trypsin-EDT Are-seeded at 50% confluence for two week at 37 °C (Al-Shammari, 2015).

**Cytotoxicity Assays**

Cytotoxic effects were determined by the MTT test. Cell lines were cultured at ($1 \times 10^4$ cells) well. After 24 hrs, the monolayer was achieved, and cells were treated with tested compound. Viability of the cell was measured after 72 hrs. by removing the medium and adding 28 µL of 2 mg/mL solution of MTT and incubating cells for 1.5 hr. at 37 °C. After that the MTT solution was eliminated, and solubilized the crystals that remaining in the wells by the addition of (130 µL) of DMSO followed by incubation at 37 °C for 15 min with shaking (Al-Shammari, 2016). The absorbency was determined on a micro plate reader at 492 nm. The percentage of cytotoxicity was determination as the following equation:-

**Inhibition rate = A-B/A *100**

Where A and B are the optical density of control and the optical density of test.

**Statistical Analysis**

Results are reported as mean ± SD of three independent replicates. Statistical analysis of data was carried out by computer using SPSS version 11.5 software. Level of significant was assessed by using the Analysis of Variance ANOVA test. The level of significance was shown using the LSD test.

## Result and Discussion

Preliminary Phytochemical Screening of *S. aromaticum* extract Based on the preliminary screening, phlobatannins, reducing sugar and steroids were absent in Ethanolic extract of *S. aromaticum*. Color change such as dark green color indicates presence of tannins and phenolics, reddish brown interface (terpenoids and glycosides), greenish (steroids), yellow to colorless (flavonoids) respectively. Formation of precipitate such as red precipitate indicates presences of phlobatannins or reducing sugar, yellow or reddish brown (alkanoids) and yellow precipitate (flavonoids) while persistence of frothing indicates presences of saponins (Table 1).

**Table 1 :** Preliminary Phytochemical Screening of *S. aromaticum* extract

| Types of Active compound | Presence |
|---|---|
| Tannins | +ve |
| Saponins | +ve |
| Phenolic compounds | +ve |
| Reducing sugar | -ve |
| Terpenoids | +ve |
| Steroids | -ve |
| Glucosides | +ve |
| Alkaloids Wagner's test | +ve |
| Flavonoids Ferric chloride test | +ve |
| Lead acetate test | -ve |

**FTIR Assay**

Results in (Figure. 2) showed that the high severe, band 3375 /cm which signify refer to (OH) groups, also band 2920- 2852 /cm refers to the incidence of asymmetrical patterns to the $CH_2$ and $CH_3$ groups of alcoholic composite. 1730 /cm band referred to incidence patterns of the ester C-O group or aromatic ketone C=O group. 1643/cm band which incidence of the aromatic carbonyl group belonging to quinine (Adorjan, 2010). 1452/cm and 1402 /cm bands refer to the incidence of $CH_2$-group, 1070 /cm band represents incidence of C-O group. 779 /cm, and 919/cm bands that represents to the incidence of pattern $CH_2$ and C=C groups, respectively (Whittaker, 2000).

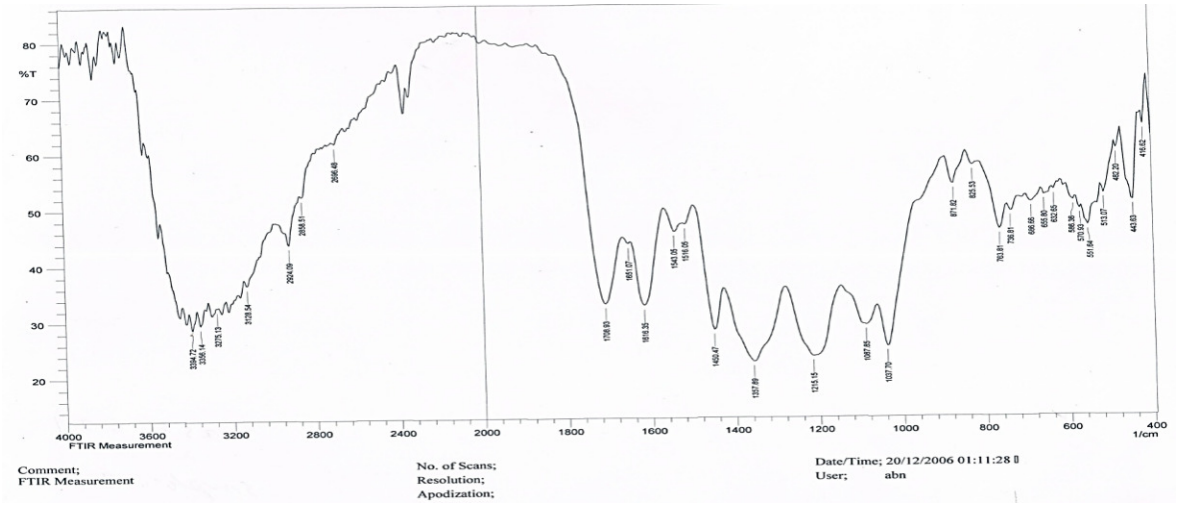


**Fig. 2 :** FTIR analysis of *S. aromaticum* crud extract

**Evaluation the Antimicrobial activity**

The effect of the alcoholic extract of the clove plant was studied on the growth of three types of pathogenic microbes two types of (Gram –ve) bacteria such as *E. coli* and *P. aeruginosa* and one (Gram +ve) *C. albicans*. There's no effect for the extract on the growth of the Yeast but its effect varied on the bacteria. The highest effect observed on the growth of *E. coli* with a diameter of zone of inhibition that reached to (22.33mm ± 0.58), followed by *P. aeruginosa* with a diameter reached to (18mm ± 0.58), finally in yeast *C. albicans* by inhibition zone reached to (8.67±1.15) (Figure. 3 and Table 2).

*S. aromaticum* constituants such as alkaloids, saponins, tannins and flavonoids obtained in this research are known to have healing activities against a number of pathogens. Therefore, the essential oil and crude extracts are hereby suggested for treatment of diseases caused *by E. coli, P. aeruginosa* and *C. Albicanss* (Usman, 2007). The inhibitory activity exhibited by *S. aromaticum* oil against the test organisms in this research might be due to the presence of several constituents such as eugenol, beta-caryophyllene, limonene, and alphaterpinolene (Chaieb, 2007). Other possible effective constituents include acetyl eugenol, methyl salicylate, iso-eugenol, methyl-eugenol, phenylacetic acid, salicyclic acid, protocatechuic acid, p-hydroxybenzoic acid, eugenin, and eugenitin (Yang, 2003). The present study detected phenolic compounds (kaempferol, rhamnetin, isorhamnetin, myricetin, quercetin, gallic acid, caffeic acid and syringic acid; (Table 1) that can also have such antimicrobial activities (Cai,1996). The broad spectrum of antimicrobial activity exhibited by *S. aromaticum* oil and the crude extract agrees with previous reports (Park, 2007 and Fu, 2007) that reported potent antifungal and antibacterial effects of *S. aromaticum* on

microorganisms due to its mechanism of action which include denaturation of proteins and reaction with cell membrane phospholipids, thus changing the membrane permeability of the microorganism.

**Table 2 :** The effect of crud extract against some pathogenic microbes each value represents the mean ± SD of 3trials

| Microorganisms | Concentrations µg / ml | | | |
|---|---|---|---|---|
| | 20 | 60 | 80 | 100 |
| *E. coli* | 14.33±0.58 | 16.33±0.58 | 20.33±0.58 | 22.33±0.58 |
| *P. aeruginosa* | 6.67±0.58 | 7.76±1.53 | 9.33±1.53 | 8.67±1.15 |
| *C. albicans* | 11.33±0.58 | 14.67±0,58 | 16.33±0,58 | 18.33±0.58 |

The present results confirm the effectiveness of *S. aromaticum* oil and crude extract against test organisms at varying inhibitory concentrations compared to antibacterial and antifungal drugs used as control. These findings justify the ethno-medicinal uses of the plant and, thus, represent an alternative source of natural antimicrobial substances for use in pharmaceutical industries and food system to prevent the growth of pathogenic bacteria and extend the shelf-life of the processed food. *S. aromaticum* constituents, such as alkaloids, saponins, tannins and flavonoids, obtained in this research are known to have curative activity against several pathogens thus the essential oil and crude extract are hereby recommended for treatment of diseases caused by *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*s since some of these organisms are known to play a vital role in invasive skin diseases, including superficial and deep follicular lesion (Usman, 2007).

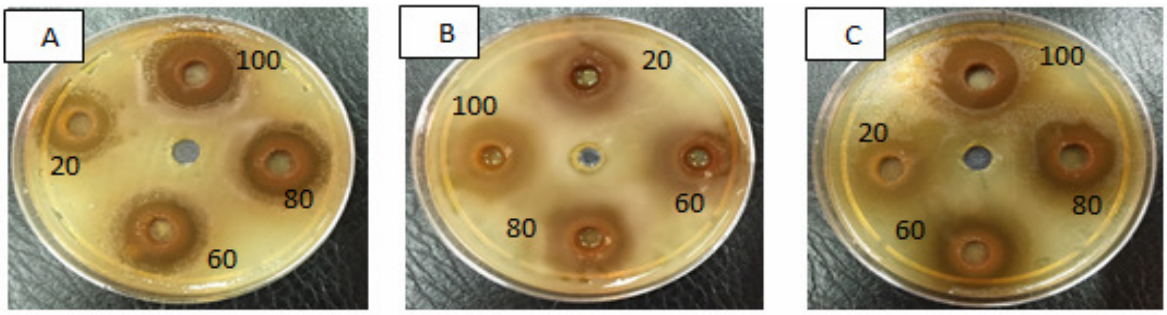


**Fig. 3 :** Antimicrobial activity for the *S. aromaticum* crud extract against (A) *E. coli*, (B) *C. albicans* and (C) *P. aeruginosa.*

**Antioxidant activity**

The antioxidant activity of clove extract in comparison with quercetin as a scavenger of the DPPH+ radical due to reduction in these radicals (Figure. 4) which shows free radical scavenging characteristics exhibited by *S. aromaticum* oil and extract have higher antioxidant activity compared with control thus indicates its ability to interact and neutralize free radicals, thus preventing them from causing damage. This implies that *S. aromaticum* can be used as dietary supplements for the prevention of diseases. Antioxidants also have many industrial uses (Dabelstein, 2007).

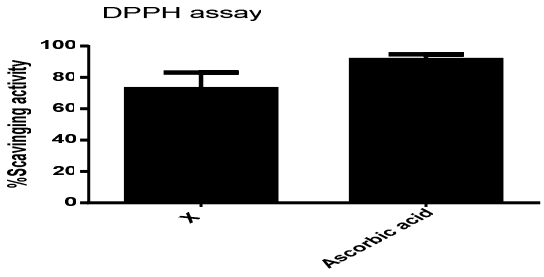


**Fig. 4 :** The antioxidant activity of *S. aromaticum* plant

**Anticancer activity of *S.aromaticum***

The cytotoxic activity was examined as shown in (Figure. 5). The results illustrated that treatment of the cells with *S.aromaticum* significantly inhibited the growth of cells in a concentration-dependent manner. The results indicate that *S. aromaticum* is considered to be a particularly valuable source of effective anti proliferative and cytotoxic agents. Crud extract of clove is able to killing cancer cells in the human body by proliferation-inhibiting and apoptosis-inducing effects. In this study, the anti-tumor effect of lung cancer cells was investigated using the crude alcoholic extract of clove plant . Anticancer drugs exert their antitumor effects against cancer cells by inducing apoptosisthat is an significant phenomenon in cancer chemotherapy (Ghassan, 2013).Compared to the typical round nuclei of the control, crud extract treated cells displayed condensed and fragmented nuclei. It was observed that level of apoptotic cell demise was maximum.

This study supports the folkloric usage of the studied plant which possesses both significant antimicrobial activity and also the effects on the cancer cell line suggested that it could be used in prevention of cancer while warranting further *in vitro* and *in vivo*

investigations to understand more about cell death mechanisms (Aggarwal, 2006).

Cancer is one of the most important causes of death in the world. Resistance to the drugs used as anticancer therapies has been recently shown which highlights the urgent need to conduct further research to discover plant-derived substances that act as anticancer. Many plants, vegetables, herbs and spices might have the potential use in medicine as a source to the prevention of cancer, which necessitates further studies of these plants to know their biological properties and therapeutic potential (Salomons, 1996).

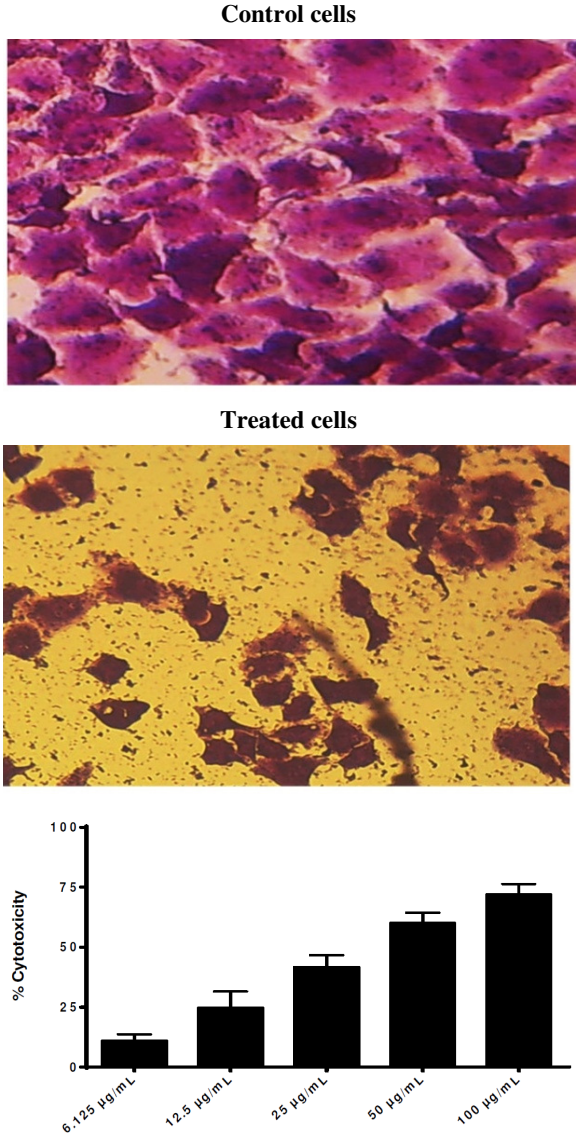


**Fig. 5 :** Cytotoxic effect of *S. aromaticum* in lung cancer cell line.

## Conclusion

It may be conclude from the present findings that *S. aromaticum* plant can be used as a probable source of innate antimicrobial compound possessing strong antioxidant, and cytotoxic potential. However, more research is needed for the identification of biologically activity for the compounds that present in this plant.

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**Conflict of interest statement**

We declare that we have no conflict of interest.

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