

## **Physiochemical Characteristic and Biological Activity of the Clove (*Syzygium aromaticum*) Bud Oil**

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### **ABSTRACT**

The present study was investigating the physiochemical and the biological activity of the Clove bud oil against the fungus (*A. niger*) and two bacteria (*E. coli* and *Staph. aureus*). The inhibition zone plate method was used for bacterial test, while the mycelia weights and radial growth methods were used for the fungal study. The A.O.S. official methods were used to determine the physiochemical properties. The studies on the physiochemical characteristics of the Clove bud oil proved that the acid value was (3.86), the saponification value was (38.27), the peroxide value was (3.83), the specific gravity was (1.043), the reflective index was (1.525), the iodine value was (182.43), and the free fatty acid was (1.94). The results of the antimicrobial tests showed that the Clove bud oil gave a complete inhibition of the radial growth of the fungus (*A. niger*) at its higher concentration and a high reduction percent at its lower concentrations. However, the mycelial fresh and dry weights of the fungus *A. niger* were completely inhibited by the Clove bud oil at its higher concentration, and clearly reduced at its lower concentrations. The inhibition zone of growth of both bacteria was larger than that of the control treatment. Clove bud oil showed clear inhibition zone when used against *Staph. aureus* compared to its use against *E. coli*. Many studies have reported that oils of different herbs and spices can yield medicinal compounds. Spices and herbs have been used for thousand of years by man in traditional medicine. However, more physiochemical characterizations need to be done and the antifungal and the antibacterial properties should be verified in any further studies on the Clove bud oil.

## INTRODUCTION

Spices and herbs contain essential oils, which are the flavouring components of extracts, and they are employed in the production of perfumes, cosmetics, toiletries, lotions, hair products, tooth pastes, and soaps. These essential oils are the basis of a number of spices flavouring and seasonings employed in food manufacturing. In many cases, oil extractives of spices are preferred to the whole or ground spices, largely because the extracts are easier to blend, the volatile oil content can be quantified, and the flavour intensity can be adjusted (Internet, 2015). In most countries preservatives (benzoic, ascorbic, acetic, propionic acids and others) and antibiotics are not permitted in foods. The need arises for non-toxic natural preservatives that could be used effectively in certain semi-processed and processed foods. Among such, natural preservatives, spices or essential oils of spices are the most important (Ismail *et al.*, 2001). Spices and herbs still have their place in medicine, particularly in China and India, where their curative virtues enjoy respect. However, in Western countries their medicinal use is more limited, but, with the revival of interest in alternative therapies since the late 20<sup>th</sup> century, the properties of herbs and spices are being reexamined (Nakatani and Nobuji, 1994). The antimicrobial activity of essential oils has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using methods that are directly comparable. Food borne illness resulting from consumption of contaminated food products with pathogenic bacteria has been of concern to public health. Controlling pathogenic bacteria would reduce food borne outbreaks and assure consumers a continuing safe, wholesome and nutritious food supply. Essential oils, which are concentrated herbal liquid extracts, inhibit the growth of wide variety of pathogenic microorganisms and many have great potential as antimicrobial agents (Mahdouani and Bakroof, 2007). The present study was conducted to investigate the physicochemical and the antimicrobial activities of the pumpkin seed oil against the fungus (*Aspergillus niger*) and two bacteria (*E. coli* and *Staphylococcus aureus*).

## MATERIALS AND METHODS

### Physical and Chemical Tests

The specific gravity, the iodine value, the moisture content and w obtained according to the A.O.C.S. Official method (1993), using a pycnometer.

The A.O.C.S. Official Method (2003) was used to determine the refractive index, using an abbe refractometer (Model ATAGO Rx -7000 $\alpha$ ) and to determine the free fatty acid which was the number of milligrams of potassium hydroxide required to neutralize the free fatty acid in one ml of oil, and to determine the acid value as well as the peroxidise value.

### Effects of the oil on bacterial growth

The cup plate method was used, using Nutrient Agar (NA). In the method 2ml of a standardized bacterial cell suspension ( $10 \times 10^5$ ) of *E. coli* or of *staphylococcus aureus* were thoroughly mixed with 200 ml of sterile molten nutrient agar, and then the medium was distributed into sterile Petri-

dishes and was left to solidify at room temperature for 24 hours. Sterile Whatman glass fiber disc (No.5) were saturated with the extract of pomegranate, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry et al (1970) and Cruickshank et al (1975). Three replicates were made for each treatment.

### **Effects of the extracts on fungal mycelial weight**

The method used was as described by Abdel-Rahim *et al.* (2002). The Potato Dextrose Broth (PDB) medium was prepared and then dispensed in 100 ml in conical flasks volume (250 ml). The oil solution was added to each flask, sterilized in an autoclave at 121<sup>0</sup>C (15-lb/in<sup>2</sup>) for 15 minutes, and then allowed to cool at room temperature, before inoculation. Each flask was inoculated by three discs (5.0 mm diameter), taken from an edge of an actively growing culture on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28– 30<sup>0</sup>C) for 8 days. After incubation mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelia mats were then dried at 80<sup>0</sup>C for 24 hours, before being reweighed. All treatments were done in triplicates.

## **RESULTS and DISCUSSIONS**

### **Physicochemical properties:**

The results of some physicochemical studies made in the present work are shown in Table (1). The refractive index of clove bud oil samples was found as 1.525 which is in the normal ranges as compared with the result reported by Gopalakrishnan *et al.*, (1982) which was (1.564) for the Zanzibar clove bud oil, and by Weiss (1997) which was (1.530), for the Madagascar clove bud oil. The refractive index *n<sub>D</sub><sup>20</sup>* was also in accordance with the range stated by the British Standard Specification (B.S.S) which was (1.528-1.538 at 20<sup>0</sup>C) as well as that stated by the Essential Oil Association (E.O.A) of U.S.A. and that determined by Ala-Aldeen *et al.*, (2005) which was 1.5310. The Specific gravity of the clove bud oil, was found as 1.043 (Table, 1). It is within the ranges reported by Reineccius (1994), for the Specific gravity of the same oil (1.038-1.060). The iodine value is the indicative of fats and oils unsaturation. Fats and oils with higher unsaturation show high iodine value. The iodine value observed for the oil sample in the present study was 182.43. This value is within the permissible range for semi-drying oils (100-300) as reported by Kyriakidis and Katsiloulis (2000). The free fatty acid and the acid values of the oil are also shown on Table (1), which were 1.94 and 5.86, respectively. The Codex Alimentarius (1969) indicated that the acids value of some oils was about 6.0. On the other hand Ebrahim (2009) reported a higher value. The higher value in his study was due to the direct addition of water to the seeds before pressing. However, in our study no water was added, that is why our values are lower.

Table (1) Some physicochemical properties of clove bud oil

No.	Characteristics	Treatments		
		1	2	Mean
1	Specific gravity	1.042	1.045	1.043
2	Refractive index	1.525	1.525	1.525
3	Iodine value	183.54	181.32	182.43
4	Free fatty acids (%)	1.93	1.95	1.94
5	Peroxide value	3.86	3.81	3.835
6	Saponification value (mg/ml)	38.21	38.33	38.27
7	Acid value	5.84	5.88	5.86

Data in Table (1) also show the peroxide value of the clove bud oil. The value (3.835) was found similar to that obtained for the fixed oil by Eka *et al.*, (2009). The Saponification value of the clove bud oil was found as 38.21. This value was in close agreement to the values reported by many investigations (Gopalakrishnan, *et al.*, 1982; Weiss, 1997; Ala-Aldeen, *et al.*, 2005).

#### Biological activities:

The present study was also investigated the biological activity of the clove bud oil against the fungus (*A. niger*) and two bacteria (*E. coli* and *Staph. aureus*). The antifungal activity was made on mycelial growth (fresh and dry weights and the radial growth of mycelia). From the results (Table 2 and 3). It is clear that the oil was highly effective in reducing both the fresh and the dry weights of the fungus (*A. niger*). It gave complete inhibition of mycelial growth at its two higher concentrations. Not only that, but even at its lowest concentration, it gave a significantly better inhibition zone than the control. In a similar work, Ansari and Shrivastave (1991), Abdel-Rahim *et al.* (1997) and Mohammed Ali (2003) reported that Clove bud oil gave a complete inhibition of mycelial growth of both *A. flavus* and *A. parasiticus*. However, Hoffman and Evans (1911) were among the earliest to describe the preservation action of cinnamon and clove oil and found them as the most effective of the different spices oils tested. In addition, cinnamon and clove oils were found to have strong antimycotic properties. The two oils were found to inhibit growth and aflatoxin formation by *Aspergillus parasiticus* (Bullerman *et al.*, 1977). The essential oils of lemon and orange have also been shown to be inhibitory to *A. niger*, *A. flavus* and *A. parasiticus* and suppressed aflatoxin production (Subba *et al.*, 1967; Alderman and March, 1976).

The effects of the clove bud oil on the inhibition zone of *E. coli* and *Staph. aureus* are also studied in present work. The effects of the clove bud oil on *E. coli* are shown in Table (4), while the effects on *Staph. aureus* are shown in Table (5). From the results it was found that the clove bud oil showed a clear inhibition zone of both bacteria. Although the oil inhibited both bacteria, but the effects were less pronounced against *E. coli* compared the other bacterium. Antibacterial activity of plant extracts are well documented (Alicia, 1981). Vlietinck *et al.* (1995) screened about 100 medicinal plants used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal and antiviral properties.

Their study showed that about 45% of the plant extracts were active against *Staph. aureus*, 2%, against *E. coli*, 16% against *Pseud. aerogenosa* and 7% against *Candida albicans*. Moreover,

about 27% of the plants tested exhibited antiviral properties. In Sumatra (Indonesia), 114 plant extracts were assayed for their antibacterial activity (Ahmed, 2002). About 82% of the extracts were active against *Staph. aureus* while 32% of them were active against *E. coli*. The plant *Garcinia cowa*, which was reported to be rich in xanthenes showed a moderate antibacterial activity against *Staph. aureus* (Pattalunget al., 1994). According to Encarnation *et al.* (1994), the flavones isolated from the medicinal plant *Cullianandra calitornia* exhibited an antibacterial activity against the two bacterial isolates tested. The extracts of plant leaves of *Tagetes minuta* were found to exhibit some activity against both Gram positive and Gram negative bacteria (Tereschuk, *et al.*, 1997). From the study it could be concluded that, the physiochemical properties of the clove bud oil were within the ranges reported earlier by different investigators. The clove bud oil has an excellent antifungal properties, it almost completely inhibited mycelial growth of the tested fungus (*A. niger*). The oil has also a good antibacterial activity. However, more physiochemical characterization need to be done and more studies needed to determine the antimicrobial activity of the clove bud oil on other organisms.

Table (2). The effect of the different concentrations of clove bud oil on the fresh and dry weight ( gm) of *A. niger*

Concentration	Fresh weight	Dry weight
0	.23	2.75
25	1.9	0.28
50	1.57	0.23
75	1.40	0.18
100	1.36	0.13

Table (3). The effect of the different concentrations of clove bud oil on the radial growth of *A. niger*.

Concentration	Days			
	2	4	6	8
0	23.5	26	28.3	33
25	5.5	6	6.2	6.6
50	5.2	5.4	5.8	6
75	5	5.3	5.6	5.8
100	5	5.1	5.3	5.4

Table (4). The effect of the clove bud oil on growth *E. Coli* (inhibition zone) in mm

Concentration	Days			
	2	4	6	8
0	5	5	5	5
25	7.4	7.8	8.1	8.1
50	9.5	9.9	10.2	10.2
75	10.6	11.4	11.5	11.5
100	12	12.8	12.8	12.8

Table(5): The effect of the clove bud oil on growth of *Staphylococcus aureus* (inhibition zone) in mm.

Concentration	Days			
	2	4	6	8
0	5	5	5	5
25	7	7.4	7.5	7.5
50	10	10.3	10.3	10.3
75	17.2	17.5	17.6	17.6
100	13.8	14	14.2	14.2

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## الخواص الفيزيوكيميائية والبيولوجية لزيت براعم القرنفل

### ملخص الدراسة

القرنفل عبارة عن براعم ذو رائحة ذكية من شجرة تنتمي لعائلة (Myrtaceae)، يتم حصاد القرنفل تجارياً في اندونيسيا، مدغشقر، زنجبار، باكستان، سيريلانكا، الهند وتتنانيا. تعتبر شجرة القرنفل من ضمن الاشجار دائمة الخضرة، يتراوح طولها ما بين (8-12) متر ، ذات أوراق عريضة ذات زهور حمراء وذات حزم طرفية. عادة ما تسقط ثمار القرنفل طبيعياً من الشجرة ثم تزرع مباشرة في الوسط الذي تنمو عليه. تم في هذا البحث دراسة الخواص الفيزيوكيميائية والنشاط البيولوجي لزيت براعم القرنفل ضد نمو الفطر (*A. niger*) واثنين من البكتيريا (*E. coli* and *Staph. aureus*). استخدمت طريقة المنطقة المثبطة في الاختبار البكتيري، واستخدمت طريقة الوزن الجاف والرطب للميسيليوم والنمو القطري ضد الفطريات. كما تم استخدام طريقة جمعية الزيت الأمريكية للكيميائيين لتحديد الخواص الفيزيوكيميائية. لقد برهنت هذه الدراسة الفيزيوكيميائية لزيت براعم القرنفل حيث وجد أن قيمة الحمض كانت (3.86)، قيمة التصبن كانت (1.043)، قيمة البيروكسيد كانت (3.83)، قيمة الكثافة كانت (1.043)، معامل الانكسار كان (1.531)، قيمة الرقم اليودي كانت (182.43)، في حين أن قيمة الاحماض الدهنية الحرة كانت (1.94). في هذه الدراسة اظهرت نتائج الاختبارات الميكروبيولوجية أن زيت براعم القرنفل اعطي تثبيط كامل للفطر (*A. niger*) في التراكيز العالية وقلل النمو بشكل كبير في التراكيز المنخفضة ، بينما في الأوزان الجافة والرطوبة اعطي زيت براعم القرنفل تثبيط كامل في التراكيز العالية واعطي تقليل واضح في التراكيز المنخفضة. تثبيط نمو البكتيريا كان أكبر مقارنة مع معامل المقارنة. زيت براعم القرنفل اعطي تثبيط واضح عندما استخدم ضد بكتيريا (*Staph. aureus*) مقارنة مع بكتيريا (*E. coli*). كثير من الدراسات اشارت الى أن عدد من الأعشاب والتوابل انتجت مركبات طبية. الأعشاب والتوابل تستخدم منذ آلاف السنين بواسطة الأنسان في الطب الشعبي. يجب عمل المزيد من الخواص الفيزيوكيميائية وكذلك دراسة المواد المثبطة للفطريات والبكتيريا في الدراسات المستقبلية على زيت براعم القرنفل.