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

Awad M. Abdel-Rahim[1], Samahir H Osman[1] and Mai A. Ali[2]

1. Center of Biosciences and Biotechnology, Fac. Of Eng. & Tech., Univ. of Gezira
2. Center of Plant Pathology, Fac. Of Agric. Sciences, Univ. of Gezira

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. aurues*). 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. aurues* 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 20th 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 out breaks and assure consumers a continuing safe, whole some and nutrition 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 agent (Mahdouani and Bakhroof, 2007).

The present study was conducted to investigate the physiochemical and the antimicrobial activities of the pumpkin seed oil against the us (*Aspergillus niger*) and two bacteria (*E. coli* and *Staphylococcus aurues).*

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 able refractometer (Model ATAGO Rx -7000α) 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 (10x105) 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 solidity 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 121^0C (15-Ib/in^2) 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– 30^0C) 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 80^0C for 24 hours, before being reweighed. All treatments were done in triplicates.

RESUTS and DISCUSSIONS

Physiochemical 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 e was also in accordance with the range stated by the British Standard Specification (B.S.S) which was (1.528-1.538 at 20Co) 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 valve 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 tu 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). Vlietincket *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. aeroqenosa* 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 *Culliandra 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
o	.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

.

REFERENCES

Abdel-Rahim, A.M; Al-Jali ,Z.I. A and Al Mismari, S.S.(1997). Factors affecting growth and aflatoxin production by the two fungi *A. flavus* and *A.parasiticus* .6th Arab congress on plant protection. ,Beirut, Lebanon,OCT.1997

Abdel- Rahim, M. A. and Mohamed Ali, R. A. (2002). Effects of plant products on fungal growth and aflatoxin production by A. flavus. and A. parasiticus. African Journal of Mycology 15: 12-20. and .parasiticus. 6th Arab congress on plant protection ,Beirut,Lebanon,OCT.1997.

Ahmed, M. M. (2002). Molluscicigdal and antimolluscicigdal activity of certain Sudanese cucurbitacea.

Ala-Aldeen, A., Ashraf, S., Suliman, M. (2005). Phytochemical Screening (*Syzygiumaromaticum).*B.SC. Thesis, Faculty of Science and Technology. Alneelain University, Khartoum, Sudan.

Alderman G.G. and Marth, E.H. (1976). Inhibition of growth and Aflatoxin production of *Aspergillusparasiticus* by citrus oils. Z. Lebensm. Unters Forsch16o:353- 358.

Alicia, S. L. (1981). Antimicrobial activity of higher Argentine plants. Fitoterapia 2:81-85.

Ansari and Shrivastave, A.k (1991). The effecting of Eucalyptus on growth and aflatoxin production by *Aspergillusflavus.* Letters APPL. Microbial. (Abstr.),13(2):75-77

A.O.C.S. (1993) . The American Oil Chemists Society. Official method reapproved (1993)

A.O.C.S. (1993). The American Oil Chemists Society. Official method revised (2003).

Barry A. L. Garica. F., Thrupp, L. D. (1970). Interpretation of sensitivity test results. Am.J. Clin-Path.53:p149.

Bullerman L. B., Lieu,F.Y. and eier, S. A. (1977). Inhibition of growth and aflatoxin production by cinnamon and clove oils.Cinnamon aldehyde and eugenol.J. Food.Sci.42:1107-1108.

Burt, S. A. and Reinders R.D.(2003). Antibacterial activity of selected plant essential oils against Esherishia coli 0157:117.Lett Appl. Microbial 36(3):162-167.

Codex Alimenyrius (1969). Fats and Oils and Related Products. Food and Agriculture Organization (FAO). Vol. 8 parts 3, Rome.

Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R. H. (1975). Medical microbiology. R. Cruickshank, R. J. P, Duguid; B. P., Marmion, R. H. Swain, (Eds), Vol. II Edinburgh,12th. Ed

Deyama, T. and Horignchi, T. (1971).''Components of essential oil of clove" Yakugaku Zasshi (Japan), 91, 1383-6 (Chem. Abs, 76, 498146).

Ebrahim, S.M.(2009). Evaluation of Ground Oil Quality Produced by Four Types Of Expeller M.Sc. in chemical Engineering, University of Gezira.

Eka B, Essien OI. Amaefule and E. Ihenacho (2009). Proximate Analysis and Physio-Chemical properites of water melon (Citrullus Iunatus) seed. Nig.J. of Biochem and Mol Biol.24(2): 6-10.

Encarnaction, R.; Ochoa, N.; Anthonill, U.; Christophersen, C. and

Nielsen, P. H. (1994). Two new flavones from *Calliandracaliforica*.J. Nat. Prod. 57(9): 1307-9.

Gildemeister, E. and Hoffmann, Fr. (1961). Die AetherischenOele, Vol. V1, Berlin: AkademieVerlag.

Gopalakrishnan, M. Menon, N. and Mathew, A.G. (1982). Changes in the composition of clove oil during maturing. J.Food Sic. Tech. 19(6)1190-192.

Gupta M.K,(2008). Basic Oil Chemistry, Practical Guide to Vegetable Oil Processing, AOCS Press, Urban,Illinos, U.S.A.

Hitokoto, H., Morozumi, S., Wauke, T., S. and Ueno, I.(1980).Inhibitory
effect of spices on growth and toxin production. fungi. Appl. Environ. Microbial, 39 : 252 - 253

Huffman,M.A. (2003). Animal self-medication and ethno-medicine: Exploration and explosion of the medication of the medicinal properties of plant.

Internet (2015). http://www.encyclopedia.com/doc/1G2-3435100100.html

Ismail, A. E.;Saad M. EL Maraghy and Abd EL Naser A. Zohri(2001).Effect of some spices and Essential oil on the growth and Aflatoxin production by IMI 89717 on liguid Medium and Miced Meat. Proceeding of the first Saudi Symposium in Food Saftey, AL Ahsa, Kingdom of Saudi Arabia.

Kyriakidis, N.B. and Katsiloulis,T.(2000). Calculation of iodine value from measurements of fatty acid methyl esters of some oils: comparison with the relevant American oil chemists society method. Journal of American Oil Chemical Society, 77: 1235-1238.

Lai PK. and Roy. J. (2004). Antimicrobial and chemo preventive properties ofherbs and species. Curr. Med. Chem:145-60.15180577 PMID.

Mahdouani and A. Bakhrouf. (2007). The chemical composition and biological activity of essential oil, Eugenia cryophyllata (Syzygiumaromaticum L. Myrtaceae): a short review. Phytother Res., 21(6): 501-506.

Mohammed –Ali E.E.(2003). The inhibitory effect of some essential oils on *Aspergillusflavus*. Thesis, University of Gezira.

Nakatani, and Nobuji, (1994). Antioxidative and Antimicrobial Constituents of herbs and spices .Spices, Herbs and Edible Fungi. Elsevier Science B. V., 251- 271.

Pattalung, P.; Thongtheeraparp, W.; Wiriyacthitra, P. and Tylor, W.C. (1994). Xanthones of *Caricinacowa*plantaMedica 60(4): 365-8.

Reineccius,G.(1994). Natural Flavouring Material. Sourcebook of flavour, 2nd.edn.Chapman and Hall, New York, p.286.

Subba,M. S. Soumithri, T. C. and Rao, R. S. (1967). Antimicrobial action of *citrus oils, J.* foodSci.,32:225-227.

Tereschuk, M .L.; Riera, M. V.; Castro, G. R. and Abdalla, L. R. (1997). Antimicrobial activity of flavonoids from leaves of *Tagetesminuta.* J. Ethnopharmacology 56(3): 227-232.

Vlietinck, A. J.; Van Hoo, L.; Totte,; Lasure, A.; Vanden, B. D.; Weiss, E. A., (1997). Essential oil crops. Victoria. Australia pp235-259.

الخواص الفيزوكيميائية 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، زنزبار، باكستان، سيريلانكا، الهند وتنزانيا. 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 الفيزوكيميائية 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 الفيزوكيميائية. لقد برهنت هذه الدراسة الفيزوكيميائية 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 المكروبيولوجية أن زيت براعم القرنفل اعطي تثبيط كامل للفطر (*A. niger*) في التراكيز العالية وقلل النمو بشكل كبير في التراكيز المنخفضة ، بينما في الأوزان الجافة والرطبة 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 اشارات الى أن عدد من الأعشاب والتوابل انتجت مركبات طبية. الأعشاب والتوابل تستخدم منذ ألاف السنين بواسطة الأنسان في الطب الشعبي. يجب عمل المزيد من الخواص الفيزوكيميائية وكذلك دراسة المواد المثبطة للفطريات والبكتيريا في الدراسات المستقبلية على زيت براعم القرنفل.