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Nutritive Value of Clove (*Syzygium aromaticum*) and Detection of Antimicrobial Effect of its Bud Oil

Abdel Moneim E. Sulieman, Iman M.O. El Boshra and El Amin A. El Khalifa
Department of Food Science and Technology, Faculty of Engineering and Technology,
University of Gezira, Wad Medani, Sudan

Abstract: The nutritional value and the antimicrobial characteristics of clove and clove bud oil were investigated. The proximate chemical composition of clove was determined as follows: moisture (10±0.006)%, fiber (20±0.1)%, ash (5.2±0.01)%, protein (1.2±0.02)%, fat (12.1±0.45)% and carbohydrates (51.5±0.02)%. The physico-chemical characteristics of clove essential oil were carried out and the acid value was found to be (3.843), saponification value (42.07), ester value (38.22), free fatty acids (1.92) and the refractive index was found to be (1.5310 at 27°C). The inhibitory effect of clove oil was detected for growth of eight microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium* (bacteria), *Candida albicans* (yeast), *Aspergillus niger* and *Rhizopus nigricans* (moulds). The results indicated that clove oil has a potent antimicrobial activity against all tested organisms. The highest antibacterial activity was found against *E. coli* (25 and 36 mm) inhibition zone diameter at lower and higher oil concentrations, respectively. The higher antimicrobial activity among all tested organisms was found against *Aspergillus niger,* it gave complete inhibition (100%). This study has shown the importance of clove and clove oil and indicated that clove and clove bud oil can be used as an antimicrobial, antiseptic and preservative agent and they can be introduced into the Sudanese food table.

Key words: Clove, chemical composition, antimicrobial activity

INTRODUCTION

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Cowan, 1999). In most countries, chemical preservatives and antibiotics are not permitted in foods. The number of antimicrobial preservatives approved for use in food is really quite small. 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 which are known to posses antimicrobial activity (Ismail *et al*., 2001).

The Arabs knew clove as *goronful*, believed to be the origin of the greck *Koryophyllon* (Weiss, 1997).

In Sudan, different kinds of spices have been used traditionally to flavour, preserve foods, to prepare perfumes and for medical treatment. Some of these spices are grown in the country and others are imported from outside the country. The most common used spices in Sudan include: pepper, onion, garlic, cardamom, ginger, clove, cinnamon, anise, mint, cumin, fenugreek and others.

Cloves are ingredients in many classic spice mixtures, with chilies, cinnamon, tumeric and other spices in the preparation of curry powder. Whole or ground cloves are frequently used to flavour cooking liquids for simmering fish, poultry and meat. They are used in the bakery industries and in the

Corresponding Author: Abdel Moneim E. Sulieman, Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan

production of sauces and pickles. Cloves have many therapeutic uses: they relieve pain, control nausea and vomiting improve digestion, protect against internal parasites and act as antimicrobial agents against fungi and bacteria cause uterine contractions and are strongly antiseptic (Burt and Reinders, 2003). In general medicine, clove is used as an agent against flatulence, stomach distension and gastro-intestinal spasm (Elujoba *et al*., 2005). The clove oil is useful for treating rheumatoid arthritis and it has analgesic, antiseptic and anti-spasmodic properties. About four grams of clove are boiled in three liters of water until half the water has evaporated. This water, taken in draughts, will slow down severe symptoms of cholera (Kalemba and Kunieka, 2003). Chewing clove with a crystal of common salt relieves the irritation in the throat and stops cough in the pharyngitis. Clove is also effective medicine for coughs caused by congested throat.

Clove bud oil is a clear, colourless to yellow mobile liquid, becoming browner with age or contamination with iron or copper, with strong characteristic sweet and spicy clove odour and a warm, almost burning and spicy flavour (Weiss, 1997). Clove oil is used in the manufacture of perfumes, soaps and detergents. Clove bud oil, has been used for a long time by Dentists; as a dressing in Dentistry, for treating minor oral wounds; as an analgesic in painful and infective diseases of the oral cavity and opharynx as well as for general hygiene (Elujoba *et al*., 2005). To validate these observations, the current study was carried out to evaluate the nutritional value of cloves and to determine the antibacterial and antifungal activity of its bud oil.

MATERIALS AND METHODS

Materials

Clove samples were obtained from Wad Medani local market, central Sudan (July, 2005). These samples which had been imported from India had a reddish brown colour, strong in aroma, hot and pungent in taste. The samples were carefully cleaned from extraneous materials.

Methods

Proximate Analysis

The dried clove buds were analyzed for the contents of moisture, protein, fiber, ash and fat according to the AOAC (1990) methods. The carbohydrates contents were calculated by difference. The experiments were repeated three times and the average and standard deviations were calculated.

Extraction of Clove Bud Oil

The clove bud oil was obtained by steam distillation technique.

Physicochemical Properties of Clove Bud Oil

The physicochemical properties of clove bud oil were determined according to the AOAC (1990) methods. These properties included: the acid value%, the saponification number, the ester value, the free fatty acid content and the refractive index.

Tested Organisms

The clove bud oil was tested against two gram-positive bacteria (*Bacillus subtilis* NCTC 8236, *staphylococcus aureus* ATCC 25923), three gram-negative organisms (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028) and three fungi (*Aspergillus niger* ATCC 9763, *Candida albicans* ATCC 7596 and the mold *Rhizopus nigricans*). The tested organisms were obtained from the Department of Microbiology, Medical and Aromatic Plants Research Institute and National Health Laboratory.

Testing of Clove Bud Oil for Antibacterial Activity

The sup-plate agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared clove bud oil extract according to the method described by Kavanagh (1972).

Testing of Clove Bud Oil for Antifungal Activity

For antifungal activity the same method for bacteria was adopted using sabouraud dextrose agar instead of nutrient agar. The fungal cultures were maintained on sabouraud dextrose agar, incubated at 25°C for 7 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended on 100 mL. sterile normal saline and the suspension was stored in the refrigerator till used.

RESULTS AND DISCUSSION

Proximate Chemical Composition

The proximate chemical composition of the dried clove buds is shown in Table 1.The contents of moisture, crude fiber, ash, protein, fat and carbohydrates of clove buds were: 10.0±0.006, 20±0.1, 5.2±0.01, 1.2±0.02, 12.1±0.45 and 51.5±0.02%, respectively. However, most of those components were in accordance with those reported for clove buds by many investigators (Purseglove *et al*., 1981; Gopalakrishnan *et al*., 1982).

Physicochemical Properties

The physicochemical properties of clove bud oil were found to be as follows: the acid value the saponification number, ester value and the refractive index of clove bud oil were found to be 42.07, 38.22, 1.92% and 1.5310 at 27°C, respectively (Table 2). These values were in close agreement to the values reported y many investigators (Gopalakrishnan *et al*., 1982; Weiss, 1997; Ela Aldeen *et al*., 2005).

Antimicrobial Activity of Clove Bud Oil

This study was conducted to determine the inhibitory activity of clove bud oil for growth of eight microorganisms, including *Escherichia coli, Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium* (bacteria), *Candida albicans* (yeast) and *Aspergillus niger* and *Rhizopus nigricans* (moulds). The average of the diameters of the growth inhibition zones obtained on the experiments is shown in Table 3 and 4.

Table 1: Proximate chemical composition of the dried clove buds

Sample	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
Dried clove buds	1.2±0.02	12.1±0.45	20±0.1	5.2±0.01	10±0.006	51.5±0.02

All results are expressed on Dry Mater (DM) basis

Table 2: Physicochemical properties of clove bud oil

Parameter	Values
Acid value	3.843
Saponification value	42.07
Ester value	38.22
Free fatty acid value (%)	1.92
Refractive index	1.5310

Table 3: The antibacterial activity of clove bud oil against the standard organisms*

				Inhibition zone diameter (mm)				
Organism	Plant extract	Solvent system	Connection	B.s	S.a	E.c	Ps.a	Sa.t
Syzygium	Buds	Methanol	1:10	22	20	25	15	20
aromaticum			2:10	24	22	36	20	25

* Standard Organisms, B.s: *Bacillus subtilis*, S.a: *Staphylococcus aureus*, E.c: *Escherichia coli*, Ps.a: *Pseudomonas aeruginosa* Sa.t: *Salmonella typhymurium*, < 15 mm: sensitive, 14-15 mm: Intermediate, > 14 mm: Resistant

Table 4: The antifungal activity of clove bud oil against the standard organisms*

Organism	Plant extract	Solvent system	Concentration	Inhibition zone diameter (mm) Ca.a	As.n	Ri.n
Syzygium aromaticum	Buds	Methanol	1:10	23	Complete inhibition zone	21
			2:10	25	Complete inhibition zone	24

* Standard Organisms, Ca.a: *Candida albicans*, As.n: *Asperillus niger*, Ri.n: *Rizopus nigricans*, < 15 mm: sensitive, 14-15 mm: Intermediate, > 14 mm: Resistant

Clove bud oil had a higher antibacterial activity against *Escherichia coli*, the inhibition growth zones diameter were 25 and 36 mm at the lower and the higher concentrations, respectively. These results agreed with that reported by Mandeel *et al*. (2003) who stated that *Escherichia coli* was sensitive to clove bud oil, with antimicrobial activity of (19 mm). The results were also in accordance with those reported by many investigators (Smith-Palmer *et al*., 1998; Hammer *et al*., 1999; Dorman and Deans, 2000). On the other hand, Krishna and Banerjee (1999) also reported that clove, cinnamon, cumin, onion and garlic inhibited the growth of *Escherichia coli*. *Escherichia coli* is a common organism found in the lower intestinal tract of man and animals. There are many types and strains of *E. coli*, a few of which are potentially pathogenic. Various strains may cause illness by a variety of infective and toxin-producing mechanisms.

The study revealed that clove oil had an antimicrobial activity of (20-22 mm) against *Staphylococcus aureus* and this result agreed with that of Smith-palmer *et al*. (2004) who stated that clove bud oil significantly decreased the production of entrotoxin A and B and alpha-toxin produced by *Staphylococcus aureus, Staphylococci* are widely distributed in the environment; they form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract. This species causes food-poisoning from entrotoxin B in foods such as cooked meats, eggs, fish, milk and milk products (e.g., ice cream).

Clove bud oil also showed antimicrobial activity of (22-24 mm) against *Bacillus subtilis* and this result agreed with Krishna and Banerjee (1999) who reported that clove have a potent antimicrobial activity against *Bacillus subtilis*. *Bacillus* species are widely distributed in nature, in soil, dust water and on vegetation. It can be found in variety of foods including cereals, spices, meat and poultry.

Clove bud oil showed antimicrobial activity of (20-25 mm) against *Salmonella typhi*. The result agreed with that reported by Nakatani and Nuboji (1994) who stated that clove, onion and nutmeg were effective against *Salmonella typhi* and *Shigella parasysenteria*. *Salmonella typhi* is a human-specific pathogen causing the systemic febrile illness (typhoid fever) (Sherburne *et al*., 2000). Clove bud oil showed the lowest antimicrobial activity among the tested bacteria (15-20 mm) against

Pseudomonas aeruginosa

Clove bud oil caused complete inhibition (100%) of growth of *Aspergillus niger* (Table 4). Hoffman and Evans, (1911) found that cinnamon and clove oils had strong antimycotic properties, the two types of oil inhibited growth and aflatoxin formation by *Aspergillus parasiticus* (Bullerman *et al*. (1977). Subba *et al*. (1967), Alderman and March (1976) and Hitokoto *et al*. (1978) studied the effect of 13 herbal drugs and 7 commercial dry condiments on growth and toxin production by several toxigenic *Aspergillus* species and found that powdered cinnamon was the most effective inhibitor. They also reported that growth and mycotoxin production were inhibited by clove, anise and cumin. Mabrouk and El Shayeb (1980) reported that clove at a concentration of 0.1% partially inhibited mould growth at concentrations of 0.5% or higher. Azzouz (1981) stated the effect of some spices, on several toxigenic species of *Aspergillus* and *Penicillum* and found that clove and cinnamon inhibited growth of all tested fungi. Azzouz and Bullerman (1982) also reported that 2% of clove or cinnamon completely inhibited growth of *A. flavus* and *A. parasiticus* for various times up to 21 days on potato dextrose agar medium. Moreover, Ahmad *et al*. (2005) found that clove oil possess strong antifungal activity against opportunistic fungal pathogens such as *Candida albicans*, *Cryptococcus neoformas* and *Aspergillus famigatus*

Candida albicans was also found to be sensitive to clove bud oil with antimicrobial activity ranged between 23-25 mm, (Table 4). Bread mould, *Rhizopus nigricans*, was found sensitive to clove bud oil with antifungal activity of (21-24 mm). Souza *et al.* (2004) studied and evaluated the *in vitro* effect of essential oils of cinnamon, clove, garlic and thyme on the mycelial growth of fungi associated with bread-making products. They reported that clove essential oil inhibited the growth of *Rhizopus* sp. at concentrations above (600 μg mL) and *Penicillum* sp. above (800 μg mL).

The results suggested that clove essential oil extracted from clove buds may provide an effective means of inhibiting the survival and growth of all tested pathogens and indicator organisms especially *Escherichia coli* (among bacteria). However, the highest inhibitory effect of clove bud oil was found against *Aspergillus niger*. The study indicated that clove and clove bud oil can be used as antibacterial, antifungal and antiseptic agent, so that they can be used as a food preservative, hence, improve shelf life of foods. Some of the tested organisms are considered as important food-borne pathogens, raising the possibility of using clove bud oil to prevent food-borne diseases. However, the application of this oil in food industry will require safety and toxicity issues to be addressed.

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