Antimicrobial activity of some plant extracts using food model media

Ahdab Abdo Elmaadawy

Department of Home Economics- Faculty of Specific Education- Zagazig University



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Department of Home Economics- Faculty of Specific Education- Zagazig University **ABSTRACT**

This study aimed to optimize the antimicrobial efficacy of plant extracts for control of foodborne pathogenic and spoilage microorganisms using food model media based on meat and milk. The extracts of 7 medicinal plants (Gambooge, Clove, Marjoram, Galangal, Thyme, Cinnamon and Marigold) were screened for their antimicrobial activities against nine standard microbial strains (S. aureus, B. cereus, L. monocytogenes, E. coli, P. aeruginosae, S. enterica, C. albicans, Rhizopus sp and A. flavus). All alcoholic extracts (except that of Marigold) inhibited all tested microorganisms. Gambooge extracts showed the best activity against all tested microorganisms producing the widest inhibition zones ranged from 13 to 40 and 8 to 25 mm against bacteria and fungi respectively, followed by Clove extracts with inhibition zones ranged from 11 to 33 and 8 to 22 mm respectively. The most susceptible bacteria among Gram-positives to the tested plant extracts were Bacillus cereus, while the least susceptible were L. monocytogenes. The most susceptible bacteria among Gramnegative bacteria were Salmonella enterica while the least susceptible were P. aeruginosa. on the other hand fungal strains showed the highest resistance more than all tested bacteria and yeast. aqueous extracts showed less activity than alcoholic extracts. When the methanolic extracts were mixed into food models the antimicrobial activity were reduced and the required minimum inhibitory concentrations (MICs) were duplicated. MICs of Gambooge extract ranged from 0.234 to 3.750, 0.468 to 6.666 and 0.877 to 7.5 mg/ml when tested on microbiological (control) media, meat model and milk model respectively. The antimicrobial activity was reduced on semi-skimmed milk model more than that on meat model and control media respectively. The results indicated that plant extracts possessing antimicrobial activity can be exploited as ideal food preservatives after taken into account the reaction and interaction between food components and extract.

Key words: Foodborne bacteria, food spoilage microbes, Antimicrobial Activity, food model, plant extracts and food preservation.

INTRODUCTION:

Food spoilage includes physical damage, chemical changes, such as oxidation, color changes, or appearance of off-flavors and off-odors resulting from microbial growth and metabolism in the product (Adams and Moss, 2008: Lorenzo et al, 2018). The spoilage of refrigerated meat is caused in part by *Pseudomonas* species which are responsible for the off-odors, off-flavors, discoloration, gas production and slime production (Oussalah *et al.*, 2006: Martin Luong et al,2020).

Foodborne pathogenic microorganisms can cause diseases in humans. The illnesses can be serious and mortal. For example, *Clostridium botulinum, Escherchia coli* and *Salmonella* can cause serious foodborne illnesses that lead to death (**Tauxe, 2002: Bintsis et al, 2017**). Illnesses caused due to the consumption of foods contaminated with pathogens such as *Listeria monocytogenes* has a wide economic and public health impact worldwide (**Gandhi and Chikindas 2007**). Foodborne pathogens also lead to an economic burden every year. According to **Scharff (2012)**, 77.7 billion dollars was lost annually to investigate foodborne illnesses associated with 31 pathogens including *Salmonella, Listeria monocytogenes*, and *E. coli* O157:H7.

Mycotoxins are secondary metabolites produced by certain fungi and can result in acute or chronic diseases in human (Sweeney and Dobson 1998: Kepinska-Pacelik and Biel, 2021). The diseases caused by mycotoxins include cancers, gastrointestinal disturbances, alteration of the immune system, and reproductive problems. Species of *Aspergillus, Fusarium,* and *Penicillium* can produce mycotoxins, such as aflatoxin, citrinin, patulin, penicillic acid, ochratoxin, and fumonisins (Bhunia 2008: Navale et al, 2021).

Although many antimicrobials are applied to foods to inhibit the growth of foodborne pathogens, there are still several concerns forcing food researchers to search for novel antimicrobials to substitute for the current synthetic compounds. Firstly, "natural" and "minimally processed" food products have become more popular among consumers. Secondly, the transfer of antibiotic resistance to pathogens. Thirdly, some antimicrobials may make the sensory properties of the products undesirable to consumers (Skandamis *et al.*, 2001; Schuenzel and Harrison, 2002: Sarron et al, 2021). There is a currently strong debate about the safety aspects of chemical preservatives since they are

considered responsible for many carcinogen attributes as well as residual toxicity (**Gutierrez** *et al.*, **2008**). Therefore, alternative sources of safe, effective and acceptable natural preservatives need to be explored.

Herbs have been known for their antimicrobial activity since ancient times. The safe use of herbs and their components has led to their current status of generally recognized as safe (GRAS) food ingredients (**Burt**, **2004 and Kisko** *et al.*, **2005: Yu et al**, **2021**).

Studies have been reported on antimicrobial properties of different plant parts and their extracts used as spices or aromatic herbs including garlic, onion, cinnamon, black pepper, thyme, sage, rosemary, basil, turmeric, cardamom, cassia, celery, clove, coriander, dill, ginger, marjoram, etc (El-Khateib and Abd El-Rahman, 1987; Hefnawy *et al.*, 1993; Hao *et al.*, 1998; Nanasombat and Lohasupthawee, 2005 and Pundir *et al.*, 2010: Parham et al,2020).

Although some studies have shown that plant extracts and essential oils are useful for reduction of pathogens associated with meat (**Mytle** *et al.*, **2006 and Ahn** *et al.*, **2007: Yousefi et al**, **2020**), others reported very low antimicrobial activity or no effect against *L. monocytogenes* or *Salmonella* when extracts and EO's were applied to beef or chicken (**Uhart** *et al.*, **2006 and Firouzi** *et al.*, **2007**). Thus, the application of plant extracts for control of foodborne pathogens and food spoilage bacteria requires the evaluation of efficacy within food products or in model systems that closely simulate food composition. In general, the efficacy of many added and naturally occurring antimicrobials may be reduced by certain food components (**Glass and Johnson, 2004: Quinto et al, 2019**).

The present study was undertaken to determine and compare the potential of some plant extracts as an antimicrobial agents against foodborne microorganisms isolated from some foods using food model media; an attempt to formulate natural food preservatives.

Materials and Methods:

1- Isolation and identification of tested microorganisms:

Different food specimens from meat, poultry, milk, vegetables and fruits products were collected and screened for the presence of food spoilage and foodborne pathogenic microorganisms on nutrient agar (for bacteria) and sabourauds dextrose agar (for fungi and yeast) according to **Jay**, *et al.*, **2005** and **Adams and Moss**, **2008**.

The purified bacterial cultures were identified and confirmed after investigating morphological and biochemical characters according to standard laboratory methods reported and recommended by Bergey's Manual of Systematic Bacteriology (Garrity *et al.*, 2005 and Vos *et al.*, 2009) and Mahon *et al.*, 2011.

The unknown isolated fungi and yeasts were identified based on macro and micro morphology, reverse and surface coloration of colonies and slide culture technique (**Pitt and Hocking, 1994**).

Six of the most common bacterial species were selected including three Gram-positive (*Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*) and three Gram-negative (*Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*), Two common fungal species were selected (*Asperigillus flavus and Rhizopus* sp) and one yeast (*Candida albicans*).

2- Plant extracts preparation:

A total of 7 Plant species were collected from various herbalists and markets in Cairo, Egypt (Table 1). They were dried in oven at 50 °C to constant moisture content, then powdered, 100g of every dried powdered plant material was soaked with 500 ml of 80% ethanol or 80% methanol or distilled boiled water (for preparing infusion extract and for preparing decoction extract, 100g of every powdered plant was decocted in 500 ml distilled water for 30 minutes) separately in a sterile conical flask for 48 hours with continuous shaking. Then it was filtered through 4 layers of muslin cloth and centrifuged for 10 min. The supernatant was collected and filtered with Whatman filter paper NO.2. The obtained extract was concentrated using a rotary evaporator (SBW-1, Shanghai Shenbo Instrument Co., China) under reduced pressure at 45°C to eliminate the solvent. The residual fraction was freeze-dried (lyophilized). Apart of each powdered extract was diluted to 15 mg/ml using 10% dimethyl sulfoxide (DMSO) as solvent (stock solutions), sterilized by filtration through bacterial filter of pore size 0.45µm using positive pressure. Then filtrate was kept at 4°C in refrigerator till use (Handa et al., 2008; Al-Daihan, 2013; Kossah et al., 2013).

3- Antimicrobial Activity of plant extracts:

The antimicrobial effects of plant extracts were determined using disc diffusion method according to Vilijoen *et al.*, (2003); Kumara *et al.*, (2009), Mohanka and Priyanka, (2014), Mueller Hinton agar (for

bacteria) and sabourauds dextrose agar (for fungi and yeasts) were sterilized by autoclaving at 121°C for 15 minutes, cooled, poured in Petri dishes and inoculated with the selected isolates by striking the swab over the surface of the medium on three directions to confirm a complete distribution. Sterile filter paper discs (Whatman No.3, 6 mm diameter & three layers) were saturated by stock solutions of each plant extract, the disks were allowed to dry for one hour, then placed on the surface of inoculated plates. The used organic solvents and distilled water disks served as negative controls. The plates were kept in refrigerator for one hour to allow better diffusion of the extract prior to incubation at 37°C/24h. for bacteria, 30°C/48h.for yeasts and 30°C/96h. for molds. After incubation, the inhibition zones formed around disks were measured in millimeter (including the diameter of the disk (6mm)). Each experiment was run in triplicates and the means were calculated.

4- Comparing the minimum inhibitory concentration (MIC) of methanolic extracts on control and food model media:

Agar dilution method was performed as described by Hammer et al., 1999; Oussalah et al., 2006 and Gutierrez, et al., 2009, but with some modifications to determine the MICs of the methanolic (the most active) extracts. Control media was Mueller Hinton agar (for bacteria) and sabourauds dextrose agar (for fungi and yeasts). Meat model media prepared from beef extract (1%) and agar (1.5%) while milk model media was made by mixing semi skimmed milk powder (1%) with agar (1.5%). Control, meat and milk model media were adjusted to pH 7.2 to separate pH effects. After autoclaving each medium was divided in sterile bottles in which each extract serially diluted to the appropriate concentrations (from 0.173 to 15 mg/ml) which poured onto a Petri dishes and allowed to solidify. Target microorganisms were previously grown (24h.) in MHB or SDB or liquid model media to allow the cells to adapt to the food environment. Plates were then seeded with the target microorganism, and incubated at the appropriate temperature (37°C/24h. for bacteria, 30°C/48h.for yeasts and 30°C/96h. for molds). The positive control consisted of control or model media inoculated with the same amount of cells but without any extract, while uninoculated plates containing the extract served as negative control. Plates were evaluated for the presence or the absence of colonies after incubation period. MIC was defined as

the lowest concentration of plant extract that completely suppressed colony growth.

RESULTS and DISCUSSION:

The antimicrobial activity of extracts from seven medicinal plant species were determined using disc diffusion method and the results were listed in table 2. Based on their inhibition zone diameter, all extracts (aqueous and alcoholic) from two plants namely, Gambooge and Clove showed the best potential antimicrobial activity against all tested microbes. All alcoholic extracts (except that of Marigold) inhibited all tested microorganisms. Gambooge extracts showed the best activity against all tested microorganisms producing the widest inhibition zones ranged from 13 to 40 and 8 to 25 mm against bacteria and fungi respectively, followed by Clove extracts with inhibition zones ranged from 11 to 33 and 8 to 22 mm respectively. On the other hand extracts of Marigold, Marjoram, Thyme, Cinnamon and Galangal showed variable antimicrobial activity. Out of all extracts the methanolic extracts of Gambooge, Clove and Marjoram respectively has the strongest potential antimicrobial activity against all tested microorganisms. Fungal species were more resistance than bacterial species and yeast.

The results of the present study showed that in general plant extracts are more effective against Gram-positive than Gram-negative bacteria (except in some cases for *Listeria monocytogenes*). This observation was reported by many authors (Nascimento *et al.*, 2000; Gibbons, 2004, Suffredini *et al.*, 2006 and Gonelimali et al,2018). The resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Ceylan and Fung, 2004).

The most susceptible bacteria among Gram-positives to the tested plant extracts were *Bacillus cereus*, while the least susceptible were *L. monocytogenes*. The most susceptible bacteria among Gram-negative bacteria were *Salmonella enterica* while the least susceptible were *P. aeruginosa*. on the other hand fungal strains showed the highest resistance more than all tested bacteria and yeast. Such results are in agreement with other data reported by (**Shelef** *et al.*, **1980; Marino** *et al.*, **1999 and Rukayadi**, *et al.*, **2013). Gupta** *et al.*, (2009) have reported that *P. aeruginosa* is the least sensitive to the tested Clove extract, which is in agreement with the results recorded in the present study. Antimicrobial and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, Coumarins, Iridoids, Lignans, Steroidals, Saponins, Xanthones, Flavonoids, Flavones, Phenols, essential oils, Steroids, Lactones and Tannins etc, and getting these chemicals out into the herbal extract depends upon the solubility of these compounds in various solvents (Cowan, 1999, Lewis & Ausubel, 2006 and Manso et al, 2022).

In general, aqueous extracts showed less activity than alcoholic extracts, which may be explained in the light of what have been reported by **De Boer** *et al.* (2005), that the same active substances are present in water extracts, but in lower concentrations and/or that some active substances were more soluble in organic solvents and not present in water extracts. Water had limited ability to extract some components from medicinal plants such as oil-based components. Alcohol extraction therefore is recommended to obtain extracts with higher antimicrobial activity.

The mode of antimicrobial action of plant extracts is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electrolyte flow, and active transport, and coagulation of bacterial cell contents (**Burt, 2004 and Saleem** *et al.*, **2010**).

When the methanolic extracts were mixed into food models the antimicrobial activity were reduced and the required minimum inhibitory concentrations (MICs) were duplicated. MICs of Gambooge extract ranged from 0.234 to 3.750, 0.468 to 6.666 and 0.877 to 7.5 mg/ml when tested on microbiological (control) media, meat model and milk model respectively (table 3), while MICs of Clove extract ranged from 0.390 to 4.444, 0.877 to 6.666 and 0.937 to 7.5 mg/ml when tested on control media, meat model and milk model respectively (table 3) and MICs of Marjoram extract ranged from 0.468 to 6.666, 1.316 to 10.000 and 1.875 to 15 mg/ml when tested on control media, meat model and milk model respectively (table 5).

The antimicrobial activity was reduced on semi-skimmed milk model more than that on meat model. This may be due to the protection action of fats, the hydrophobic antimicrobials may migrate to the fatty compounds of the foods, leaving the aqueous fraction, where the microbe develop, free of antimicrobials or may be due to the difference in complexity of the foods. In general, more complex foods are affected less by natural antimicrobial compounds. Food ingredients might serve as barriers to protect bacterial cells from inhibitory substances (**Gutierrez** *et al.*, 2009 and Rattanachaikunsopon & Phumkhachorn, 2010).

Moreover, it is supposed that the high level of fat and/or protein protect the bacteria from action of medicinal plant extracts in some way (Aureli *et al.*, 1992 and Tassou *et al.*, 1995). A reaction between carvacrol, a phenolic component of various plant extracts, and proteins have been suggested as a limiting factor in the antimicrobial activity against *Bacillus cereus* and *Listeria monocytogenes* in the presence of milk protein (Pol and Smid, 1999). Similarly, protein interaction has been suggested as a factor reducing the action of clove oil against *Salmonella spp* in diluted low-fat cheese (Smith-Palmer *et al.*, 2001).

Carbohydrates do not appear to protect bacteria from the action of medicinal plant extracts as much as fat and protein do (Shelef *et al.*, 1984). Moreover, the presence of metal ions such as monovalent cations (Na+ and K+) or divalent (Mg2+ and Ca2+) presented the inactivation effect against most of antimicrobial substances (Anderson and Yu, 2005).

It was reported that antimicrobial effect was reduced by reaction or interaction with food components and a greater concentration of medicinal plants, spices extract or their essential oils were needed to achieve the same effect in foods as in microbiological media (Shelef, 1983; Smid and Gorris, 1999). The ratio has been shown to be approximately two fold in semi-skimmed milk (Karatzas *et al.*, 2001), 10-fold in pork liver sausage (Pandit and Shelef, 1994) and 25-fold in soft cheese (Mendoza Yepes *et al.*, 1997).

Several studies have been reported on the effect of foodstuffs on microbial resistance to medicinal plants extracts and their essential oils, none appears to have quantified it or have explained the mechanism, although suggestions have been made as to the possible causes. The greater availability of nutrients in foods compared to laboratory media (control) may enable microbes to repair damaged cells faster (Gill *et al.*, 2002).

Not only are the intrinsic properties of the food (fat/protein/water content, antioxidants, preservatives, pH, salts and other additives) relevant, the extrinsic determinants (temperature, packaging in

vacuum/gas/air, characteristics of microorganisms) can also influence microbial sensitivity (**Tassou** *et al.*, **1995 and Gutierrez**, *et al.*, **2009**). **Conclusion**:

This study presents the potential use of plant extracts as an effective and safe alternative for food preservation. It also presents several factors influencing the antimicrobial activity including strains of microorganisms, extraction method and type of food. These factors need to be taken into account when plant extracts are used as food preservatives.

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Family	Scientific name	English name	Local name	Part used
Asteraceae (Compositae)	Calendula officinalis	Marigold	Azerion	Flowers
Clusiaceae	Garcinia cambogia	Gambooge	Garcinia	Fruits
Lamiaceae (Labiatae)	Origanum vulgaris	Marjoram	Bardakoosh	Aerial parts
Lamiaceae (Labiatae)	Thymus vulgaris	Thyme	Zaater	Aerial parts
Lauraceae	Cinnamomum burmannii	Cinnamon	Kerfa	Stem bark
Myrtaceae	Syzygium aromaticum	Clove	Koronfel	Floral buds
Zingiberaceae	Alpinia galanga	Galangal	Kholangan	Rhizomes

Table (1): Species of medicinal plants and plant parts tested:

Table (2): Antimicrobial activity of the tested alcoholic and aqueous extracts against 9 tested microbial markers:

		Inhibition zone (mm) *								
Plan t Spec ies	So l.	S. enter ica	P. aerugi nosa	E. coli	B. cereus	S. aureu s	L. monocyt ogenes	A. flavu s	Rhizop us	C. albica ns
	E M	19 22	15 18	17 20	20 25	19 22	17 19	11 10	8 12	20 19
Alpinia galangal	W W D	12	8	0	13	8	0	0	0	19
₩ 8	W I	13	11	8	16	10	0	0	0	11
0 f	Ε	16	12	15	18	12	13	0	0	0

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	Μ	20	10	20	23	15	15	0	0	0
	W D	0	0	0	0	0	0	0	0	0
	W I	0	0	0	0	0	0	0	0	0
m	E	20	15	15	22	16	16	8	8	15
mu inii	Μ	17	17	20	25	18	20	10	10	19
Cinnamomum burmannii	W D	10	0	0	11	8	0	0	0	8
Cinr bu	W I	11	0	0	12	10	9	0	0	10
	Ε	31	19	25	36	34	32	15	17	23
ia gia	Μ	35	22	29	40	36	35	18	20	25
Garcinia cambogia	W D	21	15	16	21	17	13	12	10	15
G ca	W I	25	17	18	25	19	15	14	8	17
	Ε	23	19	21	25	24	20	11	10	16
II II	Μ	27	22	24	27	27	22	13	15	19
Origanum vulgaris	W D	13	10	11	13	10	0	0	0	8
Or v	W I	15	11	12	16	10	8	0	0	11
_	Ε	26	22	23	30	28	24	11	13	20
m	Μ	30	25	26	33	31	29	14	16	22
Syzygium aromaticum	W D	16	12	14	17	14	11	8	9	11
Sya	W I	18	13	16	19	16	13	10	10	13
	Ε	19	15	15	18	20	11	8	8	21
S S	Μ	17	19	20	23	18	14	10	10	19
Thymus vulgaris	W D	10	0	0	11	8	0	0	0	0
T	W I	11	8	0	14	10	0	0	0	10

*Inhibition zones include the paper disc diameter (6 mm), Values calculated as means of triplicates. **Sol.**: solvent. **E**: Ethanol extract, **M**: Methanol extract, **WD**: Water decoction extract , WI: Water infusion extract. 0: No inhibition zone.

Table (3): The minimum inhibitory concentration (mg/ml) ofGambooge methanolic extract on laboratory and food model media:

Microorganism	Control media	Meat model	Milk model
Salmonella enterica	0.877	1.316	1.875
Escherichia coli	0.937	1.975	2.962
Pseudomonas aeruginosa	1.316	1.875	3.750
Bacillus cereus	0.234	0.468	0.877
Staphylococcus aureus	0.390	0.937	1.875
Listeria monocytogenes	0.585	1.316	2.962
Candida albicans	0.877	1.316	1.875
Rhizopus sp	2.962	3.750	4.444
Asperigillus flavus	3.750	6.666	7.500

Table (4): The minimum inhibitory concentration (mg/ml) of Clovemethanolic extract on laboratory and food model media:

Microorganism	Control media	Meat model	Milk model
Salmonella enterica	0.937	1.875	2.962
Escherichia coli	1.316	2.962	3.750
Pseudomonas aeruginosa	1.975	2.962	4.444
Bacillus cereus	0.390	0.877	0.937
Staphylococcus aureus	0.468	0.937	1.316
Listeria monocytogenes	0.877	1.316	2.962
Candida albicans	0.937	1.875	3.750
Rhizopus sp	3.750	4.444	6.666
Asperigillus flavus	4.444	6.666	7.500

Table (5): The minimum inhibitory concentration (mg/ml) ofMarjoram methanolic extract on laboratory and food model media:

Microorganism	Control media	Meat model	Milk model
Salmonella enterica	1.316	1.975	2.962
Escherichia coli	1.975	2.962	3.750
Pseudomonas aeruginosa	1.975	2.962	4.444
Bacillus cereus	0.468	1.316	1.875
Staphylococcus aureus	0.585	1.975	2.962
Listeria monocytogenes	0.877	1.316	1.975
Candida albicans	1.875	1.975	2.962
Rhizopus sp	6.666	7.500	10.000
Asperigillus flavus	6.666	10.000	15.000

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