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PHYTOCHEMICAL INVESTIGATION AND COMPARISON OF ANTIMICROBIAL SCREENING OF *CLOVE* AND *CARDAMOM*

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ABSTRACT

The aim of present study was to investigate the phytochemical screening and to compare the antimicrobial activity of oils of Clove bud and Cardamom. Clove bud was successively extracted by steam distillation and isolated with Dichloromethane. The phytochemical analysis revealed the presence of alkaloids, glycoside, steroids, carbohydrates, terpenoids, tannins and phenolic compound. The dichloromethane extract was chromatographed over silica Gel (60-120) and eluted with pure toluene, toluene: Dichloromethane (9:1), toluene: Dichloromethane (8:2), toluene: Dichloromethane (7:3), fraction were monitored by T.L.C. similar fractions were combined and concentrated .eleven fractions were obtained and were labelled as f1, f2, f3 to f11. Cardamom fruit was successively extracted with petroleum ether. The phytochemical analysis revealed the presence of alkaloids, glycoside, steroids, protein, carbohydrates, terpenoids, tannins and phenolic compound. The Petroleum ether extract was chromatographed over silica Gel (60-120) and eluted with pure Benzene, Benzene: chloroform (9:1), Benzene: chloroform (8:2), Benzene: chloroform (7:3), Benzene: chloroform (6:4), Benzene: chloroform (5:5), Benzene: chloroform (4:6), and with pure chloroform. Fractions were monitored by T.L.C. similar fractions were combined and concentrated. Fourteen fractions were obtained were labelled as fcd1, fcd2 to fcd14. Antimicrobial activity was performed by Disc diffusion method on the *staphylococcus aureus* (+ve), *Escherichia coli* (–ve), *Pseudomonas aerugenosa* (-ve) bacteria and was found that cardamom and clove extract both were similar active for *Pseudomonas aerugenosa* (-ve) but cardamom was more active for *E. coli* than clove extracts.

INTRODUCTION: Clove (*Eugenia caryophyllata* Thunb.) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China. Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavouring agent and antimicrobial material in food [1, 2]. For example, clove oil was effective against *L. monocytogenes* and *S. Enteritidis* in tryptone soya broth (TSB) and cheese [3]. The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability [4]. Essential oils are well known inhibitors of microorganisms.

Clove oils are natural preservative and flavouring substances that are not harmful when consumed in food products. There have been a number of reports of substances in each of clove oils that inhibit the growth of molds, yeasts and bacteria. Clove oil added at 2% in potato dextrose agar (PDA) completely inhibited the growth of seven mycotoxigenic molds (*A. flavus, A. parasiticus, A. ochraceus, Penicillium sp*. M46, *P. roqueforti, P. patulum*, and *P. citrinum*) for various times up to 21 days [5].

Similarly reported that cinnamon oil and clove oil could separately inhibit many other microbes including *Lactobacillussp., Bacillus thermoacidurans, Salmonella sp., Corynebacteri-ummichiganense, Pseudomonas striafaciens, Clostridium botulinum, Alternaria sp., Aspergillus sp., Canninghamella sp., Fusarium sp., Mucor sp., and Penicillium sp*.[6]. The essential oil, isolated from the fruits of *E. cardamomum* showed antimicrobial [7]. The objective of the research was to study the inhibitory effects of cardamom and clove oils, added singly and in various combinations on growth of bacteria.

MATERIALS AND METHODS:

Plant materials: The clove bud (eugenia caryophyllus) and cardamom (amomum subulatum) which was collected from Sanjeev pharmaceutical pvt. Limited, Lucknow district of U.P. in the month of April 2008 was authenticated by the plant taxonomist Dr. A. K. Sharma, Department of botany, M. M. P. G. College, Modinagar (U.P.). Bacterial and fungal strains were obtained from the I.T.S. paramedical college Delhi-Meerut road Muradnagar Ghaziabad (U.P.). Which were obtained already from the institute of microbial Technology Chandigarh, India.

Nutrient Medium:

Beef extract	: 500mg
Yeast extract	: 1.5gm
Peptones	: 5 gm
Agar	: 7.5gm.
Distilled water	: 500ml

Sterilization: The sterilization of media, culture tubes pipettes and other materials were done by autoclaving at 15lb/sq. inch pressures for 30 min.

Standard Drug: Amikacin (A): 30 µg/ ml.

Preparation of Drug Sample: Clove and cardamom extract was dissolved in DMSO solvent and make different concentration sample such as100 µg/ml, 200µg/ml and 1mg/ml

Bacterial Stock Culture: A loopful of bacterial strain were transferred to nutrient medium and incubated overnight at 37 ^{0}C. The number of colony forming unit were found to be 10^3 per ml.

Method employed for antimicrobial studies:

Disc diffusion method: Antimicrobial study was determined by disc diffusion method [8].The nutrient agar plates were prepared by pouring 20 ml of molten media into sterile Petri plates. The

plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compounds were loaded on 5mm discs. The loaded discs were placed on the surface of medium and the compounds were allowed diffuse for 5 minutes and the plates were kept for incubation at 37 °C for 24 hr for bacteria with yeast peptone and agar and medium. At the end of incubation, inhibition zones formed around the discs were measured after 18 hr, 20 hr, and 48 hr.

Extraction of clove oil: The clove buds was dried at room temperature and prepare a fine powdered with grinder. The fresh fine powdered of clove buds (50gm) are packed in 500 ml R.B.F. and set up steam distillation apparatus.

Procedure: Add 300ml of distilled water and boiling chip to the flask. Mix well with a glass stirring rod and add 300ml distilled water in another round bottom flask which is indicated as 2 and set up a steam distillation unit. Use the 250ml iodine flask as receiver and kept ice at the bottom of flask so that volatile oil not evaporates water is allowed to run in condenser so that distillate can be condensed. Heat the 500 ml round bottom flask with heating mantle in which distilled water are kept and adjust the temperature of heating mental to maintain the distillation rate of approximately one drop every 3-5 second. Add the water to the round bottom flask at 10-min intervals to keep the water level at the mark. Stop the distillation when approximately 100 ml of distillate.

Isolation of clove oil: Take 100 ml of distillate immediately after end of extraction and allow the receiver to cool at room temperature. Carefully pour the distillate from the receiver into a 125 ml separating funnel. Add 10 ml of saturated Sod. Chloride solution by using Pasteur pipette, carefully rinse the condenser neck of the flask with 5ml of dichloromethane. Swirl the flask gently to dissolve the remaining clove oil. Add the dichloromethane to the distillate in the separating funnel. Cap the separating funnel and gently swirl the contents for five min. After the pressure has been ventured, shake the contents vigorously to mix the two layers. Swirl the separating funnel. At the same time, gently cap it the separating funnel with your index finger to force the cap and allow the layers to separate. Drain the lower dichloromethane into a 50 ml Erlenmeyer flask, making certain that none of the aqueous layer is transferred to the flash.

Rinse the condenser and the receiver with a second 5ml portion of dichloromethane. Transfer the rinsed to the separately funnel. Repeat the extraction of the aqueous layer. Drain out the second dichloromethane extract from the separately funnel and combine it with the first one in the 50ml Erlenmeyer flask. Repeat the rinsing and extraction process with a third 5ml portion of dichloromethane. Combine the third extraction in the same 50ml Erlenmeyer flask. Add approximately 0.5 g of anhydrous sodium sulphate to the flask containing the dichloromethane exacts. Stopper the flask. Allow the extracts to dry for 5 min. Use three additional 2ml portion of dichloromethane to rinse the sodium sulphate and ensure complete transfer of the clove oil to the beaker. Assembled a simple distillation apparatus using the 50-ml round bottom flask as the pot. Add a boiling chip. Use a 40 C sand bath or a hot water bath to obtain the mass of the clove oil.

Isolation and Extraction of cardamom oil:

Hot Continuous Extraction/Soxhletion: The use of commercially available soxhlet extract or convenient way to prepare crude plant extracts. This procedure is used mainly pure solvent. In this method the material to be extracted is placed in a thimble made of cellulose or cloth in a central compartment with a siphoning device and side

arm both connected to the lower arm. The solvent is placed in lower compartment and a reflux condenser is attached above the central sample compartment. Note that each component of the set up (solvent, sample compartment and reflux condenser) is a separate item of glass ware which is assembled together with a appropriate contents, to make the complete apparatus. The solvent in the lower container (usually round-bottomed flask) is heated to boiling, and vapour passes through the side arm up into the reflux condenser here the vapour liquefies and drip in to the thimble containing the material to be extracted. The warm solvent percolate through the material and the wall of thimble and the extracted gradually collect in the central compartment. Once the height of the extract reaches the top of the siphon, entire liquid in the central compartment flows through this and back in to the lower solvent container. The process is then repeated.

In this method, the extract collect in the lower vessel, gradually becoming more and more concentrated. Assuming that no volatile substance is present, the vapour raising from the heated extract is pure solvent vapour and the so liquid dripping in the material from the condenser is essentially pure solvent, through derived from the extract. Thus, although a relatively small volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The soxhlet process is useful for the exhaustive extraction of plant material with a particular solvent, e.g. for defatting or where 100% yield of a particular component is desired, e.g. Hexane, chloroform, methanol and water.

However it is necessary to dry the plant material in between changes of solvent to prevent carry-over traces of previous. Solvent into the next one varying size of soxhlet apparatus are available to suit the scale of operation.

Preliminary phytochemical screening:

Colour test of clove oil: Three different tests to determine the Eugenol in Solution.

1. **Bromine test**: When a reaction occurs the yellow colour disappears and a clear solution is obtained.

2. **Permanganate test**: the solution is originally purple, and when it reacts with a double bond, or eugenol, it loses the purple colour and forms a precipitate.

3. **Phenol test**: If there is an OH^- group, then the originally yellow solution will turn very dark.

Qualitative Tests Procedure for Clove: The clove oil obtained from steam distillation followed by extraction with Dichloromethane were subjected to preliminary qualitative tests for the presence of Carbohydrate, Protein, Steroid, Glycosides, Alkaloids, Terpenoid and steroid [9].

1. Test for Carbohydrates:

- **Molish Test:** To about 2ml of extracts few drops of α-naphtha (20% in ethyl alcohol) was added. Then about 1ml of conc. H_2SO_4 was added along the side of the test tube- reddish violet ring at the junction of the two layer s appeared in the presence of carbohydrates.

- **Reduction of Fehling's Solution:** 10 ml of Fehling solution (copper sulphate in alkaline condition) were added to the concentrated extracts and heated on a steam bath. Brick-red precipitates indicated the presence of carbohydrate.

1. Test for Proteins:

- **Biuret Test:** To 3 ml of extracts was added 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears.

- **Million's Test:** Mixed 3 ml extracts with 5ml Million's reagent. White ppt. warm ppt. Turns brick red or the ppt. dissolves giving red coloured solution.

2. Test for Steroid

- **Salkowski Reaction:** To 2 ml of extracts was added 2 ml of chloroform and 2ml of concentrated H_2SO_4 shake well. Chloroform layer shows greenish yellow fluorescence.

- **Libermann-Bruchard Reaction:** The extracts were evaporated to dryness and the residues were extracted with petroleum ether and acetone. The insoluble residues left after extraction were dissolved in chloroform and few drops of acetic anhydride were added along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to blood red color indicates the presence of sterols in the extracts.

3. **Test for Glycosides:** About 2 ml of extracts were taken separately and subjected to the following tests:-

- **Keller- Killani Test:** 1 ml of glacial acetic acid containing traces of $FeCl_3$ and 1ml of concentrated H_2SO_4 were added to the extracts carefully. A reddish- brown colour is formed at the junction of two layer and the upper layer turns bluish green in presence of glycosides.

- **Borntrager's Test:** 1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the extracts. A reddish colour was formed in the presence of glycosides.

- **Legal Test:** Concentrated extracts were made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added to the solution. Presence of blue colour solution indicated the presence of glycosides in the extracts.

4. **Test for Alkaloids:** 5 ml of the alcoholic extracts of each extracts were evaporated to dryness the alcoholic residue were taken in 5ml of 2% hydrochloric acid, saturated with sodium chloride and filtered. The filtrates were taken with alkaloids reagent like:-

- **Dragendroff's Test:** To 2-3 ml filtrate, add few drops Dragendroff's reagent-Orange brown ppt. is formed.

- **Wagner's Test:** To 2-3 ml filtrate with few drops Wagner's reagent gives reddish brown precipitate.

Thin layer chromatography: 50 gm of silica gel G was weighed and 100 ml of distilled water was added and shake to make a homogeneous slurry. This slurry was poured into TLC applicator. This was adjusted to 0.25 mm thickness. 20 carrier plates were laid together in a row and coated with silica gel by drawing the applicator. The plates were allowed to dry at room temp and then dried at 110 ^{0}C for 30 minutes in hot air oven.

Solvent system:

Toluene : Dichloromethane (9:1)

Detecting reagent : Vanillin sulphuric acid reagent (in which 1% Ethanolic Vanillin + 1% Ethanolic sulphuric acid)

Qualitative Tests for Cardamom: The cardamom oil obtained from steam distillation followed by hot soxlate extraction with petroleum ether were subjected to preliminary qualitative tests for the presence of Carbohyd-rate, Protein, Steroid, Glycosides, Alkaloids, Terpenoid and steroid [9].

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Thin layer chromatography: 50 gm of silica gel G was weighed out and shaken to form a homogeneous suspension with 100 ml of distilled water. This suspension was poured into TLC applicator. Which was adjusted to 0.25 mm

thickness.20 carrier plates were laid together in a row and coated with silica gel by drawing the applicator. The plates were allowed to dry at room temp and then dried at 110^0 C for 30 minutes in hot air oven.

Solvent system : Benzene: chloroform (7:3)
Detecting reagent : Iodine vapour

RESULT AND DISCUSSION: Extraction of clove bud, *Eugenia caryophyllus* was done by steam distillation and isolation with the dichloromethane. Then obtained extract had subjected to phytochemical analysis which revealed the presence of alkaloids, glycoside, steroids, carbohydrates, terpenoids, tannins and phenolic compound (**table 1**).

TABLE.1: DATA FOR PHYTOCHEMICAL SCREENING OF CLOVE OIL & CARDAMOM OIL

CLOVE OIL		CARDAMOM OIL	
Tests	Dichloromethane extract	Tests	Dichloromethane extract
Carbohydrates		**Carbohydrates**	
1)Molisch test	+	1)Molisch test	+
2)Fehling's test	-	2)Fehling's test	-
Proteins		**Proteins**	
1)Biuret test	-	1)Biuret test	+
2)Million's test	-	2)Million's test	-
Terpenoids		**Terpenoids**	
Liebermann Burchard test	+	Liebermann Burchard test	+
Steroids and sterols		**Steroids and sterols**	
1) Salkowsky test	+	1) Salkowsky test	-
2) Libermann Burchard test	+	2) Libermann Burchard test	+
Glycosides		**Glycosides**	
1)Killer-Killani test	+	1)Killer-Killani test	+
2)Borntrager's test	+	2)Borntrager's test	+
3)Legal test	+	3)Legal test	+
Test for saponin	-	**Test for saponin**	-
Alkaloids		**Alkaloids**	
1)Dragendroff's test	+	1)Dragendroff's test	+
2)Wagner's test	+	2)Wagner's test	+
Tannins and Phenolic compounds		**Tannins and Phenolic compounds**	
1) $FeCl_3$ (5%)	+	1) $FeCl_3$ (5%)	+
2) Br_2 water	+	2) Br_2 water	+
3) Potassium dichromate	+	3) Potassium dichromate	+
Test for flavonoids		**Test for flavonoids**	
1) Ammonia test	-	1) Ammonia test	-

+ indicates presence; - indicates absence

After phytochemical analysis extract were subjected to column chromatography and eluted with pure toluene, toluene: Dichloromethane (9:1), toluene: Dichloromethane (8:2), toluene: Dichloromethane (7:3), yielded eleven compounds and were labelled as f1, f2, f3 to f11. Extraction of cardamom, *Amomum subulatum* was done with petroleum ether. Thus obtained extract were subjected to phytochemical analysis which showed the presence of alkaloids, glycoside, steroids, protein, carbohydrates, terpenoids, tannins and phenolic compound (table 1). After phytochemical analysis extract were subjected to column chromatography and eluted with pure Benzene, Benzene: chloroform (9:1), Benzene: chloroform (8:2), Benzene: chloroform (7:3), Benzene: chloroform (6:4), Benzene: chloroform (5:5), Benzene: chloroform (4:6), and with pure chloroform yielded fourteen compounds and were labelled as fcd1, fcd2 to fcd14 (**Table 2** and **3**).

UV and IR spectra of compounds are characteristics of functional groups present.UV spectra of clove compounds (f1-f11) showed absorption at 205.6 nm, 220nm, 280nm, 212nm, 221nm, 267nm,218nm, 259nm, 218nm, 258nm, 217nm, 259nm, 217 nm, 259nm, 216nm, 259nm, 216nm, 259nm, 216nm, 259nm, 216nm, 259nm, 220 nm. UV maximum absorption of clove compound first at 228nm could be attributed to the $-NH_2$ group.

UV spectra of cardamom compounds showed absorption at 253nm, 203nm, 220nm, 248nm, 252nm, 224nm, 252nm, 224nm, 224nm, 223nm, 226nm, 224nm, 215nm, 249nm, 224nm, 224nm. UV maximum absorption of cardamom compound first at 253nm could be attributed to the benzene.

The I.R. spectrum of clove compounds had a band at 3518 cm^{-1} which was attributed to absorption by association –OH group and band at 3418 cm^{-1}. The later could be assigned to 2852 cm^{-1} (C-H) vibration, 1713cm^{-1} (C=O) saturated aldehyde, 1608 cm^{-1} (C=C) alkene, 1512 cm^{-1} (C=C) aromatic, 3458 cm^{-1} (O-H) alcoholic and Phenolic. The I.R. spectrum of cardamom compounds had a band at 3440 cm^{-1} which was attributed to absorption by association –OH group (phenol). The latter could be assigned to 2922 cm^{-1} (C-H) vibration, 2852 cm^{-1} (C-H) vibration,1744 (α-keto ester), 1734cm^{-1} (C=O) saturated aldehyde, 1261 cm^{-1} (O-H) secondary alcoholic.

Antimicrobial activity of clove and cardamom extract was performed by Disc diffusion method on the *staphylococcus aureus* (+ve), *Escherichia coli* (–ve), *Pseudomonas aeruginosa* (–ve) bacteria and found that petroleum ether extract of cardamom were more active than clove extract and clove compound and is less active as compare to standard compound Amikacin as antibacterial which was shown in **table 4**.

TABLE 2: I. R. DATA OF CLOVE FRACTIONS (F1-F11)

Fractions		Spectral data of Clove fraction
F1	I. R. data	3518 (O-H), 2938 (-CH_3), 2441 (C-H), 1638 (C=C), 1608 (N-H,C=C), 1513 (C=C)
F2	I. R. data	3523 (O-H), 2937 (C-H), 1608 (C=C), 1513 (C=C), 1431 (C-H)$_b$, 1367(C-H)$_b$, 1267 (C-O), 1234 (C-O & O-H)
F3	I. R. data	3531 (0-H), 2924 (C-H), 2853 (C-H) ,1764 (ester stretching), 1605 (C=C), 1512 (C=C), 1459 (C-H)$_b$ 1369 (C-H), 1268 (C-O)
F4	I. R. data	3448 (O-H),2922 (C-H),5852 (C-H), 2367 (C-H)$_b$,1633 (C=C)
F5	I. R. data	3458 (O-H), 2922 (C-H), 5852 (C-H), 1648 (C=C)
F6	I. R. data	2921 (C-H), 2852 (C-H),1737 (C=O), 1511 (C=C) ,1459 (C-H)$_b$
F7	I. R. data	2923(C-H), 2853 (C-H), 1713(C=O), 1604 (C=C),1512 (C=C)
F8	I. R. data	2923 (C-H), 2853 (C-H), 1713 (C=O), 1512 (C=C)
F9	I. R. data	2924 (C-H), 2853(C-H), 1743(α- keto ester)
F10	I. R. data	2924 (C-H), 2854 (C-H), 1743 (α- keto ester)
F11	I. R. data	3448 (O-H), 2927 (C-H), 2859 (C-H), 1711 (C=O), 1456 (C-H), 1371 (C-H)

TABLE 3: I.R. DATA OF CARDAMOM FRACTION (FCD1-FCD)

Fractions		Spectral data of Cardamom fraction
Fcd1	I. R. data	2962(C-H), 2924(C-H), 2853(C-H), 1463(C-H)$_b$, 1261(O-H) & (C-O)$_b$
Fcd2	I. R. data	2961(C-H), 2922(C-H), 2852(C-H), 2360(C-H)$_b$, 1261(O-H)$_b$ & (C-O)
Fcd3	I. R. data	2923(C-H), 2857(C-H), 1743(α-keto ester), 1461(C-H)$_b$, 802(C-H)$_b$
Fcd4	I. R. data	2924(C-H), 2854(C-H), 2360(C-H)$_b$, 1746(α-keto ester), 1464(C-H)$_b$, 1164(C-N)
Fcd5	I. R. data	3440(O-H), 2924(C-H), 2853(C-H),2358(C-H)$_b$, 1795(α- keto esters), 1965(C-H)$_b$,
Fcd6	I. R. data	3449(O-H), 2922(C-H), 2853(C-H), 2362(C-H)$_b$, 1744(α- keto esters) 1462(C-H)$_b$
Fcd7	I. R. data	2955(C-H), 2922(C-H), 2852(C-H), 1732(C=O), 1463(C-H)$_b$,1186(C-H), 1080(C=S), 966(C-H)$_b$
Fcd 8	I. R. data	2922(C-H), 2853(C-H), 2364(C-H)$_b$, 1746(α- keto esters), 1462(C-H)$_b$, 1379(C-H)$_b$, 1187(C-N), 966(C-H)$_b$
Fcd 9	I. R. data	2923(C-H), 2853(C-H), 1745(α- keto esters), 1463(C-H)$_b$, 1186(C-N), 1080(C=S), 967(C-H)$_b$
Fcd10	I. R. data	2923(C-H), 2853(C-H), 2364(C-H)$_b$, 1711(C=O), 1554(N-H)$_b$, 1461(C-H)$_b$
Fcd11	I. R. data	3449(O-H), 2924(C-H), 2853(C-H), 1740 (α- keto esters), 1459(C-H)$_b$, 1509(C=C)
Fcd12	I. R. data	2919(C-H), 2851(C-H), 2659(O-H), 1702(C=O), 1461(C-H)$_b$,
Fcd13	I. R. data	3432(N-H), 2924(C-H), 2854(C-H), 1725 (cyclic γ-lactams, 1019(C-X
Fcd14	I. R. data	3439(N-H), 2920(C-H), 2851(C-H), 2356 (C-H)$_B$, 1734 (C=O), 1460 (C-H)

TABLE 4: ANTI BACTERIAL ACTIVITY AFTER 18 HRS AND 48 HRS

Sample	Concentration (mg/disc)	Zone of inhibition (in mm) after 18 hr			Zone of inhibition (in mm) after 18 hr		
		Gram negative Bacteria		Gram positive Bacteria	Gram negative Bacteria		Gram positive Bacteria
		E. coli	*P. aerugenosa*	*S. aureus*	*E. coli*	*P. aerugenosa*	*S. aureus*
Amikacin	30 µg/disc	20	21	19	20	21	19
Petroleum ether extract of cardamom	100 µg/ml	7	8	6	7	8	6
	200 µg/ml	7	8	8	7	9	8
	1 mg/ml	8	10	9	8	10	9
Clove oil extract	100 µg/ml	5	7	9	7	7	9
	200 µg/ml	6	8	9	7	7	9
	1 mg/ml	8	8	11	10	8	11
Clove oil fraction	100 µg/ml	6	10	6	8	8	7
	200 µg/ml	7	8	6	8	8	8
	1 mg/ml	6	9	7	7	9	7
Mixture of clove oil & cardamom	1mg/ml	6	5	5	6	5	8

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REFERENCES:

1. Huang Y, Ho SH, Lee HC, Yap YL: Insecticidal properties of eugenol, isoeugenol and methyleugenol and their effects on nutrition of Sitophilus zeamais Motsch. (Coleoptera: Curculionidae) and Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae).Journal of Stored Products Research 2002; 38:403–412.
2. Velluti A, Sanchis V, Ramos AJ, Marı´n S: Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by Fusarium proliferatum in maize grain. International Journal of Food Microbiology2003; 89 :145–154.
3. Smith-Palmer A, Stewart J, Fyfe L: Antimicrobial properties of plant essential oils and essences against five important food borne pathogens. Letters in Applied Microbiology1998; 26:118–122.
4. Wenqiang G, Shufen L, Ruixiang Y, Shaokun T, Can Q: Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. Food Chemistry 2007; 101: 1558–1564.
5. Azzouz MA, Bullerman LB: Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. Journal of Food Protection 1982; 45: 1298– 1301.
6. Matan N, Rimkeeree H, Mawson AJ, Chompreeda P, Haruthaithanasan V, Parker M: Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. International Journal of Food Microbiology 2007; 107: 180 – 185.
7. Jamal A, Javed K, Aslama M, Jafri MA: Gastroprotective effect of cardamom, *Elettaria cardamomum* Maton. Fruits in rats. Journal of Ethnopharmacology 2006; 103:149–153.
8. Awerbuch TE, Lustman L, Piret JM: A Numerical Method to Determine Minimal inhibitory concentration (MICs) of antibiotics directly disc diffusion susceptibility test. Journal of Microbiological method 1989; 9:1-7.
9. Khandelwal KR:Book of practical Pharmacognosy. Nirali Prakashan Pune 2004:149-153

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