

**WELCOME TO
MY
PRESENTATION**





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TITLE

**STUDY ON NUTRITIONAL COMPOSITION, BIOACTIVE
COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF THE
CLOVE (*Syzygium aromaticum*)**



**DEPARTMENT OF APPLIED CHEMISTRY AND CHEMICAL TECHNOLOGY
FACULTY OF FOOD SCIENCE & TECHNOLOGY
CHATTOGRAM VETERINARY & ANIMAL SCIENCES UNIVERSITY**

OUTLINE OF THE PRESENTATION



- **Background**
- **Aims & Objectives**
- **Materials & Methods**
- **Results & Discussion**
- **Conclusions**
- **Recommendations & Future Perspectives**

BACKGROUND

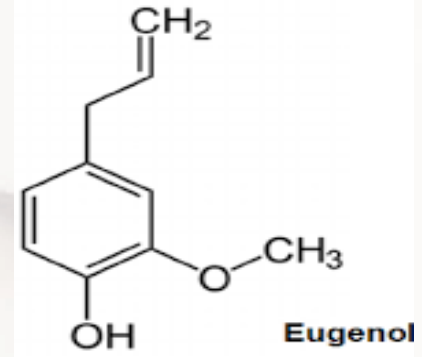


- Clove (*S. aromaticum*) is one of the most valuable spices (Phytother. Res. 21, 2007).
- Cloves are a very useful herb that has many uses for the body and the aroma.
- Clove is native of Indonesia but nowadays is cultured in several parts of the world (Phytother. Res. 21, 2007).
- The term ‘Clove’ is derived from the ‘Clou’ (French word) and the ‘Clout’ (English word) (Chaieb et al., 2007a).
- This plant represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate (D. Francisco et al., 2014).

BACKGROUND



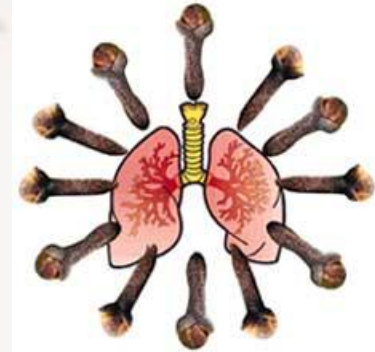
- Main components of clove bud oil such as eugenol, caryophyllene were identified by GC-MS (Nazrul et al., 2010).
- The high levels of eugenol contained in clove essential oil responsible for strong antimicrobial activity (Huang et al., 2002; Velluti et al., 2003).
- Eugenol act as a natural antioxidant.
- Antioxidants are effective for inhibiting different human diseases (Zengin and Baysal, 2014).



BACKGROUND



- Clove has been used as food preservative and for many medicinal purposes (Parle and Khanna, 2011).
- Cloves can effectively prevent the lung cancer as well as the skin cancer (Parle Milind et al., 2011).
- Cloves benefit the diabetic patients by controlling the blood glucose levels. (Chaieb K et al., 2007).



BACKGROUND



➤ Clove is used as strength against gastro-intestinal spasm, stomach distension and flatulence (Elujoba et al., 2005).



BACKGROUND



- Clove essential oil used as an antimicrobial, anticancer, antibiotics (David & Gordon, 2012).
- The proximate chemical composition of clove as follows: moisture 10%, fiber 20%, ash 5.2%, protein 1.2%, fat 12.1% and carbohydrates 51.5% (Abdel M. 2007).
- Clove has physical, mental and emotional health benefits.
- Cloves represent one of the Mother Nature's premier antiseptic (Parle Milind et al., 2011).

HEALTH BENEFITS OF CLOVES



Anti-Bacterial

Anti-fungal

Antiseptic

Cancer Prevention

Powerful germicidal

Cardiovascular Health

Reduce Hypertension

Cholera Prevention

Mosquito Repellent

Blood Purifier

Indigestion

Toothache

Headaches

Nausea



AIMS & OBJECTIVES

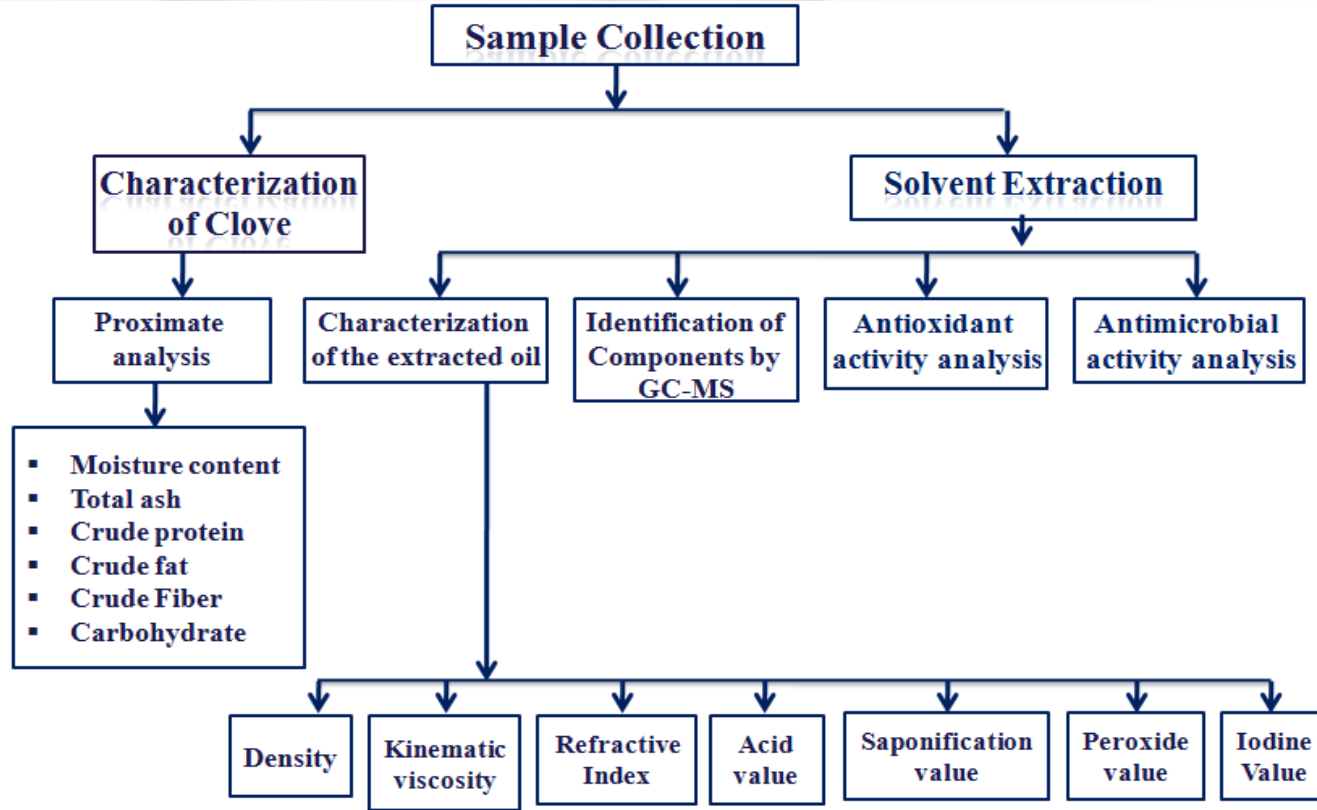


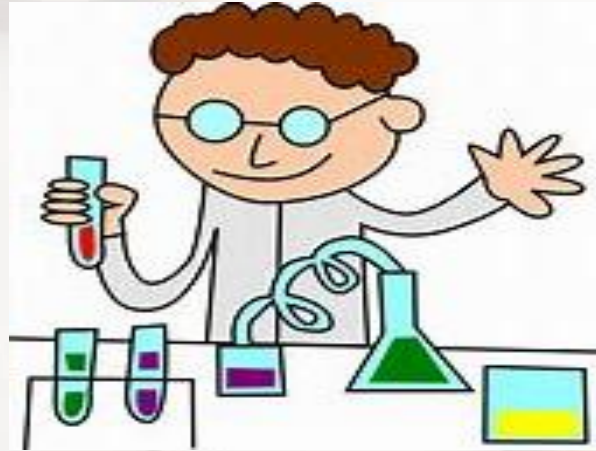
- Extraction and proximate analysis of three brands clove.
- To quantify the bioactive compounds (phenolic content, flavonoid content and anthocyanin content) and antioxidant activity (AOA) of clove essential oil.
- To determine antimicrobial activity of essential oil of clove.
- To identify the unknown compounds in clove oil by GC-MS.



EXPERIMENTAL DESIGN

EXPERIMENTAL DESIGN





MATERIALS & METHODS

Extraction of essential oil of clove



Experimental work was conducted using solvent extractor. In solvent extraction process, petroleum ether was used as a solvent.



Clove powder



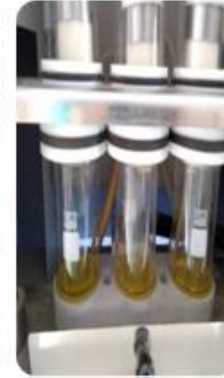
Measuring



Immersion



Washing



Recovery



Oil collection

Physicochemical properties of CEO



Determination of Acid Value

- The no. of mg of KOH needed to neutralize the free fatty acids present in 1g of oil.
- Acid Value of oil samples were determined by standard method described in AOAC (2016) for oils and fats.

Determination of Saponification Value

- The weight of KOH expressed in mg, required to saponify of 1 g of the oil or fat.
- Saponification values of oil samples were estimated according to AOAC (2016).



Titration with 0.1N KOH



Adding indicator

Physicochemical properties of clove essential oil



Determination of Peroxide Value

- ✓ The content of peroxide oxygen per 1 kilogram of fat or oil.
- ✓ Peroxide value was determined according to AOAC Official Method.



Titration with unknown $\text{Na}_2\text{S}_2\text{O}_3$ solution

Determination of Iodine value

- ✓ Iodine value is expressed in g of iodine absorbed by 100 g of oil.
- ✓ Iodine value was determined according to AOAC (2016) Official Method.



Titration with 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ solution



Proximate composition analysis



Determination of moisture:



Weighting sample



Keeping oven at 105°C for 24 hr



Placed in desiccators

DETERMINATION OF ASH



Weighting sample



burne



placed in muffle furnace



Weighting burned sample



placed in an oven



DETERMINATION OF CRUDE FIBER



Taking sample



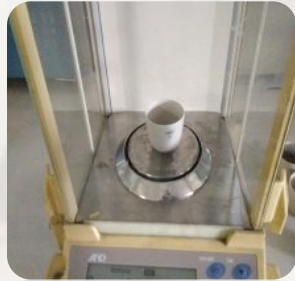
Adding H_2SO_4



Boiling



Washing



Weighting



Muffle furnace



placed in an oven



Taking residue

DETERMINATION OF PROTEIN



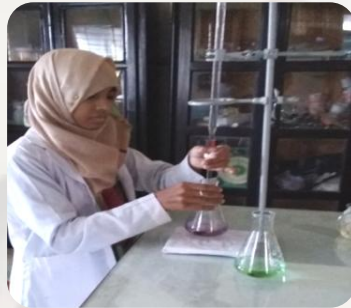
Digestion



Adding boric acid



Adding beaker



Titration



Taking beaker

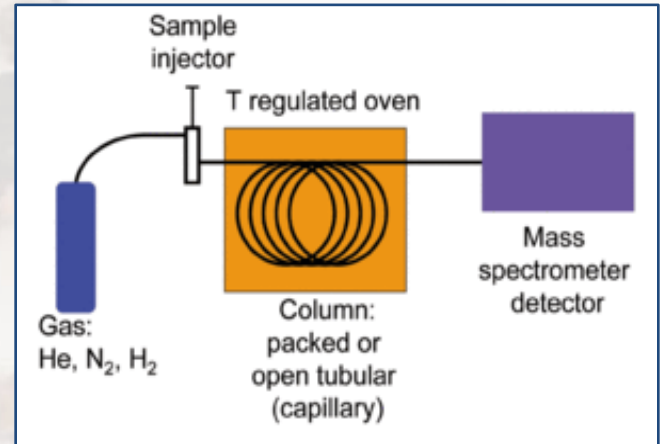


Distillation

GC-MS ANALYSIS



- The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (He).
- The sample flows through the column and compounds mixture are separated by virtue of their relative interaction with the coating of the column (stationary phase) and the carrier gas (mobile phase).



ANTIOXIDANT ACTIVITY (AOA)



- Antioxidant capacity of the extracts was determined using DPPH assay.
- The absorbance was read at wavelength 517 nm using UV-VIS spectrophotometer (UV-2600, Shimadzu Corporation, USA).
- Trolox used as standard.
- TEAC composite (Trolox equivalent antioxidant capacity) was used for the calibration standard curve.

DETERMINATION OF ANTIOXIDANT ACTIVITY (AOA)

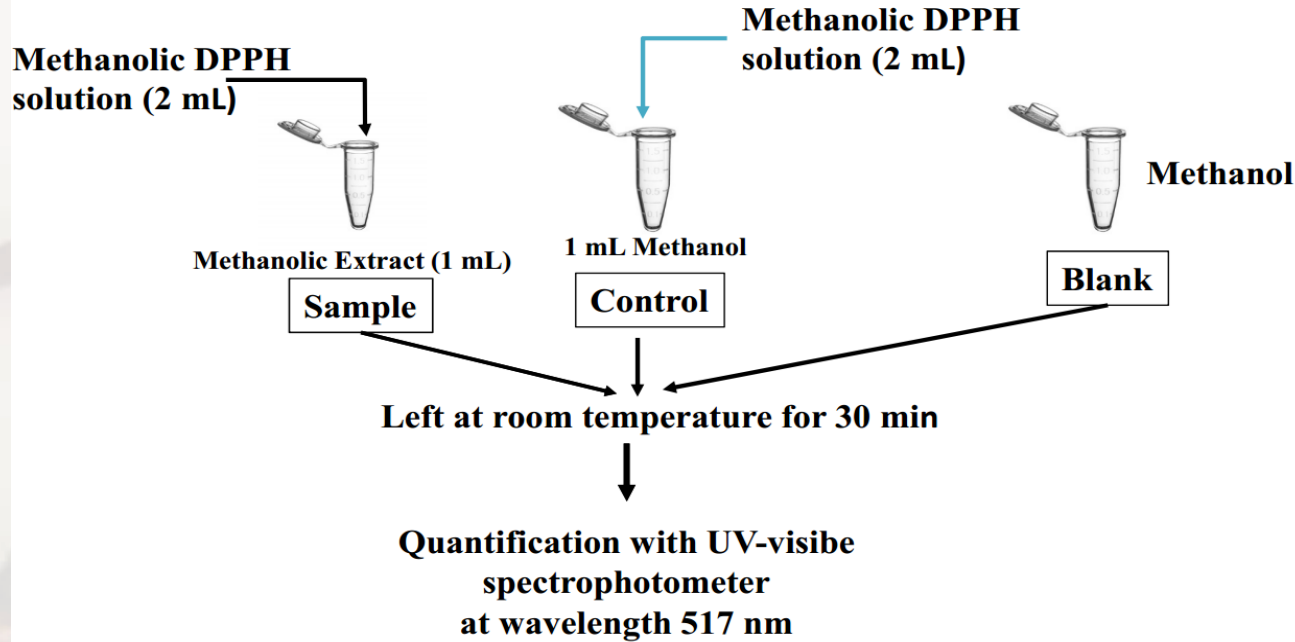
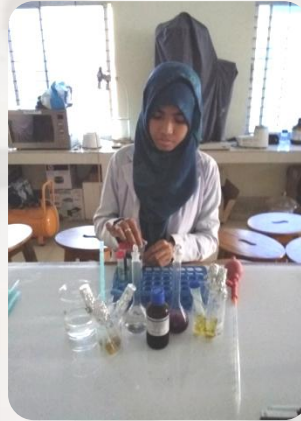


Figure : Antioxidant activity (AOA) determination procedure

DETERMINATION OF ANTIOXIDANT ACTIVITY (AOA)



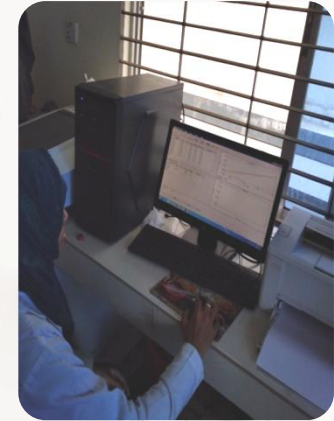
Adding methanolic
DPPH solution



Left for 30 min



UV-Visible
spectrophotometer



Quantification with
UV-visible
spectrophotometer

BIOACTIVE COMPOUNDS

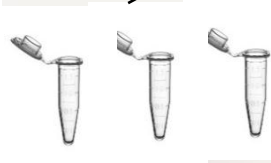


- ✓ The term "bioactive" is consisting of two words: *bio-* and *-active*.
- ✓ A bioactive compound is just a constituent that has a biological action.
- ✓ Bioactive compounds are not nutrients they are contained in foods or their constituents.
- ✓ "Bioactive compounds" are essential and non-essential composites that occur in nature.
- ✓ Bioactive compounds like total flavonoids content (TFC), total phenolic content (TPC) and total anthocyanin content (TAC).

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)



**Stock solutions
of Extract**



**Standard solutions
of gallic acid**

**Adding
Diluted FC
reagent (1.5
mL)**

**Left
for 3
min**

**Adding 1.5
mL of sodium
carbonate (75
g/L) solution**

**Left
for
60 min**

**Quantification
with UV-visibe
spectrophotome
ter at
760 nm**

TOTAL FLAVONOIDS CONTENT



Total Flavonoids Content (TFC) of the clove oil samples were determined by using the aluminum chloride colorimetric process reported by Chang et al. (2002) with slight modifications.



Reagents



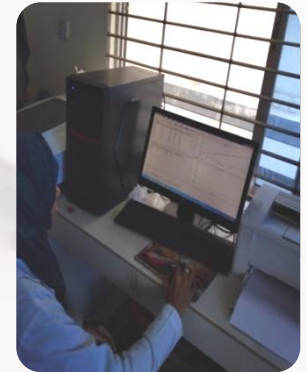
FC reagent



Na_2CO_3 solution



Quantification with UV-visible spectrophotometer



ANTIMICROBIAL ACTIVITY



- Antibiotic as originally defined was a chemical substance produced by various species of microorganisms that was capable of inhibiting the growth or kill the microorganism .
- The ability that a drug kills or suppresses the growth of microorganisms.
- Protect against pathogenic insects, bacteria, fungi or protozoa.
- Volatile gas combination of cinnamon and clove oil inhibit growth of spoilage fungi, yeast and bacteria.



CULTURE SENSITIVE TEST AT MULLER HINTON AGAR



Sterile saline solution



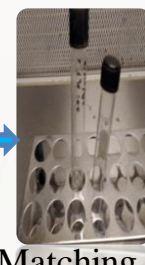
Bacterial culture



1 loop bacterial culture



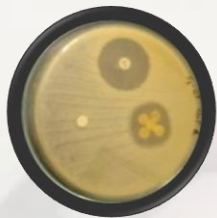
Vortex mixture



Matching with Mac Farland



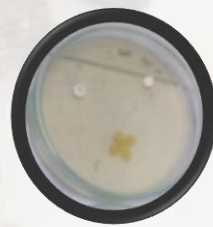
Mueller Hinton Agar



After incubation at 37°C for 24 h



incubation at 37°C



CST of CEO on M-H Agar



Streaking



RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PROPERTIES OF THREE BRANDS CLOVE ESSENTIAL OIL



Sample ID (n = 3)	Acid value (mg KOH/g oil)	Saponification value (mg /g oil)	Peroxide value (meq O ₂ /kg oil)	Iodine value (g I ₂ /100 g of oil)
CI _s	5.213 ± 0.01 ^c	37.257 ± 0.01 ^c	4.610 ± 0.01 ^c	51.457 ± 0.01 ^a
CI _d	6.087 ± 0.01 ^b	40.670 ± 0.01 ^a	6.667 ± 0.01 ^a	49.507 ± 0.01 ^c
CS _I	6.457 ± 0.01 ^a	38.056 ± 0.01 ^b	5.127 ± 0.01 ^b	50.157 ± 0.01 ^b

DENSITY FOR THREE BRANDS CLOVES ESSENTIAL OIL



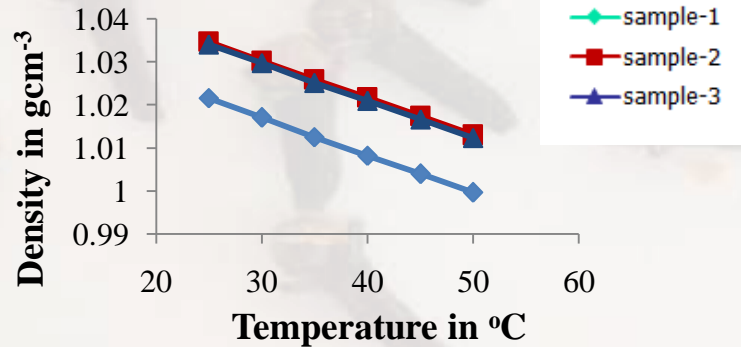
Density (ρ) for three brands cloves essential oil are measured at six different temperatures between 25°C and 50°C maintaining 5°C interval.

The following are the observations regarding density (ρ):

At a particular temperature, ρ of the cloves essential oil decreases in the order:

CId > CS1 > CI1

The ρ vs. T curves for three brands CEO follow a similar trend - ρ decreasing almost linearly with the temperature.



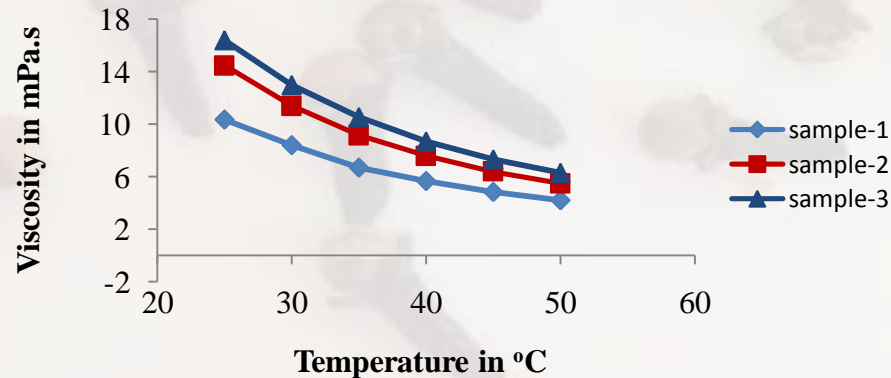
VISCOSITY FOR THREE BRANDS CEO



Viscosity (η) for three brands cloves essential oil are measured at six different temperatures between 25°C and 50°C maintaining 5°C interval.

At a particular temperature, η of the cloves essential oil decreases in the order: CS1 > CId > CIs

The η vs. T curves for three brands cloves essential oil follow a similar trend - η decreasing slowly with the temperature.



PROXIMATE COMPOSITION OF THREE BRANDS CLOVE



Sample ID	% Moisture	% Ash	% Protein	% Fat	% Fiber	% CHO	Energy (Kcal/g)
CI _s	12.287 ± 0.01 ^b	4.877 ± 0.01 ^c	4.213 ± 0.01 ^b	9.817 ± 0.01 ^c	16.694 ± 0.01 ^a	52.210 ± 0.01 ^a	313.71 ± 0.01 ^b
CI _d	13.653 ± 0.01 ^a	5.177 ± 0.01 ^b	4.127 ± 0.01 ^c	10.187 ± 0.01 ^b	15.821 ± 0.01 ^c	51.53 ± 0.01 ^c	312.35 ± 0.01 ^c
CS _I	11.553 ± 0.01 ^c	5.253 ± 0.01 ^a	4.557 ± 0.01 ^a	11.187 ± 0.01 ^a	15.875 ± 0.01 ^b	51.587 ± 0.01 ^b	325.23 ± 0.01 ^a

Phytocomponents identified in clove essential oils



Hit#:1 Entry:66605 Library:NIST17-1.lib

SI:79 Formula:C₁₅H₂₄O CAS:0-00-0 MolWeight:220 RetIndex:1531

CompName:trans-Z.-alpha.-Bisabolene epoxide \$S\$ 4-[(1Z)-1,5-Dimethyl-1,4-hexadienyl]-1-methyl-7-oxabicyclo[4.1.0]heptane # \$S\$

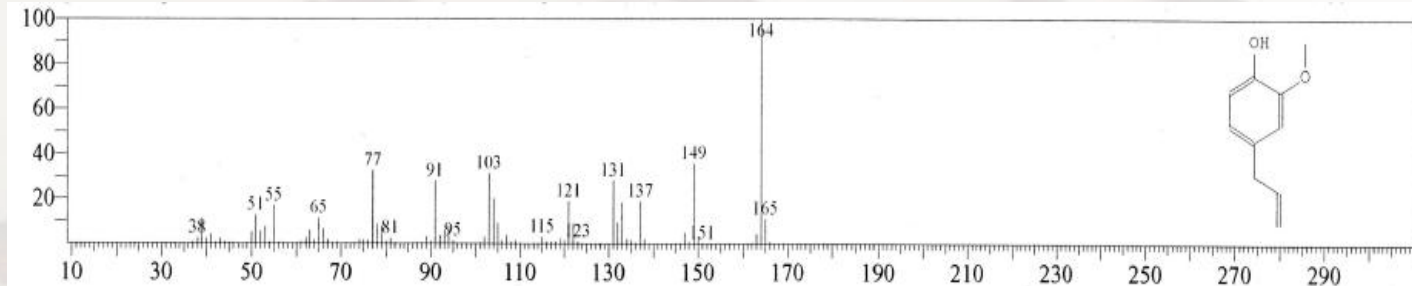
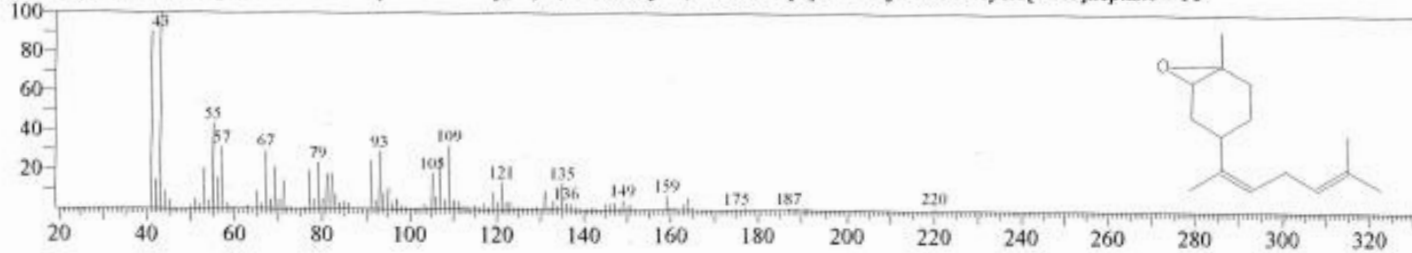
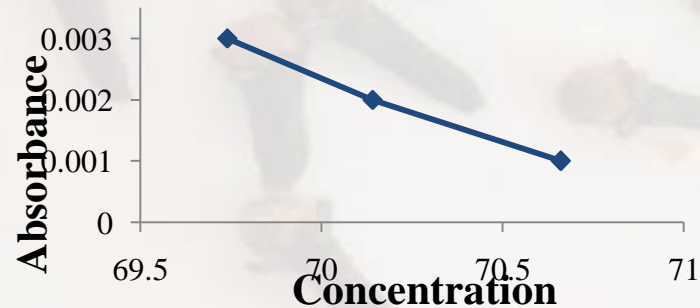


Figure: MS spectra of the Eugenol (CId)

Concentration and absorbance of sample solution for AOA



Sample ID	Type	Conc. (ppm)	WL 517.0	Wgt. Factor
CI _s	EAI _s	70.66 ± 0.01^a	0.001	1.000
CI _d	EAI _d	70.14 ± 0.01^b	0.002	1.000
CSI	EAS	69.74 ± 0.01^c	0.003	1.000



Concentration & absorbance of sample Solution for bioactive compounds



Sample ID	Type	TFC		TPC		TAC		Wt Factor
		Conc. (ppm)	WL 415nm	Conc. (ppm)	WL 760nm	Conc. (ppm)	WL 520nm	
CI _s	EAI _s	244.36 ± 0.01 ^b	1.603	159.44 ± 0.01 ^a	1.725	16.071 ± 0.01 ^c	0.233	1.000
CI _d	EAI _d	161.13 ± 0.01 ^c	1.057	142.82 ± 0.01 ^b	1.854	18.156 ± 0.01 ^a	0.338	1.000
CSI	EAS	321.95 ± 0.01 ^a	2.112	135.11 ± 0.01 ^c	1.912	17.942 ± 0.01 ^b	0.326	1.000

BIOACTIVE COMPOUNDS



Results of total flavonoids, total phenolics and total anthocyanin contents of three brands clove essential oil.

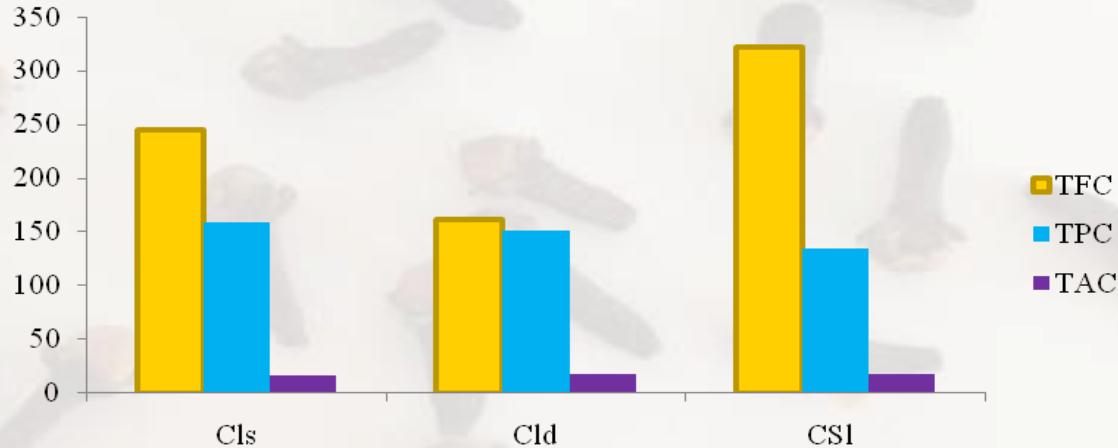


Figure: Bioactive Compounds of ClS, ClD & CS1 sample at different concentrations

Zone of inhibition (mm) showing the antimicrobial activity of CEO



Test Organism →		<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Pseudomonas aeruginosa</i>		
Sample ID	COE (µg/ml)	Essential oil (mm)	(+) control: Ciprofloxacin (mm)	(-) control: SSS + FP (mm)	Essential oil (mm)	(+) control: Ciprofloxacin (mm)	(-) control: SSS + FP (mm)	Essential oil (mm)	(+) control: Ciprofloxacin (mm)	(-) control: SSS + FP (mm)
CI _s	100	26	30	Nz	25	Nz	Nz	Nz	36	Nz
CI _d	100	25	28	Nz	24	Nz	Nz	Nz	27	Nz
CSI	100	24	29	Nz	27	Nz	Nz	Nz	33	Nz

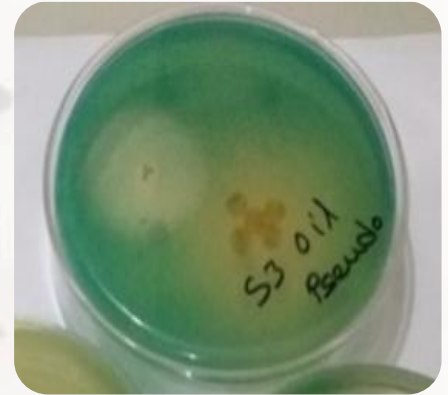
CST OF CLOVE ESSENTIAL OIL



CST of *Staph. aureus* isolate
by disc
diffusion method (DDM) for
CEO



CST of *E. coli* isolate
by DDM for CEO



CST of *P. aeruginosa*
isolate
by DDM for CEO

DISCUSSION



- ❖ This study includes that clove powder has high amount of carbohydrate, dietary fiber and low in fat.
- ❖ Density (ρ) and Refractive Index (n_D) were decreasing almost linearly with increasing temperature but viscosity (η) decreasing slowly with increasing temperature.
- ❖ Analysis using GC-MS was found to be the best method to identify even the minor components.
- ❖ Phenolic compounds are known to have antioxidant and antimicrobial properties.
- ❖ The high concentration of eugenol in buds oil makes it potentially useful in the medicines because they exhibit antibacterial and antioxidant properties.



CONCLUSIONS



- In this study, I investigated the proximate analysis, physicochemical properties, phytochemicals, antioxidant activity, bioactive compound and antimicrobial activity of the essential oil isolated from *S. aromaticum* buds of Indonesia (CIs), India (CI_d) and Sri Lanka (CS_l).
- Eugenol is the main component of clove essential oil.
- The study implied that clove and clove oils can be used as antibacterial and antiseptic agent.
- Cloves can be used as a food preservative due to improve shelf life of foods.

Recommendations and future perspectives



- ✓ I will give a chance and long period of time, minerals and broad microbial analysis may be considered for further analysis.
- ✓ Chemical constituents, antifungal and antimutagenic activities may be considered for further analysis.
- ✓ Clove bud essential oil Eugenol application for anti-oxidation.
- ✓ Investigation of temperature, solvent type and other factors.

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ACKNOWLEDGEMENTS



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Department of Microbiology





Thanks To All

Do You Have Any Questions

