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Studies on analgesic, antidiarrheal, and antimicrobial effects of clove (*Syzygium aromaticum*)

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CERTIFICATE

We the under signed certify that on November the 4th 2012 Abdel Hamid Mohammad Senussi was examined for his thesis entitled “Studies on analgesic, antidiarrheal, and antimicrobial effects of clove (*Syzygium aromaticum*)”.

The thesis has been accepted in partial fulfillment for the requirements for the degree of Master of Science in Pharmacology

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I hereby certify that the work embodied in this dissertation is the result of my own investigations except where reference has been made to published literature.

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DECLARATION

I hereby declare that this work has not already been accepted for any degree and is not been concurrently submitted in candidacy for any degree.

Candidate's signature……

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DEDICATION

This work is dedicated to my parents, my wife, and my son Osama

Abdel Hamid M. Senussi

SUMMARY

In the present work, antidiarrheal, analgesic and antimicrobial effects of clove aqueous extract (CAE) and clove oil (CO) were studied.

In *in vitro* studies CAE as well as CO inhibited the rhythmic contractions of rabbit isolated intestine; CO was more effective.

CAE and CO also inhibited Ach-induced contractions of rabbit isolated intestine; CO was more potent. However, $BaCl_2$-induced contractions of rabbit isolated intestine was not blocked by CO but blocked by verapamil. In *in vivo* experiments, CO given orally inhibited charcoal meal transit in mice; the inhibition by 0.3 ml CO was equivalent to 10mg/kg dose of atropine. Oral administration of CO prolonged the duration of onset of diarrhea induced by castor oil in mice and decreased the diarrheal episodes. These observations indicate that CO have a potent antispasmodic and antidiarrheal effect.

Orally administered CAE as well as CO have shown dose-dependent analgesic action in acetic acid-induced writhing in mice; CO was more potent. Intraperitoneal administration of CAE also showed analgesic action. Naloxone could not reverse the analgesic action of CO but reversed that of morphine. This indicates that analgesic action of CO is not mediated through opioid receptors.

The *in vitro* antimicrobial studies revealed that clove oil inhibited the growth of staphylococcus aureus, klebsiella, E. coli and proteus but was not effective against pseudomonas aeruginosa. CAE was not effective against any of these bacteria. Ceftriaxone, oxacillin, nalidixic acid, ampicillin, kanamycin, fusidic acid,

erythromycin and pipercillin discs were used as positive control. Ceftriaxone was found to be effective against all the above bacteria except staphylococcus aureus which was inhibited only by fusidic acid. Klebsiella was also inhibited by kanamycin and nalidixic acid whereas proteus was inhibited by kanamycin but not nalidixic acid. Pseudomonas aeruginosa was also inhibited by pipercillin but not by ampicillin and nalidixic acid. CO effectively inhibited the growth of candida albicans. The minimum inhibitory concentration (MIC) of clove oil was determined for candida albicans, E. coli and klebsiella which was 1.87, 3.75 and 7.5 µl respectively.

The clinical study on very few patients showed that clove oil effectively eradicated tinea pedis and tinea versicolor infection after 2-3 weeks topical application.

The present work convincingly showed that clove oil has a good potential to be used as analgesic, antidiarrheal, antibacterial and antifungal. However, more work is required to establish its efficacy and safety.

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LIST OF ABBREVIATIONS

AA : Acetic acid

Ach : Acetycholine

AMP : Ampicillin

$BaCl_2$: Barium chloride

CAE : Clove aqueous extract

CEF : Ceftriaxone

CO : Clove oil

COX : Cyclooxygenase

E : Erythromycin

FA : Fucidic acid

FDA : Food and Drug Administration

GRAS : Generally Recognized As Safe

i.p. : Intraperitoneally

IC : Indian clove oil (from market)

INDO : Indomethacin

IUCN : International Union for Conservation of Nature

KAN : Kanamycin

KOH : Potassium hydroxide

MORPH : Morphine

NA : Nalidixic acid

NSAIDs : Non-steroidal anti-inflammatory drugs

OO : Olive oil

OXA : Oxacillin

PG_s : Prostaglandins

PIP : pipercillin

s.c. : Subcutaneously

Verap : Verapamil

WHO : World Health Organization

CONTENTS

Ch. 1 INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last few decades, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in many parts of the world. From fossil records, the mankind has used plants as medicines at least since the Middle Paleolithic age around 60,000 years ago (Solecki and Shanidar, 1975).

Most of the drugs that were used until early 1800s were herb-based or extraction of ingredients from botanical sources (Rick, 2009).

Worldwide, there are more than hundred twenty prescription drugs which were made from plants, and the global market for such drugs was estimated at 43 billion dollar. Moreover, there are still growing global demands for such herbal-based medicines (Arihan and Özkan, 2007).

The plant kingdom continues to hold many species of plants with good medicinal substances which have yet to be discovered. Large numbers of plants are constantly being screened for their possible pharmacological use particularly for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, anti-fertility, cytotoxic, antibiotic and anti-Parkinsonism properties (Evans, 2008).

In Britain, a national telephone survey of a nationally representative sample of 1204 people found that around 7% of those contacted had used herbal medicines in the previous year (Ernst and White, 2000). In another cross-sectional study, over 5000 randomly selected people in England were sent a postal questionnaire on their use of herbal medicine. Twenty percent of the

respondents had used an over-the-counter herbal medicines in the previous 12 months (Thomas, 2001).

Pharmaceutical companies are much interested in traditional herbal medicine which otherwise would cost them a lot of money on screening. Plant-derived drugs based on traditional knowledge have benefited the pharmaceutical companies greatly, and indigenous knowledge of plants has played a significant role in drug development. Out of 10,000 molecules only one will emerge as a new drug. It nearly needs 15 years to be experienced and it is very expensive as it costs 800 million dollar for a single drug to be developed. This interest by companies from the more developed countries has led them to the knowledge of the indigenous peoples of the less developed countries, and converted medicinal herbs into pharmaceutical products without providing any payment to the providers of the knowledge. (Arihan and Özkan, 2007).

The development of drugs of plant origin involves botanical identification of the plant, cultivation and post-harvest procedures, extraction procedures, standardization of extracts and pharmaceutical formulation. This means that development of herbal-based medicines are in the hands of personnel from different specialties. The production of drugs from plants needs the co-operation of a big team of horticulturists, botanists, ecologists, taxonomists, phyto-chemists, pharmacists, pharmacologists, pharmaceutical specialists, marketing and distribution specialists, etc. Nowadays, the developed countries are turning towards the use of traditional medicinal systems that involve the use of herbal drugs and remedies. At the present time, about 1400 herbal

preparations are being used, according to a survey in Member States of the European Union. Herbal preparations are popular and are of significance in primary health care system in Belgium, Germany, France, and the Netherlands. Such popularity of healthcare plant-derived products has been traced to their increasing acceptance and use in the cosmetic industry as well as to increasing public costs in the daily maintenance of personal health and well being. Examples of such beauty-oriented products are skin tissue regenerators, anti-wrinkling agents and anti-age creams. Most dermaceuticals are derived from algal extracts that are rich in minerals and the vitamin B group. Skincare products such as skin creams, skin tonics, etc. derived from medicinal plants are grouped together as dermaceuticals. Amongst the poor, cures and drugs, derived from plants, constitute the main source of healthcare products (Hota, 2007).

Some researchers surveying the use of spice and their medicinal properties around the world, concluded that spices serve the purpose of reducing food-borne disease. Research papers have shown that the preservative properties of spices against food spoilage is due to the presence of antimicrobial substances that led to the elimination of pathogenic organisms in food preparations (Hota, 2007).

One of the well-known spices is clove. Clove has a very old association with human history. The first indication for the use of fragrant clove comes from the Chinese physician who wrote that during ancient Chinese Han dynasty (lasting from 207B.C. to 220 A.D.), the court visitors to the emperor were required to hold

cloves in their mouths, while they addressed the emperor which was just to save the ruler from the bad breath of the visitors (Andaya, 1993).

Clove was known in Europe by the fourth century, although was very expensive. The spice islands were occupied by the Portuguese at the beginning of sixteenth century, but they were expelled by the Dutch in 1605. The Dutch made every effort to secure the monopoly on clove trade. The Dutch eventually gained complete monopoly on clove trade by destruction of every viable clove tree population in all the islands except those on Dutch colonized islands and in these islands, they devoted large areas for plantation of clove trees. The Dutch monopoly of clove trade was broken in 1770 when the French managed to cultivate the tree in their colonized islands (Madagascar, Zanzibar and elsewhere). As cloves were grown in different geographical regions, other areas of the world began to produce huge quantities of cloves. The island of Zanzibar, which belongs nowadays to Tanzania in Eastern Africa, has been a major producer of cloves for many decades. The clove tree grows so well in the island of Zanzibar to the extent it is also known as “Island of cloves”. The islands of tropical countries are also major production centers for cloves such as Caribbean island of Jamaica, the south Asian island Sri Lanka and the countries of Malaysia, Brazil, and of course Indonesia (Evans, 2008).

Cloves were traded by Muslim sailors and merchants during the Middle Age in the profitable Indian Ocean trade. The famous Arabian traveler and writer Ibn Battutah (1304-1368) mentioned the clove in his book Rihla “the journey”. The clove also has been mentioned by even the famous One Thousand and One

Nights characters such as Sinbad the Sailor is known to have bought and sold cloves. Clove may be the third most traded spice all over the world after black pepper and paprika (Attokaran, 2011).

Cloves belong to family known as Myrtaceae. Clove is the dried flower buds of *Syzygium aromaticum* and also known by different names including (*Eugenia caryophyllus, Caryophyllus aromaticus, Eugenia aromatica*), a tree 10-20 meter high which is indigenous to Molucca islands of Indonesia. Because this plant originated from Molucca islands, these islands are also known as “clove islands” (Bisset, 1994).

The word clove is derived from French word “clou” which in French means nail as the dried buds look like little nails. It is known by different names in different countries. In Indonesia, it is known as “cengkeih” or “cengkih”, in India “laong” or “lanvang” in Arabic “kronfol” in Turkey “carenfil” and in Spanish “clavo”.

The clove tree (Figure 1) is an evergreen tree which grows only in tropical climate. Clove tree when fully grown can go up to thirty to forty feet. The tree has a pyramidal shape. It has broad brighter green leaves and shiny leaves. The leaves have visible dots containing the aromatic substances. These aromatic substances are released when the leaves are crushed. The stem is covered with a smooth grayish bark. The stem divides into large branches at the end of which the tiny yellow flowers grow in loose-clusters. The flower buds are at first of pale color, after which they develop into a bright red color, when they are ready for collection. The flower buds are picked by hand or beaten by the tree. These

flower buds are then dried in sun over a mat for few days until they turn into dark brown colored spice known as “clove”.

FIGURE 1

CLOVE TREE (*Syzygium aromaticum)*

The plant requires a tropical climate with 150 – 300 cm of rainfall per year as in Indonesia, Zanzibar, Madagascar, Sri Lanka, and Southwest India. The tree prefers rich loamy soil with high humus. In the middle of rainy season, the hot and humid atmosphere disperses the fragrance of clove trees all over the islands where they grow. The characteristic strong aromatic fragrance produced by the

living tree is mainly from the glands dotting the smooth shiny leaves and also from the flower buds when they flower. It was observed that there were absence of epidemics on the islands where cloves are cultivated, which were attributed to the medicinal scents from the tree that were believed to be due to its strong antiseptic properties. It was also observed that after the Dutch destroyed the cloves on some islands in the early seventeenth century in order to secure monopoly on clove trade, inhabitants of those islands started suffering from epidemics and many died (Attokaran, 2011).

The cloves are collected twice a year between August and December. If flower buds are left too long on the tree, the buds open and the petals fall, leaving blown cloves, which later become fruits known as “mother cloves”. Cloves are imported in bales covered with matting from strips of coconut leaves.

When harvested, clove is 10-17.5 mm long and consist of a long calyx, terminating into four spreading sepals and four unopened petals which form a small ball in the center (Kim et al. 1998). The clove tree does not produce the spice until aged about 5 years, and can carry on increasing its yield until it is reaching the full bearing age of 20 years old. The tree then continues to bear for about 100 years. The yield of a mature tree is about 3-4 kg fresh buds. When these are dried, the weight reduced to about one kg (Chomchalow, 1996 and Bown, 2001).

Macroscopic character of clove:

Cloves are 10-17.5 mm long (Figure 2) the Penang and Amboyna varieties are largest and plumpest and are most esteemed. They are in such demand in the

East that relatively very small quantity of them reach other parts of the world. The Zanzibar variety is of good quality although smaller and leaner than the Penang variety and are of blackish brown rather than reddish brown color.

Cloves have a strong fragrance and spicy pungent aromatic taste. Cloves sink in freshly boiled and cooled water. This property is used to distinguish the cloves which have been exhausted of volatile oil which float in water (Evans, 2008).

FIGURE 2

CLOVE FLOWER BUDS

Microscopic characters of cloves*:*

The hypanthium, in the region below ovary, shows in transverse section a heavy cuticularized epidermis in which occur stomata. Within this, there is a zone of parenchymatous cells containing numerous oil glands arranged in two or three intermixed layers. Within the oil gland layer, there is a zone of cells with thickened walls. The ground tissue of this zone contains cluster crystals of calcium oxalate.

The xylem is composed of 3-5 lignified spiral vessels. The hypanthium, in the region of ovary, shows epidermis and oil glands. Within this, there is a zone of cells with very strong thick cellulose walls, forming the wall of the ovary.

The sepals and petals have a simplified leaf structure. The mesophyll parenchyma contains calcium oxalate and embeds numerous oil glands. The epidermis of the sepals shows stomata. The epidermis of petals is devoid of stomata and is composed of very irregular cells.

The stomata are composed of filaments and anther. The filaments shows an epidermis of longitudinal cells and parenchyma containing numerous oil glands (Evans, 2008).

One of the most well-known traditional medicinal use of clove is applying clove oil to relieve toothache. This use was reported for the first time in France in1640 in “Practice of Physic” (Barceloux, 2008).

In early 1900s, smoking clove cigarettes (kreteks) has started in Indonesia and later spread to the world. Kreteks are made up of 60-85% tobacco and 15-40%

clove buds. Originally, they claimed to relieve chest problems such as asthma, although later was proved not a safe alternative to tobacco smoking (Polzin, 2007).

The pharmacological properties and the medicinal value documented for cloves are associated with the volatile essential oil, which is made up mainly of eugenol. The essential oils are volatile, not greasy, not saponifiable in contrast with fatty oils. Naturally, the essential oils play a vital role in the attraction of insects to promote the spread of pollens and seeds or to repel other harmful insects. In addition, essential oils may also act as antibacterial, antiviral, antifungal, insecticidal, herbicidal, or have feeding deterrent effects against herbivores by reducing their appetite for such plants. Essential oils have also an important role in allelopathic communication between plants (Ibrahim et al. 2001 and Bakkali et al. 2008).

The essential oils and the extracts of various species of edible and medicinal plants, herbs, and spices contain very potent natural biologically active agents. They have a complex composition, containing from a few to several hundred substances, especially hydrocarbons and oxygenated compounds. Both hydrocarbons and oxygenated compounds are responsible for the characteristic odors and flavors, which are formed by aromatic plants as secondary metabolites (Ebrahimi et al. 2008). Essential oils and their components are widely used in medicine as constituents of different medical products, and in the food industry as flavoring additives. Beyond their antimicrobial properties in food systems, they

may be regarded as an additional intrinsic determinant to increase the safety and shelf life of foods and in cosmetics as fragrances (Bakkali et al. 2008).

The yield of essential oil from clove is 15-20%, which means that the yield of essential oil from one tree will be 150-200 ml as the dried flower buds procured from one tree is only up to one kg. The leaves yield is up to 2% while the stem gives about 5% essential oil (Jirovetz et al. 2006).

As the clove flower buds contains up to 20% essential oil, which is made up mainly of eugenol, so the pharmacological properties is usually attributed to eugenol (European Medicines Agency, 2011).

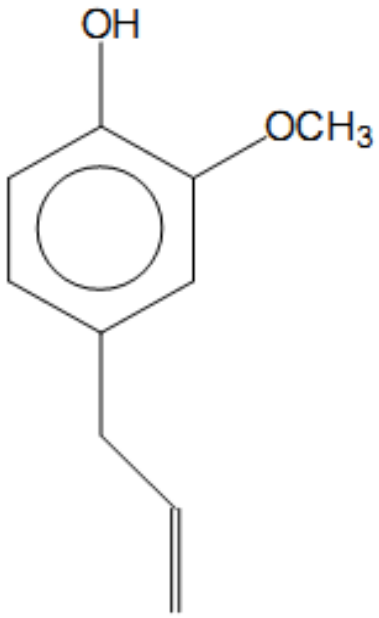
The main constituent of clove oil is eugenol (derived from the plant name *Eugenia caryophyllata*), which makes from 45-90% and in addition it contains acetyl eugenol (4-15%), β-caryophyllene (5-14%), chavicol, acetyl salicylate and humulenes (Zheng et al.,1992; Blaschek et al., 2008).

Eugenol was the first component of an essential oil proved to be of significant germicidal and sedative properties used in dentistry, and today is still in use (Markowitz et al., 1992).

Eugenol (Figure 3) is 4-allyl-1-hydroxy-2-methoxybenzene, a natural phenolic constituent of clove oil, and other plants including cinnamon, basil, and nutmeg (Yukio et al., 2003). In 1929 eugenol was isolated and commercial production started in USA in 1940s (Barceloux , 2008).

FIGURE 3

CHEMICAL STRUCTURE OF EUGENOL



eugenol

Although eugenol is most abundant in clove, it was also extracted from other aromatic plants such as: nutmeg (*Myristica fragrans*); allspice (*Pimenta dioica*); bay leaf (*Laurus nobilis*) Japanese star anise (*Illicium anisatum*) (Table 1) and (Figure 4) (Barnes et al., 2007).

Eugenol can also be produced synthetically, however, it is almost extracted from the essential oil via steam distillation. Commercial eugenol is derived from clove oil, cinnamon (*Cinnamomum zeylanicum; Cinnamomum loureirii*) leaf oil, or basil

(*Ocimum basilicum*) through a process known as steam distillation and afterward it will be refined (Bedoukian, 1986; Barceloux , 2008).

TABLE 1

ESSENTIAL OIL CONTENT AND EUGENOL CONTENT OF DIFFERENT PLANTS

Plant	Essential oil content	Eugenol content in the essential oil
Clove buds	15-16%	80-90%
Clove stem	5%	90-95%
Clove leaves	2%	82-88%
Cinnamon bark	1%	46%
Cinnamon leaf	1.5-2%	90%
Basil	≤1%	53%
Allspice	0.7-2.9%	65-95%
Nutmeg	6-12%	1%

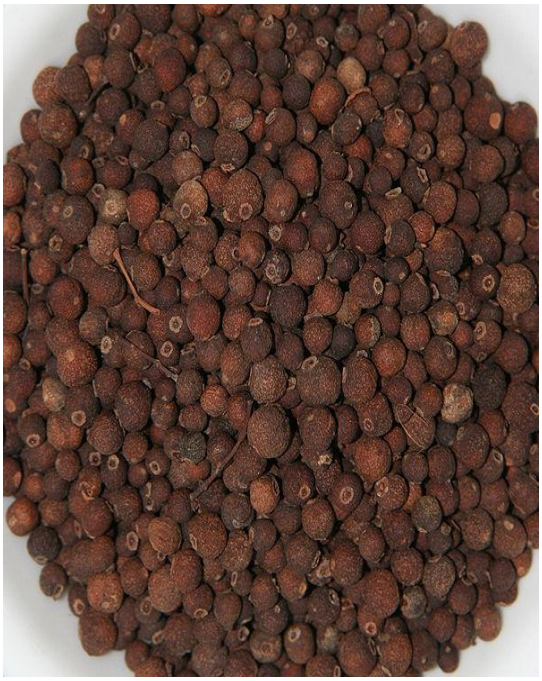
(Barnes et al., 2007)

FIGURE 4

SOME OTHER PLANTS WHICH CONTAIN EUGENOL : A=BASIL B=ALLSPICE C=NUTMEG D=CINNAMON

A

B



C

D

Eugenol is an allyl chain-substituted guaiacol (4-allyl-2-methoxyphenol) (Figure 3). Eugenol is weakly acidic and slightly soluble in water, but readily soluble in organic solvents. Eugenol is clear to slightly yellowish liquid with characteristic pleasant clove smell . It is extraordinarily versatile molecule with diverse usage applications for example eugenol is used in production of cosmetics and synthesis of soap, detergents, and perfumes (Bedoukian, 1986; Barceloux, 2008; Good guide. 2012).

Several documented reports were issued regarding the effect of eugenol in dental pain as well as the analgesic and anti-inflammatory effects in animal models (Diaz and Sembrano, 1985; oztürk and ozbek, 2005; Kurian et al., 2006; Daniel et al., 2009; Dohi et al.,1991; Ozeki, 1975; Park et al., 2006; Li et al., 2007; Inoue et al., 2012; Ferland et al., 2012; Filiciotto et al., 2012).

Selzer (1992) and Gerosa et al. (1996) claimed that, the zinc oxide eugenol cement is widely used in dentistry for indirect pulp capping, and as a temporary filling and root canal sealer. Eugenol has antioxidant and anti-inflammatory properties. However, eugenol at high concentrations has been reported to have some cytotoxic effects.

There have been several reports regarding the antimicrobial effect of eugenol. Singh et al. (2007) documented the inhihitory effect of eugenol against Gram positive and Gram negative bacteria and Ali et al. (2005) pointed to the antibacterial effect of eugenol against H. pylori.

Nascimento et al. (2000) showed that among seven different spice essential oils, eugenol, which was extracted from cloves, showed the highest antimicrobial activity against Gram positive and Gram negative bacteria.

Nazrul Islam et al. (1990) revealed that clove would be a good antibacterial drug against multiple drug resistant Shigella and Vibrio cholera.

Devi et al. (2010) said eugenol kills salmonella by destroying bacterial membrane, and thus less likely for the bacteria to develop resistance.

He et al. (2007) proved that eugenol is effective against candida albicans.

Chami et al (2004) proved the effect of eugenol as prophylaxis and treatment of vaginal candiasis is immunosuppressed rats.

Benencia and Courrges (2000) showed *in vitro* antiviral activity of eugenol against herpes simplex virus (HSV-1 and HSV-2). They reported that eugenol has synergistic action when combined with acyclovir against those viruses.

Ueda-Nakamura et al. (2006) showed essential oil of *Ocimum gratissimum* (rich in eugenol) and pure eugenol were shown to have leishmanicidal activity.

Saad et al. (2004) stated that eugenol has hypotensive effect in both anesthetized and conscious rats indicating its effect may be probably through an active vascular relaxation. They said this effect explains the traditional use of some essential oil for treatment of hypertension.

Nishijima et al. (1999) stated that eugenol when tested on rabbit arterial tissue, inhibits voltage-dependent calcium channels, block the calcium extrusion mechanism, and inhibit the contractile machinery.

Nagababu and Lakshmaiah (1997) demonstrated that eugenol inhibited lipid peroxidation in rat liver microsomal lipid liposomes.

Ardjmand et al. (2006) showed that eugenol depress synaptic transmission in rat hippocampal slices.

Prasad et al. (2005) showed that clove has insulin-mimetic action in hepatocytes and hepatoma cells, which means that it could be used for the treatment of diabetes.

Banerjee et al. (2006) showed that clove infusion has apoptosis-enhancing and proliferation-inhibiting effect against benzo[a]pyrene-induced lung carcinogenesis by modulating the balance between pro- and anti-apoptotic factors and also by regulating some growth-promoting genes.

Tajuddin et al. (2003) suggested that use of 50% ethanolic extracts of nutmeg and clove have sexual behavior enhancing effect in male mice. Thus the experimental findings substantiate the claim of Unani physicians that the nutmeg and clove are clinically useful to improve sexual function in males.

Beside these medical uses there are other uses of clove (eugenol) in other fields like agricultural uses to control postharvest decay in crops (Amiri et al., 2008 and Combrinck et al., 2011).

Due to the global concern of chemical pollution, eugenol was also used as insecticidal. The protective and repellent effect of eugenol against beetles was investigated and documented (Obeng-Ofori and Reichmuth, 1997).

1-1 AIMS AND OBDICTIVES OF THE PRESENT WORK

From the available reports, it appears that clove oil is frequently used as analgesic in toothache and also used in several other medical conditions as folklore. However, the systemic scientific studies are still lacking. Therefore, the present work was undertaken to elucidate the mechanism of its analgesic action and to scientifically establish its antidiarrheal and antimicrobial effects.

Ch. 2 MATERIAL AND METHODS

2-1 Experimental animals:

Swiss albino mice and rabbits were used. Mice were bred at the Central Animal House of Faculty of Medicine, Benghazi University. The rabbits were purchased from local market. The animals were provided with standard diet and water provided *ad libitum* . The mice were divided into groups (6-8 each).

2-2 Chemicals and drugs:

Acetic acid	(Sigma chemical company, USA)
Acetylcholine (Ach)	(Sigma chemical company, USA)
Activated charcoal	(BDH chemical Ltd. Poole, England)
Ampicillin 10 µg disc	(Oxoid Ltd. Hampshire, England)
Atropine	(Sigma chemical company, USA)
Barium chloride	(Sigma chemical company, USA)
Calcium chloride	(Sigma chemical company, USA)
Castor oil	(purchased from local market)
Ceftriaxone 30 µg disc	(Oxoid Ltd. Hampshire, England)
Erythromycin 10 µg disc	(Oxoid Ltd. Hampshire, England)
Fucidic acid 10 µg disc	(Oxoid Ltd. Hampshire, England)
Glucose	(Riedel-De Haen AG,. Seelze, Germany)
Gum acacia	(BDH chemical Ltd. Poole, England)
Indomethacin	(Sigma chemical company, USA)
Kanamycin 30 µg disc	(Oxoid Ltd. Hampshire, England)

Loperamide	(JANSSEN-CILAG, BELGIUM)
Magnesium chloride	(BDH chemical Ltd. Poole, England)
Market clove oil (30%)	(GOPALDAS VIRSAM & CO.Ltd. India)
Morphine	(Vifor Pharma Ltd. Geneva, Switzerland)
Nalidixic acid 30 µg disc	(Oxoid Ltd. Hampshire, England)
Naloxone	(Sigma chemical company, USA)
Olive oil	(purchased from local market)
Oxacillin 1 µg disc	(Oxoid Ltd. Hampshire, England)
Pipercillin 30 µg disc	(Oxoid Ltd. Hampshire, England)
Potassium chloride	(Fluka chemie AG. Buchs, Switzerland)
Potassium hydroxide	(BDH chemical Ltd. Poole, England)
Sodium bicarbonate	(BDH chemical Ltd. Poole, England)
Sodium chloride	(Farmitalia Carlo Erba, Milano, Italy)
Sodium dihydrogen phosphate	(Sigma chemical company, USA)
Verapamil	(Sigma chemical company, USA)

2-3 EXTRACTION OF CLOVE FLOWER BUDS:

Dried clove flower buds were purchased from local market. They were subjected to :

(a) aqueous extraction

(b) volatile oil distillation

2-3-A Aqueous extraction of clove:

Ten grams of clove flower buds were crushed into small pieces in a mixer and put in 50 ml of distilled water for 24 hours. Occasional stirring was done during this period. It was then filtered through Whatman filter paper. The filtrate thus collected and the volume was made up to 50 ml by adding distilled water. This extract hereafter referred to as “clove aqueous extract” was kept in the refrigerator and used in the experiments.

2-3-B Extraction of essential oil from clove:

The clove was subjected to steam distillation for extraction of its essential oil. The idea of steam distillation is based on the fact that when two immiscible liquids, one of them is water, will co-distill at a temperature that is lower than the boiling points of the individual components. This is because the total vapor pressure of an immiscible liquid mixture is the sum of the individual vapor pressures that each component would exert if it were a pure liquid. The higher total vapor pressure leads to a lower boiling point for the mixture than for either single component. When total vapor pressure equals the atmospheric pressure, the

mixture boils. Since many essential oils contain liquids that may not survive heating at higher temperatures, steam distillation is an especially advantageous technique for isolating them because their immiscible mixture with water boils below 100°C.

The volatile essential oil was extracted by steam distillation using Clevenger's apparatus (Figure 5). Clove buds (300 grams) were put in 2 liter round bottom flask (distillation flask) to which 1200 ml of distilled water was added. The level of water in the flask was marked by a marker, so that lost water during distillation can be replenished to prevent it from drying. The mixture was heated until distillation began. Occasionally water level was checked and added more water if necessary. The distillate was collected in a conical flask. Then the distillate was transferred to the separating funnel to separate water from volatile oil. This oil hereafter referred to as "clove oil" was kept in the refrigerator in tightly closed glass container for the use in experiments. For making different dilutions of clove oil, it was mixed with olive oil.

In some experiments, clove oil (30%) manufactured in India for toothache was purchased from local market also used; which hereafter is referred to as "Indian clove oil".

FIGURE 5

CLEVENGER'S APPARATUS FOR EXTRACTION OF ESSENTIAL OIL.

2-4 PHARMACOLOGICAL STUDIES OF CLOVE EXTRACTS ON THE ISOLATED INTESTINE OF RABBIT:

Rabbits weighing between 2.5 kg to 3.5 kg were killed by stunning and cutting the throat. The abdomen was opened and jejunum portion of intestine was quickly removed and was put in a Petri dish filled with Tyrode solution containing 8 grams of sodium chloride; 0.2 gram of potassium chloride; 0.2 calcium chloride; 0.1 gram of magnesium chloride; 0.05 gram sodium dihydrogen phosphate; 1 gram sodium bicarbonate; and 1 gram glucose per liter . The lumen of the intestine was gently washed with Tyrode solution by using a pipette. Then the intestine was cut into small pieces of approximately 2 cm length. Both ends of each piece were tied with thread. It was then mounted in isolated tissue bath filled with Tyrode solution and bubbled with carbogen gas. The preparation was maintained at 37°C through a thermostat (Figure 6). The procedure was according to Williamson et al. (1996).

The drug solutions were tested by directly adding to the isolated tissue bath and the effects were recorded through an isotonic transducer. The effects of different doses of clove aqueous extract and clove oil were studied. The effects of clove aqueous extract and clove oil on acetylcholine and barium chloride induced contractions of intestine were also studied.

FIGURE 6

ISOLATED TISSUE BATH

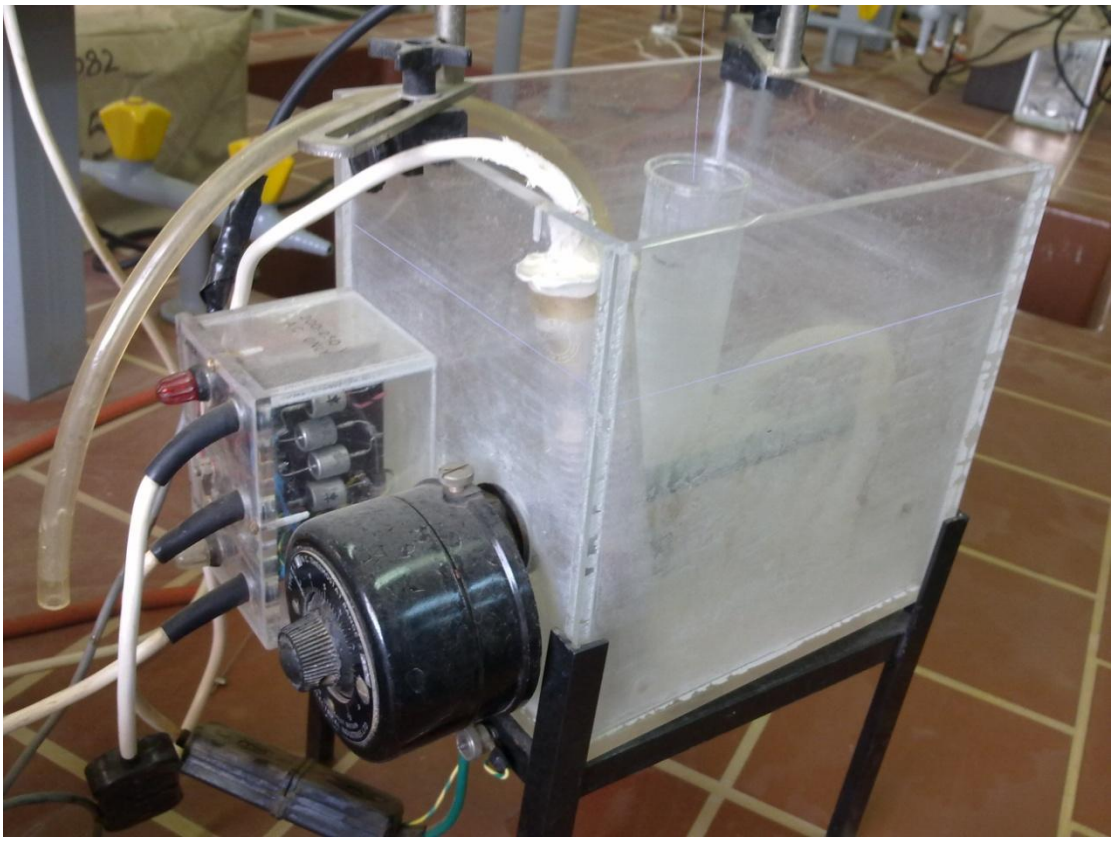


FIGURE 7

OSCILLOGRAPH WASHINGTON (400 MD 2C)

2-5 STUDY OF EFFECT OF CLOVE EXTRACTS ON SMALL INTESTINE TRANSIT BY CHARCOAL MEAL TEST:

The effect of clove extract on small intestine transit was studied in mice of either sex weighing between 25 gm and 30 gm. The mice were fasted overnight when they were allowed only to water *ad libitum*. Mice were divided into groups of six animal in each group. The vehicle control group were administered olive oil. The test group received different doses of clove oil. The positive control group received 10 mg/kg atropine orally. Five minutes after treatment, mice were given 0.2 ml of charcoal meal (5% activated charcoal in 5% gum acacia) by oral route. All animals were sacrificed after 30 minutes of administering the charcoal meal. The abdomen were opened and the small intestine along with stomach and caecum were gently removed and spread on white paper. The distance from pylorus to ileocaecal junction was measured, and the distance of charcoal movement from pylorus was measured. The results were expressed as percentage of distance travelled by charcoal (Williamson et al., 1996).

2-6 STUDY OF ANTIDIARRHEAL EFFECT OF CLOVE EXTRACTON CASTOR OIL-INDUCED DIARRHEA IN MICE:

Mice of either sex weighing between 25-28 gm were used. They were divided into groups of 5-6 animals each. Each animal was placed in an individual cage, the floor of which was lined with white paper which was replaced every hour. Diarrhea was induced by administering 0.3 ml of castor oil to the mice. The

vehicle control group received olive oil. The test groups of mice received different oral doses of clove oil. The positive control group received loperamide 2 mg/kg orally. Forty minutes after treatment, mice were administered 0.3 ml of castor oil orally. Onset of diarrhea, and number of diarrheal episodes were recorded for each animal for a total of 4 hours.

2-7 STUDY OF THE ANALGESIC ACTION OF CLOVE EXTRACT USING ACETIC ACID-INDUCED WRITHING IN MICE:

Mice of either sex weighing 25-30 gm were used. Writhing assessed according to the procedure prescribed by Siegmund et al. (1957). Pain was produced in mice by injecting 0.2 ml of 1% acetic acid intraperitoneally which produced irritation in the peritoneal cavity leading to pain which was expressed by animal by showing writhing (writhing is elongation of the mouse body with arching of the back proceeded by inward rotation). The number of writhes were counted to make the test quantitative. Mice were divided into groups of 5-8 animals in each group. The vehicle control groups received distilled water orally or intraperitonealy for clove aqueous extract and olive oil orally for clove oil. The test groups were administered different doses of clove aqueous extract or clove oil orally or intraperitoneally. The positive control groups received indomethacin (5 mg/kg intraperitoneally) or morphine (5 mg/kg subcutaneously). Thirty minutes after drug administration, the mice were administered acetic acid intraperitoneally. In other group of animals, naloxone (1mg/kg intraperitoneally) was injected 15 minutes before injecting clove oil, indomethacin, and morphine. The number of

writhes were counted for 15 minutes starting from acetic acid administration. The percentage of writhing inhibition was calculated.

2-8 ANTIMICROBIAL STUDIES OF CLOVE EXTRACTS :

2-8-A *IN VITRO* STUDIES:

I. PROCUREMENT OF MICROORGANISMS :

Gram negative bacteria including : E. coli; Klebsiella; Proteus; and Pseudomonas aeruginosa, and one gram positive bacteria staphylococcus aureus, and one fungus candida albicans were isolated from human patients and identified by department of microbiology. These microorganism samples were kept in a refrigerator and used to inoculate the media during the study on clove extracts. Whenever the source plates were found to be contaminated, they were discarded and another fresh samples were collected.

II. PREPARATION OF NUTRIENT AGAR PLATES :

Nutrient agar plates were prepared from nutrient agar powder which contained beef extract 3 gm; sodium chloride 8 gm; peptone 5 gm ; and agar 12 gm per liter. Beef extract provides carbohydrates, peptone helps in controlling pH, and agar acts as a solidifying agent. Nutrient agar powder (28 gm) was soaked in 1000 ml of distilled water in 2 liter flask for 10 minutes. It was autoclaved at 121°C for 15 minutes, and cooled to about 47°C, and poured into 90 mm Petri dishes about $1/4^{th}$ deep. The Petri dishes were allowed for about 30 minutes to solidify and stored in a refrigerator at 3°C upside down.

III. PREPARATION OF SABOURAUD DEXTROSE AGAR PLATES :

Sabouraud dextrose agar plates were prepared from sabouraud dextrose agar powder which contains peptone 10 gm; glucose 40 gm; and agar 15 gm per liter. Sabouraud dextrose agar powder (61.5 gm) was soaked in 1000 ml of distilled water in a 2 liter flask. The flask then was boiled with frequent stirring. It was allowed to cool to around 47°C. Then the content was poured in 90 mm Petri dishes 1/4th deep. The Petri dishes were then allowed for 30 minutes to solidify and stored in a refrigerator at 3°C upside down.

IV. STUDY OF ANTIBACTERIAL ACTIVITY OF CLOVE EXTRACT :

The procedure of agar-well diffusion method was done according to the description of Valgas et al. (2007). The nutrient agar plates were inoculated by disposable sterile cotton swabs. The microorganisms from source Petri dishes were taken on a cotton swab and was inoculated and spread on nutrient agar media of fresh Petri dishes. Then wells of 7 mm diameter were made by using a borer. The distance between two wells were 20-30 mm. These wells were filled with different doses of clove aqueous extract, clove oil, or olive oil (vehicle control for clove oil). Commonly used and effective antimicrobial discs were used as positive control. Then these Petri dishes were put in an incubator at 37°C for 24 hours. After 24 hours of incubation, the Petri dishes were examined for the growth of microorganisms. The inhibitory zone was measured around the wells and around the antimicrobial discs in mm by using a scale.

V. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL FOR BACTERIA :

The minimum inhibitory concentration (MIC) is the minimum antimicrobial concentration at which no microbial growth occurs. Serial two fold dilution of clove oil with the use of olive oil was done 2, 4, 8, 16, 32, and 64 times dilution. 30 µl of the each dilution was put in one of six wells which were dug into the Petri dishes containing bacteria. 30 µl olive oil used as negative control. Then the Petri dishes were put in incubator at 37°C for 24 hours. Ceftriaxone disc was used as positive control. The results were observed after 24 hours by measuring the zone of inhibition with a scale in mm. The MIC was calculated for E. coli and Klebsiella.

VI. STUDY OF ANTICANDIDAL ACTIVITY OF CLOVE OIL :

The Sabouraud dextrose agar plates were inoculated by disposable sterile cotton swabs. The candida albicans from source Petri dish were taken on a cotton swab and was spread onto Petri dishes containing Sabourad dextrose agar. A borer was used to dig a 7 mm diameter wells. Each well was filled with 30 µl clove oil, or olive oil (vehicle control). The results were observed after 24-48 hours after incubation at 37°C by measuring the zone of inhibition around the wells with a scale .

VII. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF CLOVE EXTRACT FOR CANDIDA ALBICANS :

Serial 2 fold dilution of clove oil in olive oil as 2, 4, 8, 16, 32, and 64 times was prepared. Seven wells were dug into Sabouraud dextrose agar plates. Each well was filled with 30 µl of clove oil of different dilution and one for olive oil (vehicle control). The results were observed 48 hours after incubation at 37°C by measuring the zone of inhibition around the wells with a scale in mm.

2-8-B *IN VIVO* (CLINICAL STUDY) OF CLOVE OIL AS AN ANTIUNGAL IN SOME COMMON SKIN FUNGAL INFECTION (DERMATOPHYTOSIS):

This clinical study was conducted in the outpatient department of dermatology clinic in Alfwaihat Clinic, Benghazi. Out of 14 patient who agreed to participate in this clinical trial, only 3 patients regularly applied the clove oil. All patients were diagnosed with superficial skin fungal infection (dermatophytosis). The 3 cases were two women with tinea pedis (athlete's foot) and one man with pityriasis versicolor (tinea versicolor). The diagnosis were made by clinical examination and by potassium hydroxide (KOH) examination under light microscope of the skin scrapings. All patient were advised to apply 15% clove oil twice daily for two weeks. The reason for defaulted patients is that ; two patients complained of the smell and the mild irritation produced by clove oil and the rest just did not follow up.

2-9 STATISTICAL ANALYSIS

The result are presented as the mean ± standard error of the mean (S.E.M). The test of significance was done by paired student's t-test. P values < 0.05 were considered as significant, P value < 0.01 as highly significant and P value< 0.001 as very highly significant.

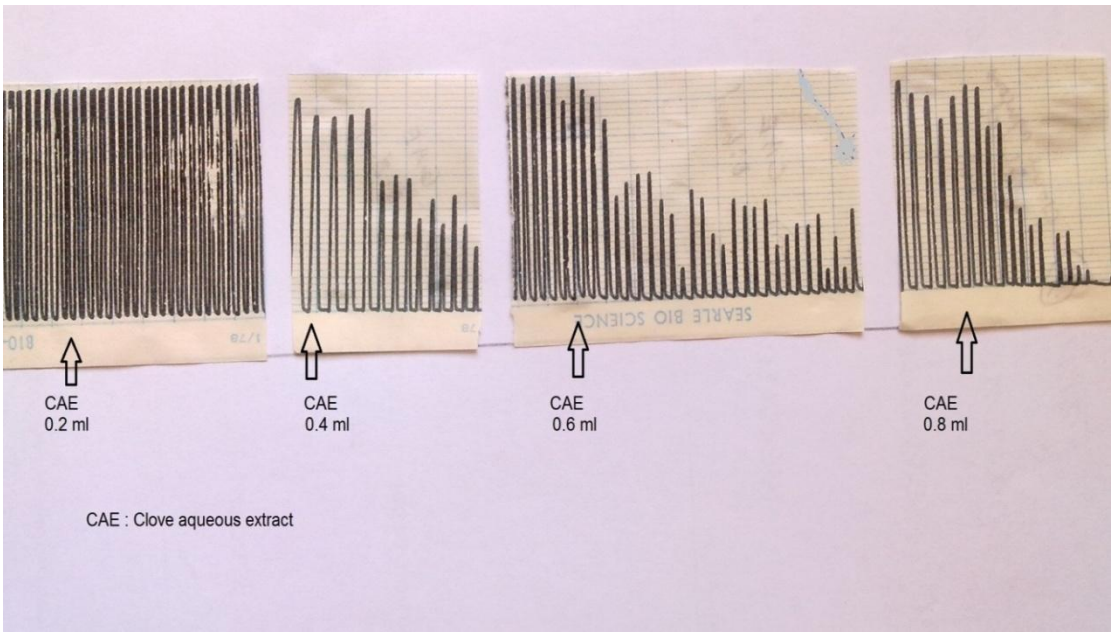
Ch. 3 RESULTS

3-1 EFFECT OF CLOVE AQUEOUS EXTRACT ON RHYTHMIC CONTRACTIONS OF RABBIT ISOLATED INTESTINE :

Isolated intestine showed consistent rhythmic contraction (Figure 8). Clove aqueous extract 0.2 ml did not show any appreciable effect on this rhythmic contractions. However, when the dose was increased to 0.4 ml, there was clear inhibition of the intestinal contractility. The inhibitory action of clove aqueous extract on rhythmic contractions was dose-dependent between 0.4 to 0.8 ml doses (Figure 8).

FIGURE 8

EFFECT OF DIFFERENT DOSES OF CLOVE AQUEOUS EXTRACT ON RHYTHMIC CONTRACTION OF RABBIT INTESTINE

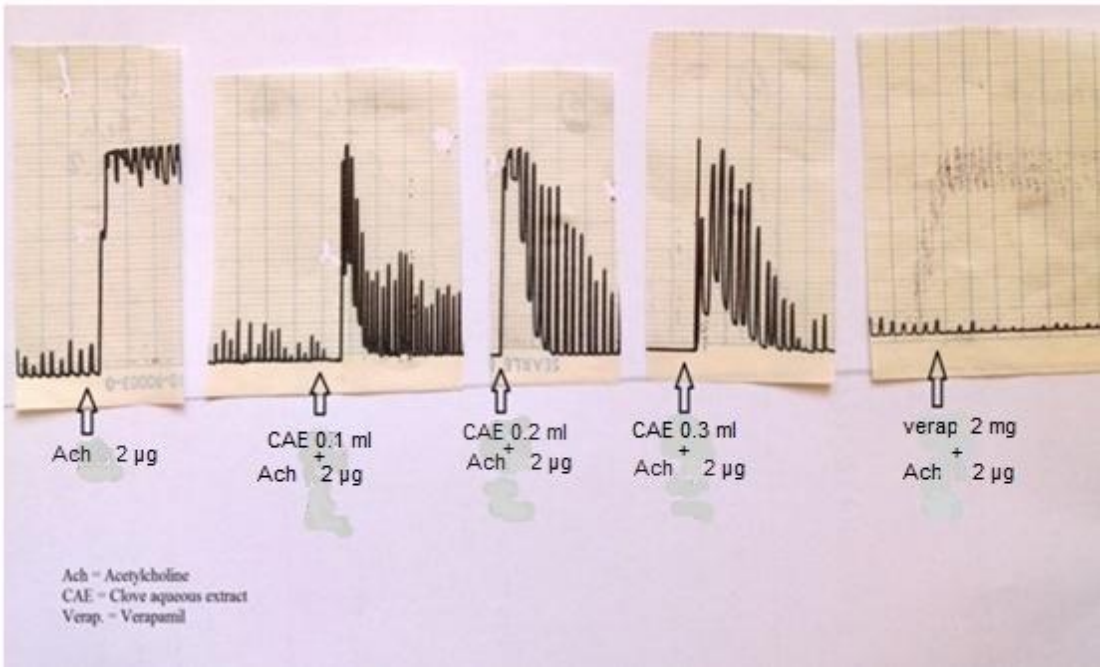


3-2 EFFECT OF CLOVE AQUEOUS EXTRACT ON Ach-INDUCED CONTRACTIONS OF RABBIT ISOLATED INTESTINE:

When 2 µg of Ach was used, it showed contraction of isolated intestine (Figure 9). When 0.1 ml of clove aqueous extract was used before adding 2 µg of Ach, there was inhibition of the Ach-induced contraction (Figure 9). This inhibition was dose-dependent, 0.3 ml of clove aqueous extract showed greater inhibition than with 0.2 ml (Figure 9). In comparison, when 2 mg verapamil used before 2 µg Ach, there was complete inhibition of Ach-induced contraction (Figure 9).

FIGURE 9

EFFECT OF CLOVE AQUEOUS EXTRACT ON Ach-INDUCED CONTRACTION OF RABBIT INTESTINE

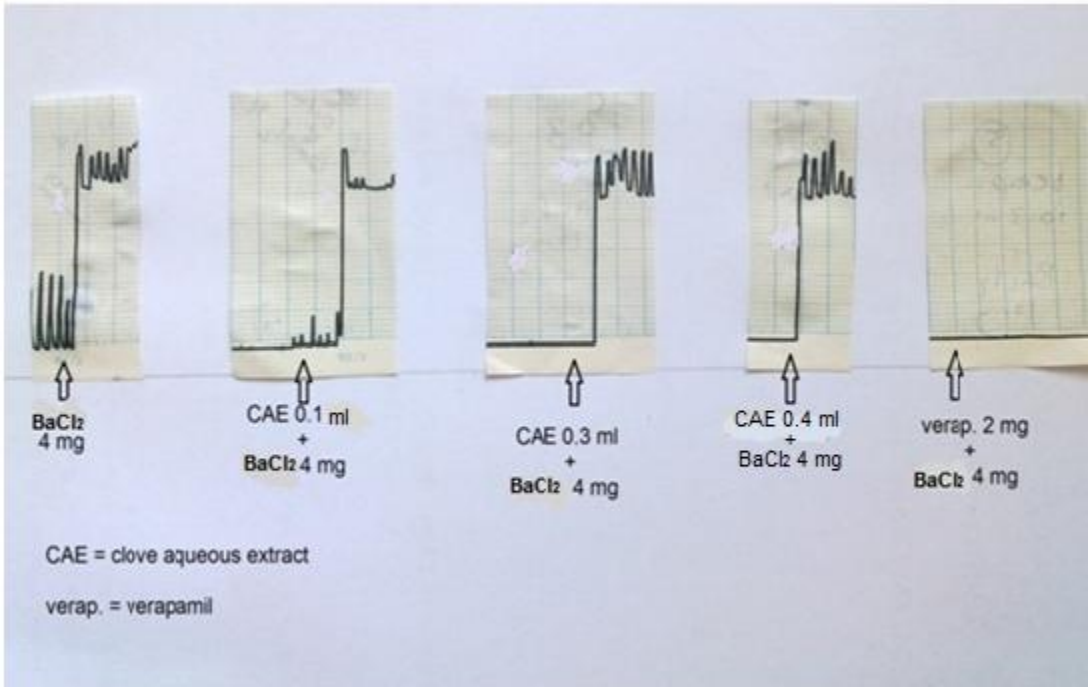


3-3 EFFECT OF CLOVE AQUEOUS EXTRACT ON $BaCl_2$-INDUCED CONTRACTIONS OF RABBIT ISOLATED INTESTINE :

Four mg of $BaCl_2$ produced contraction of the rabbit intestine when used alone. When different doses (0.1, 0.3, and 0.4 ml) of clove aqueous extract were added to the isolated intestine before adding 4 mg $BaCl_2$, they did not inhibit $BaCl_2$-induced contraction. However, when 2 mg of verapamil was added before 4 mg $BaCl_2$, there was complete inhibition of $BaCl_2$-induced contraction (Figure 10).

FIGURE 10

EFFECT OF CLOVE AQUEOUS EXTRACT ON $BaCl_2$-INDUCED CONTRACTIONS OF RABBIT ISOLATED INTESTINE

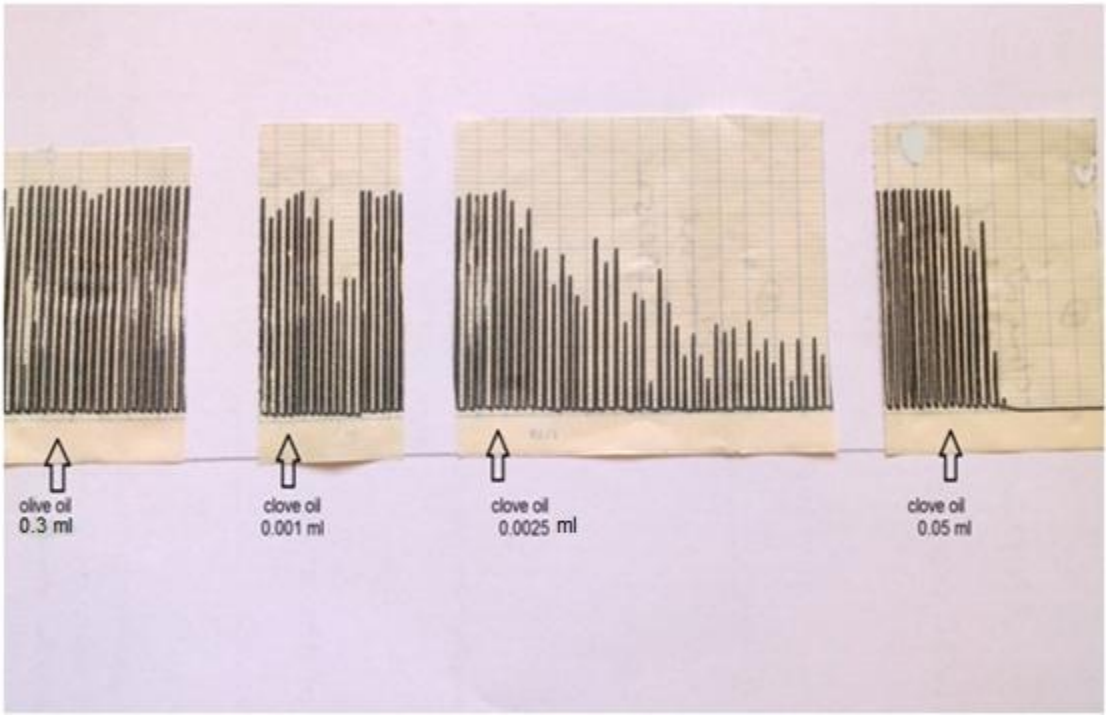


3-4 EFFECT OF CLOVE OIL ON RHYTHMIC CONTRACTIONS OF RABBIT ISOLATED INTESTINE:

The isolated rabbit intestine showed consistent rhythmic contractions which was not affected by addition of 0.3 ml olive oil (vehicle control). However, there was dose-dependent inhibition of intestinal rhythmic contractions when clove oil was added. Clove oil (0.0025ml) showed more effect than 0.001 ml, but 0.05 ml of clove oil showed complete relaxation of intestinal rhythmic contractions (Figure 11).

FIGURE 11

EFFECT OF CLOVE OIL ON RHYTHMIC CONTRACTION OF RABBIT ISOLATED INTESTINE

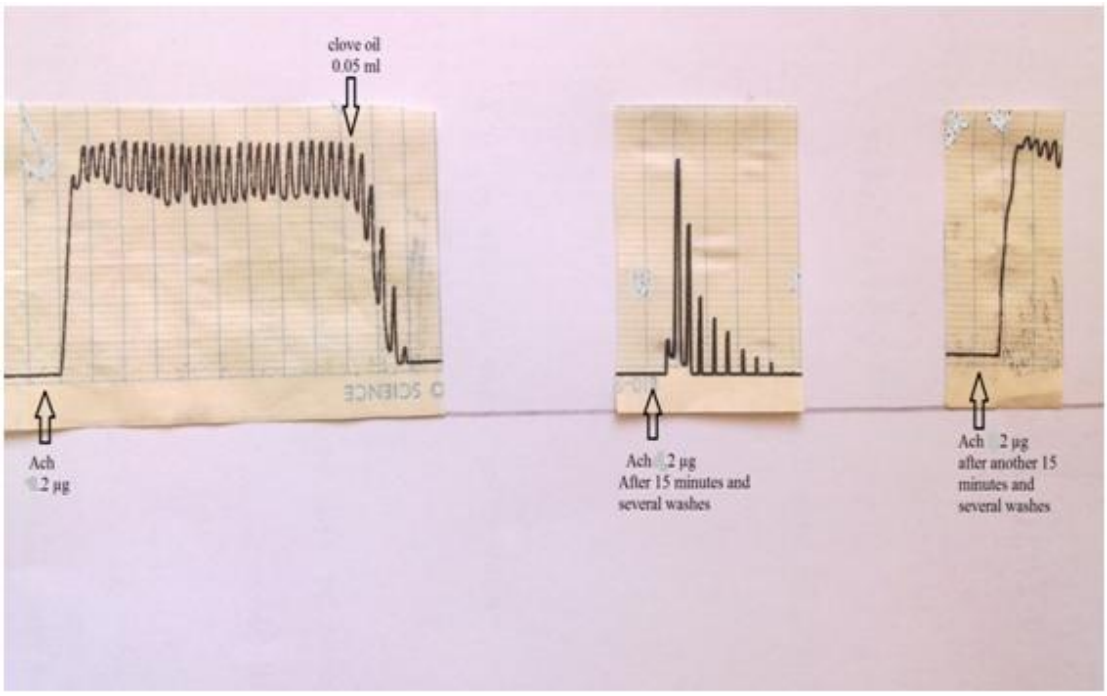


3-5 EFFECT OF CLOVE OIL ON Ach-INDUCED CONTRACTIONS OF RABBIT ISOLATED INTESTINE :

Two µg Ach showed contraction of rabbit's isolated intestine (Figure 12). A volume of 0.05 ml clove oil showed rapid inhibition of Ach-induced contraction (Figure 12). After 15 minutes and several washes 2 µg Ach showed contraction, but the recovery was not complete. After another 15 minutes and several washes, 2 µg Ach showed almost complete recovery (Figure 12).

FIGURE 12

EFFECT OF CLOVE OIL ON A Ach-INDUCED CONTRACTION OF RABBIT ISOLATED INTESTINE



3-6 EFFECT OF CLOVE OIL ON SMALL INTESTINE TRANSIT OF CHARCOAL MEAL IN MICE :

Results in (Table 2) and (Figure 13) showed that the movement of charcoal in small intestine in control group was 48.14 cm. Clove oil in all three doses studied inhibited the intestinal transit of charcoal in a dose-dependent manner. 0.1, 0.3, and 1.0 ml of clove oil given orally reduced the movement of charcoal meal in mice to 26.5, 12.28, and 2.71 cm respectively which were very highly significant ($P < 0.001$). Oral dose of atropine (10mg/kg) also reduced the movement of charcoal meal to 10.85 cm which was very highly significant ($P < 0.001$) lower than control volume (48.14 cm) and comparable to the effect of 0.3 ml clove oil.

TABLE 2

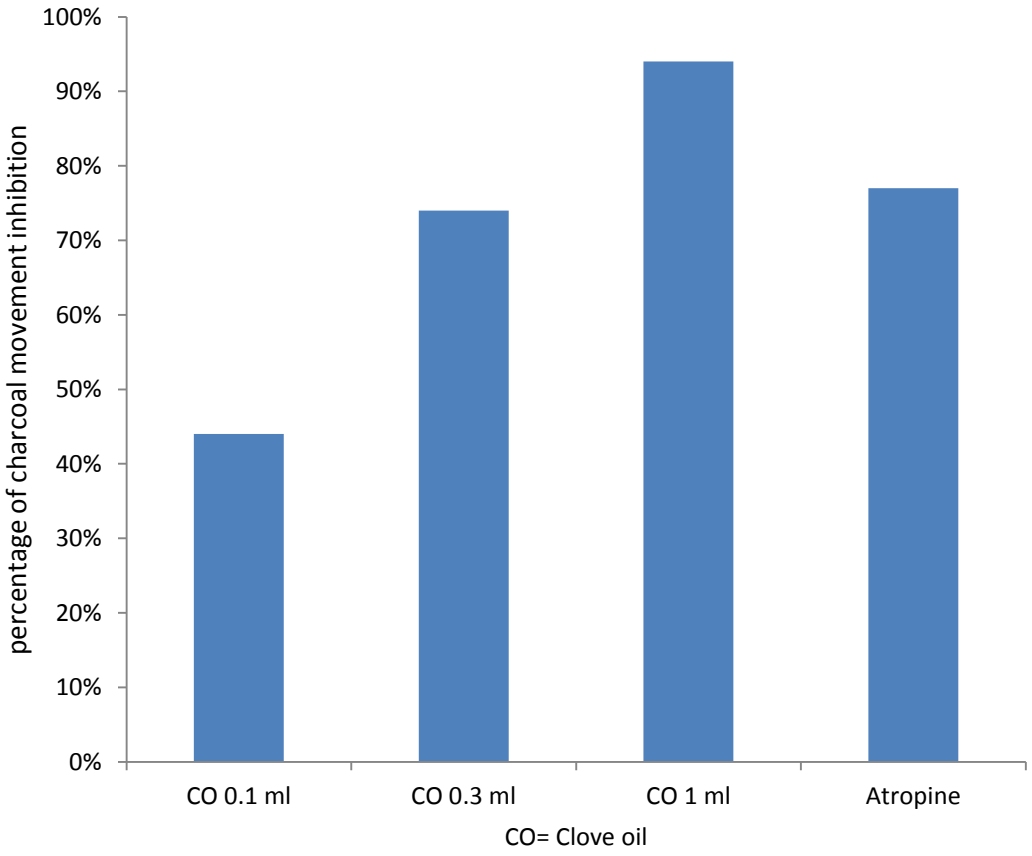
EFFECT OF CLOVE OIL ON CHARCOAL MEAL TRANSIT OF SMALL INTESTINE IN MICE

Drug	Dose	No. of mice	Movement of charcoal as percent of intestinal length from pylorus to caecum (cm)
Control	Olive oil	7	48.14 ±1.92
Clove oil	0.1 ml orally	6	26.5 ±3.26***
	0.3ml orally	7	12.28 ±1.32***
	1 ml orally	7	2.71 ±1.32***
Atropine	10 mg/kg orally	7	10.85 ±1.29***

*** very highly significant (P<0.001)

FIGURE 13

PERCENTAGE OF INHIBITION OF CHARCOAL TRANSIT BY CLOVE OIL IN MICE INTESTINE



3-7 EFFECT OF CLOVE OIL ON CASTOR OIL-INDUCED DIARRHEA IN MICE:

Results are presented in (Table 3) (Figure 14 and 15). All the mice in control group had diarrhea within 30 minutes after administration of castor oil and the average number of diarrhea episodes were 7.28 ± 0.25. Pretreatment with clove oil increased the period of onset of diarrhea and reduced the diarrheal episodes in a dose-dependent manner. Clove oil in doses of 0.1, 0.3, and 1.0 ml increased the period of onset of diarrhea to 47.5, 83.0, and 192.4 minutes respectively compared with the control (26.7 minutes) which were very highly significant ($P<0.001$) and reduced the diarrheal episodes to 6.14, 2.33, and 0.85 respectively compared with the control value of 7.28. A group of mice which received higher dose (0.3 ml) had no diarrhea; in these animals the onset of diarrhea was considered as 4 hours (240 minutes) which was the maximum observation period. The positive control group which received loperamide (10 mg/kg) did not produce any wet stool which was very highly significant ($P<0.001$).

TABLE 3

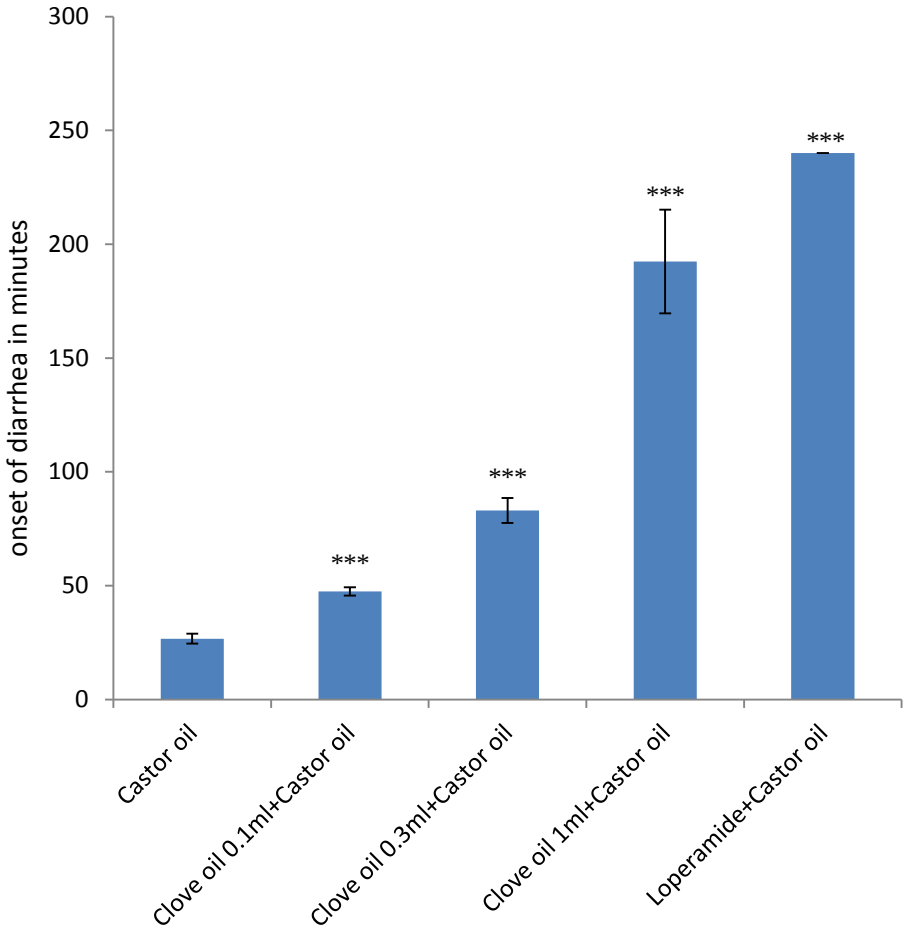
EFFECT OF CLOVE OIL ON CASTOR OIL-INDUCED DIARRHEA IN MICE

Drug	Dose	No. of animals	Onset of diarrhea (minutes) within 4 hours	No. of diarrheal episodes in 4 hours
Castor oil	0.3ml/mouse orally	7	26.71 ±2.20	7.28 ±0.52
Clove oil + Castor oil	0.1 ml orally 0.3 ml orally	6	47.5 ±1.87***	6.14 ±0.73
Clove oil + Castor oil	0.3 ml orally 0.3 ml orally	6	83 ±5.53***	2.33 ±0.76***
Clove oil + Castor oil	1.0 ml orally 0.3 ml orally	7	192.42 ±22.8***	0.85 ±0.45***
Loperamide + Castor oil	10 mg/kg orally 0.3 ml orally	6	240***	0***

*** very highly significant (P<0.001)

FIGURE 14

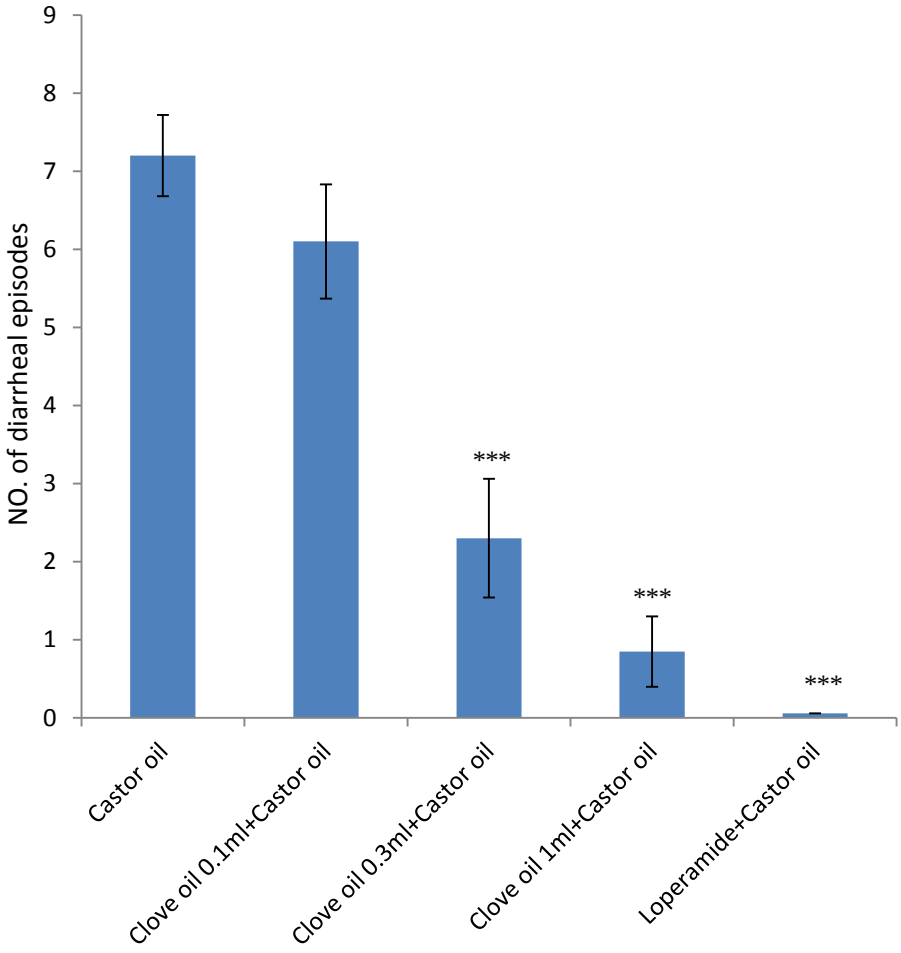
HISTOGRAM SHOWING THE EFFECT OF CLOVE OIL ON ONSET OF CASTOR OIL-INDUCED DIARRHEA



*** very highly significant

FIGURE 15

HISTOGRAM SHOWING THE EFFECT OF CLOVE OIL ON THE NUMBER OF DIARRHEAL EPISODES IN CASTOR OIL-INDUCED DIARRHEA IN MICE.



*** very highly significant

3-8 EFFECT OF CLOVE EXTRACT ON ACETIC ACID-INDUCED WRITHING IN MICE :

Acetic acid injected intraperitoneally (i.p.) produced irritation and pain in peritoneal cavity which was manifested in animals by showing writhing. Acetic acid alone produced an average of 12.33±1.92 writhes. Clove aqueous extract in 0.1 and 0.2 ml doses given intraperitoneally 30 minutes prior to acetic acid reduced this number of writhes to 3.83 and 1.71 and the percentage of inhibition were 68% and 86% respectively. When the same doses (0.1 and 0.2) ml of the aqueous extract were given orally, the inhibitory effect on writhing was comparatively less than the i.p. administration; the percentage of inhibition were 44% and 65% respectively. On the other hand, clove oil given orally produced higher inhibition in the writhes than clove aqueous extract given in the same doses by the same route of administration as their percentage of inhibition were 83% and 87% respectively (Table 4). Indomethacin and morphine used as positive controls also inhibited the writhings and morphine had greater inhibitory potency (90%) than indomethacin (49%).

The effects of naloxone on acetic acid-induced writhing and on analgesic action of clove oil, indomethacin, and morphine are presented in (Table 5). Naloxone on its own had no effect on acetic acid induced writhing. Administration of naloxone before clove oil also did not affect the analgesic action of clove oil. The average number of writhes with naloxone was 1.71±0.47 (85% inhibition) as compared to mice which did not recieve naloxone 1.5±0.61 (87% inhibition) which were statistically not significant ($P > 0.05$). On the other hand, the analgesic action of morphine was significantly reversed by naloxone.

TABLE 4

EFFECT OF CLOVE EXTRACT ON ACETIC ACID-INDUCED WRITHING IN MICE

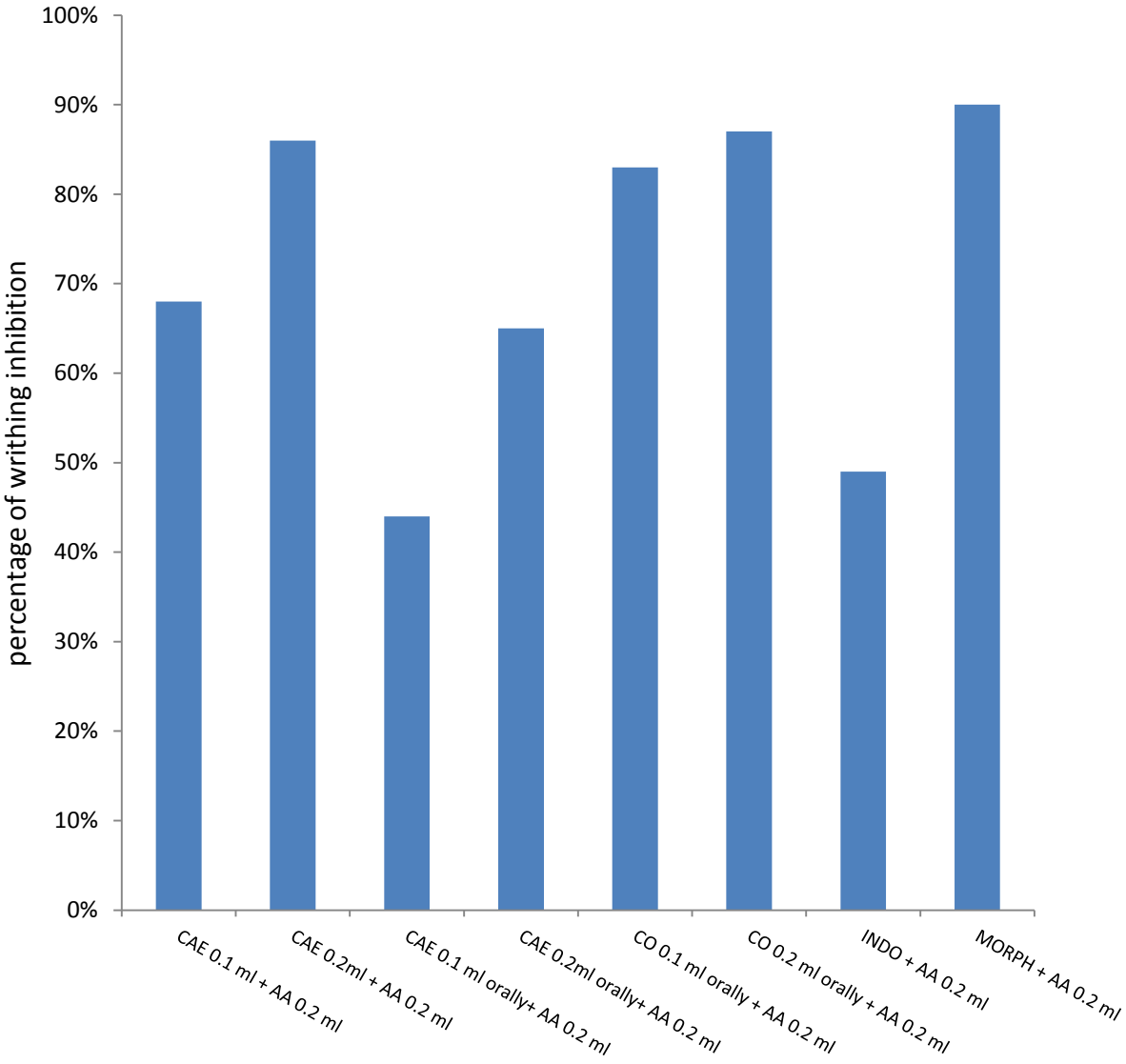
Drug	Dose per mouse or kg	Route of administration	No. of animals	No. of writhings	% of inhibition of writhings
1% Acetic acid	0.2 ml	i.p.	9	12.33 ±1.92	___
CAE + 1% Acetic acid	0.1 ml 0.2 ml	i.p. i.p.	6	3.83 ±0.70***	68.94%
CAE + 1% Acetic acid	0.2 ml 0.2 ml	i.p. i.p.	7	1.71 ±0.80***	86.13%
CAE + 1% Acetic acid	0.1 ml 0.2 ml	Oral i.p.	7	6.85 ±0.63**	44.44%
CAE + 1% Acetic acid	0.2 ml 0.2 ml	Oral i.p.	7	4.28 ±0.64***	65.29%
Clove oil + 1% Acetic acid	0.1 ml 0.2 ml	Oral i.p.	5	2 ±0.54***	83.78%
Clove oil + 1% Acetic acid	0.2 ml 0.2 ml	Oral i.p.	6	1.5 ±0.61**	87.83%
Indomethacin + 1% Acetic acid	5mg/kg 0.2 ml	i.p. i.p.	5	6.2 ±0.66**	49.72%
Morphine + 1% Acetic acid	5 mg/kg 0.2 ml	i.p. i.p.	6	1.16 ±0.54***	90.59%

CAE=clove aqueous extract s.c.=subcutaneously i.p.=intraperitoneally

** highly significant (P<0.01) *** very highly significant (P<0.001)

FIGURE 16

THE PERCENTAGE OF WRITHING INHIBITION BY CLOVE EXTRACTS



CAE=clove aqueous extract AA=acetic acid 1% CO=clove oil INDO=indomethacin 5mg/kg MORPH=morphine 5mg/kg

TABLE 5

EFFECT OF NALOXONE ON ANALGESIA PRODUCED BY CLOVE OIL

Drug	Dose per mouse or kg	Route of administration	No. of animals	No. of writhings	% inhibition of writhing
1% Acetic acid	0.2 ml	(Intraperitoneal) i.p.	9	12.33 ±1.92	___
Clove oil + 1% Acetic acid	0.2 ml 0.2 ml	Oral i.p.	6	1.5 ±0.61	87%
Indomethacin + 1% Acetic acid	5mg/kg 1% acetic acid	i.p. i.p.	5	6.2 ±0.66[a]	49%
Morphine + 1% Acetic acid	5 mg/kg 0.2 ml	i.p. i.p.	6	1.16 ±0.54[c]	90%
Naloxone + 1% Acetic acid	1mg/kg 0.2 ml	i.p. i.p.	5	13 ±2.00	0%
Naloxone + Clove oil + 1% Acetic acid	1mg/kg 0.2 ml 0.2 ml	i.p. Oral i.p.	7	1.71 ±0.47	85%
Naloxone + Indomethacin + 1% Acetic acid	1mg/kg 5mg/kg 0.2 ml	i.p. i.p. i.p.	5	5.8 ±0.73[b]	52%
Naloxone + Morphine + 1% Acetic acid	1mg/kg 5mg/kg 0.2 ml	i.p. s.c. i.p.	5	11.4 ±1.16[d]	7.5 %

Difference between “a” and “b” not significant (P>0.05). Between “c” and “d” very highly significant (P<0.001)

3-9 ANTIMICROBIAL STUDIES:

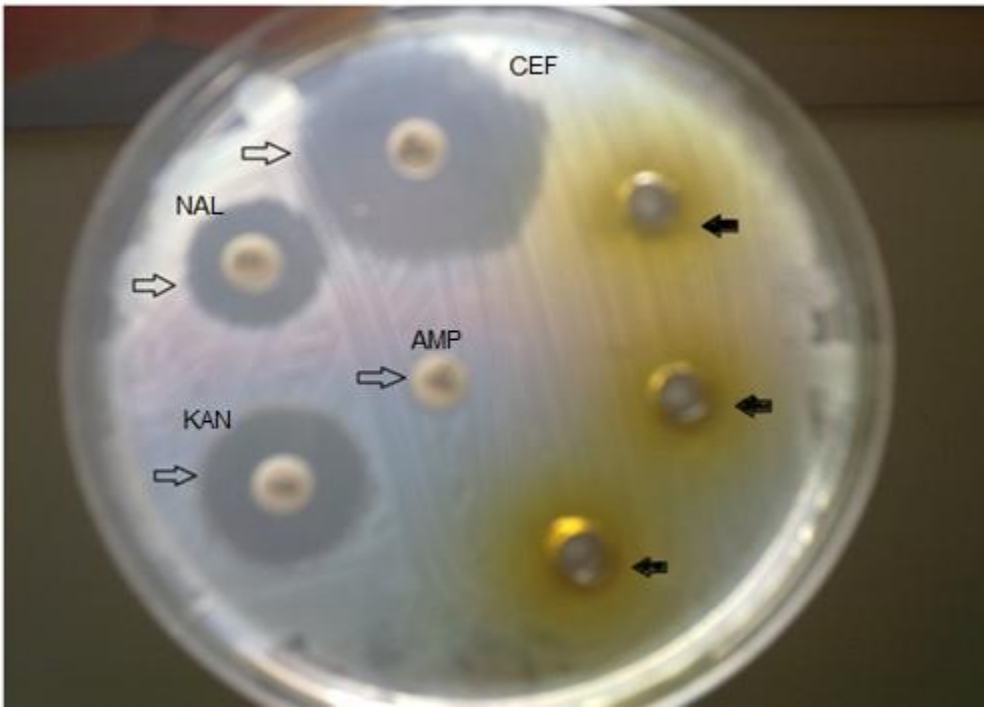
3-9-A ANTIBACTERIAL STUDIES :

i. Effect of clove aqueous extract on growth of E. coli and staphylococcus aureus :

Clove aqueous extract 50 µl showed no inhibitory effect against any of those bacteria. On the other hand, antibiotic discs fusidic acid (10 µg) produced 22 mm zone of inhibition, while ampicillin, oxacillin, and pipercillin showed no effect against staphylococcus aureus. Regarding E. coli, ceftriaxone (30 µg), kanamycin (30 µg), and nalidixic acid (30 µg) produced 30, 20, and 15 mm respectively and ampicillin showed no effect (Figure 17and 18).

FIGURE 17

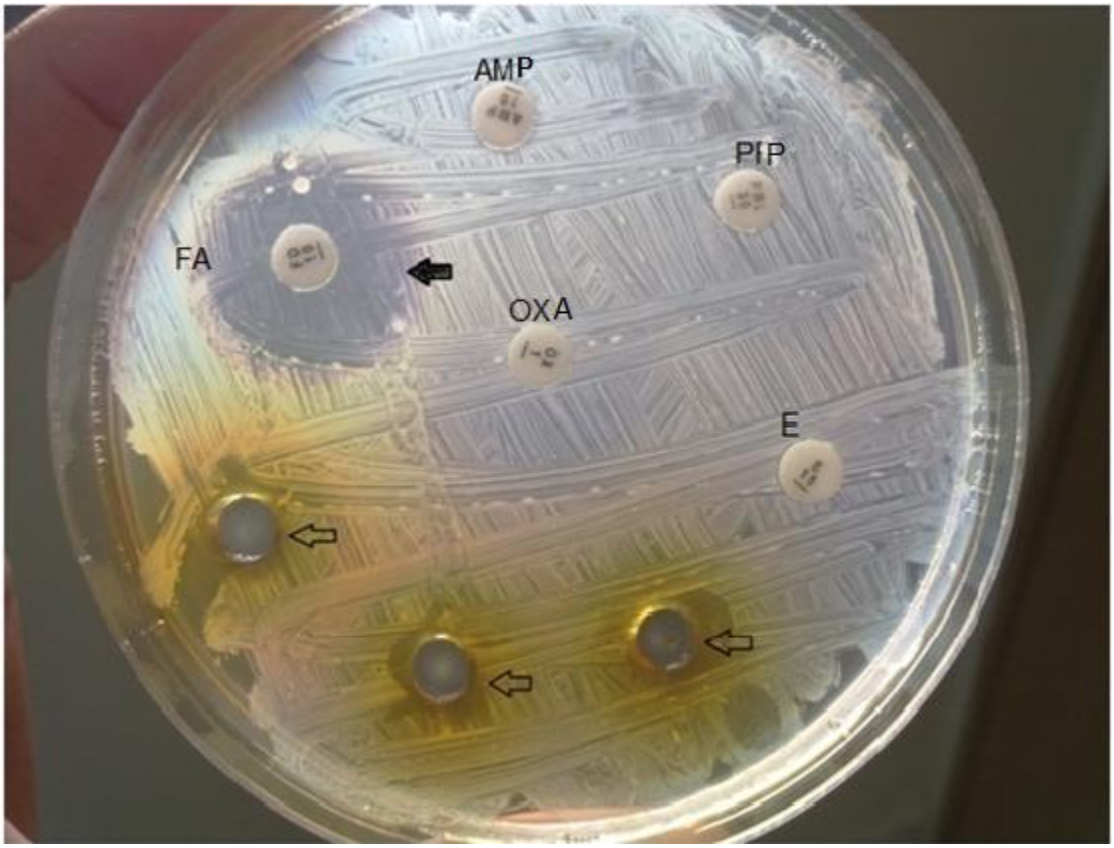
EFFECT OF CLOVE AQUEOUS EXTRACT ON GROWTH OF E. COLI



Solid arrows (wells filled with clove aqueous extract) have no effect on E. coli, and the empty arrows represents different antibiotic discs. (AMP) ampicillin disc in the middle has no effect, while (CEF) ceftriaxone, (KAN) kanamycin, and (NAL) nalidixic acid showed antibacterial effect.

FIGURE 18

EFFECT OF CLOVE AQUEOUS EXTRACT ON GROWTH OF STAPHYLOCOCCUS AUREUS



Empty arrows are clove aqueous extract which showed no effect. Solid arrow showed the only effective antibiotic disc (FA) fucidic acid, while (AMP) ampicillin, (OXA) oxacillin, (PIP) pipercillin, and (E) erythromycin had no effect.

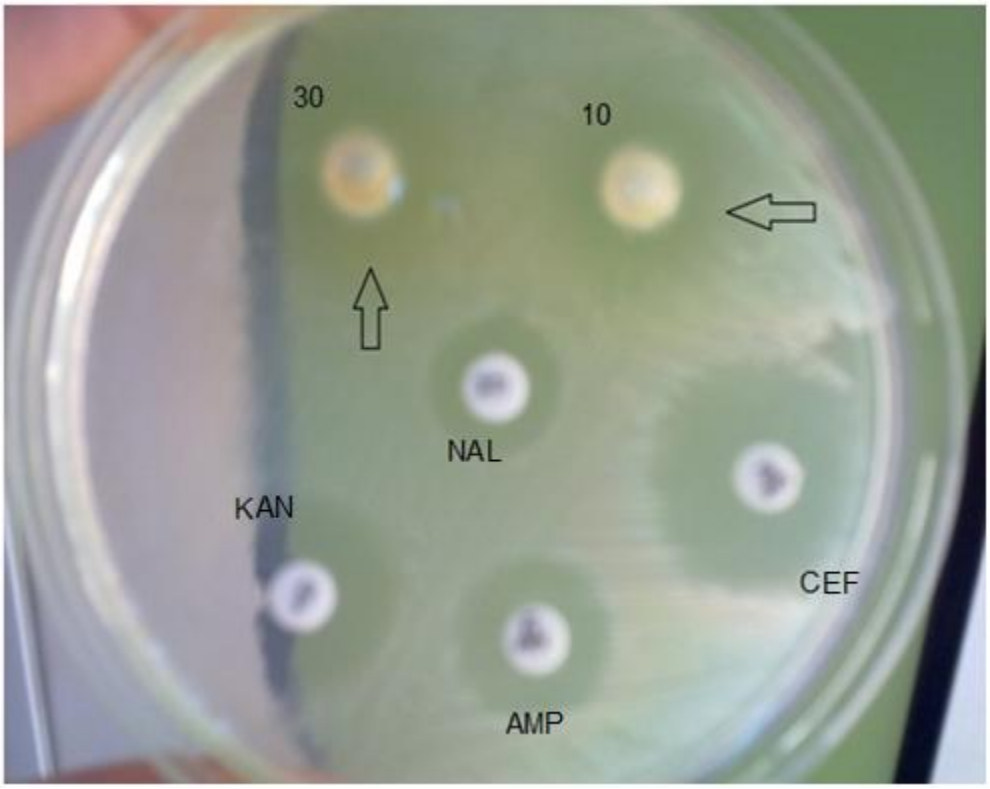
ii. Effect of different doses of clove oil on growth of E. coli on nutrient agar plate :

Clove oil showed dose-dependent inhibition of the growth of E. coli (Table 6) (Figure 19 and 20). Clove oil in doses of 2, 3, 10, and 30 µl produced 8.4, 11.0, 11.8 and 24.8 mm of inhibitory zones respectively. Olive oil (30 µl) used as vehicle for diluting clove oil was completely devoid of antimicrobial activity. Ceftriaxone (30 µg), kanamycin (30 µg), nalidixic acid (30 µg) and ampicillin (10 µg) discs used as positive control were found to inhibit the growth of E. coli producing inhibitory zone of 25.5, 18.7, 13.5, and 15 mm respectively. The inhibitory effect of (30 µg) of ceftriaxone was maximal which was comparable to the inhibitory effect produced by 30 µl of clove oil. The inhibitory effect of 10 µg ampicillin was also comparable to 10 µl of clove oil. The inhibitory effect of (30 µg) kanamycin and (30 µg) nalidixic acid were less than 30 µg of ceftriaxone or 30 µl of clove oil (Table 6) (Figure 19 and 20).

In some experiments, Indian clove oil which was available in the market for use in toothache was also studied. 30 µl dose of this product was found to produce inhibitory zone of 31.6 mm which was more than 30 µl of clove oil distilled in our laboratory (Table 6) and (Figure 20).

FIGURE 19

EFFECT OF CLOVE OIL ON GROWTH OF E. COLI



Arrows indicate wells filled with 10 and 30 µl of clove oil which showed clear inhibition zone. Different antibiotic discs (CEF) ceftriaxone, (KAN) kanamycin, (NAL) nalidixic acid, and (AMP) ampicillin also showed inhibitory zones.

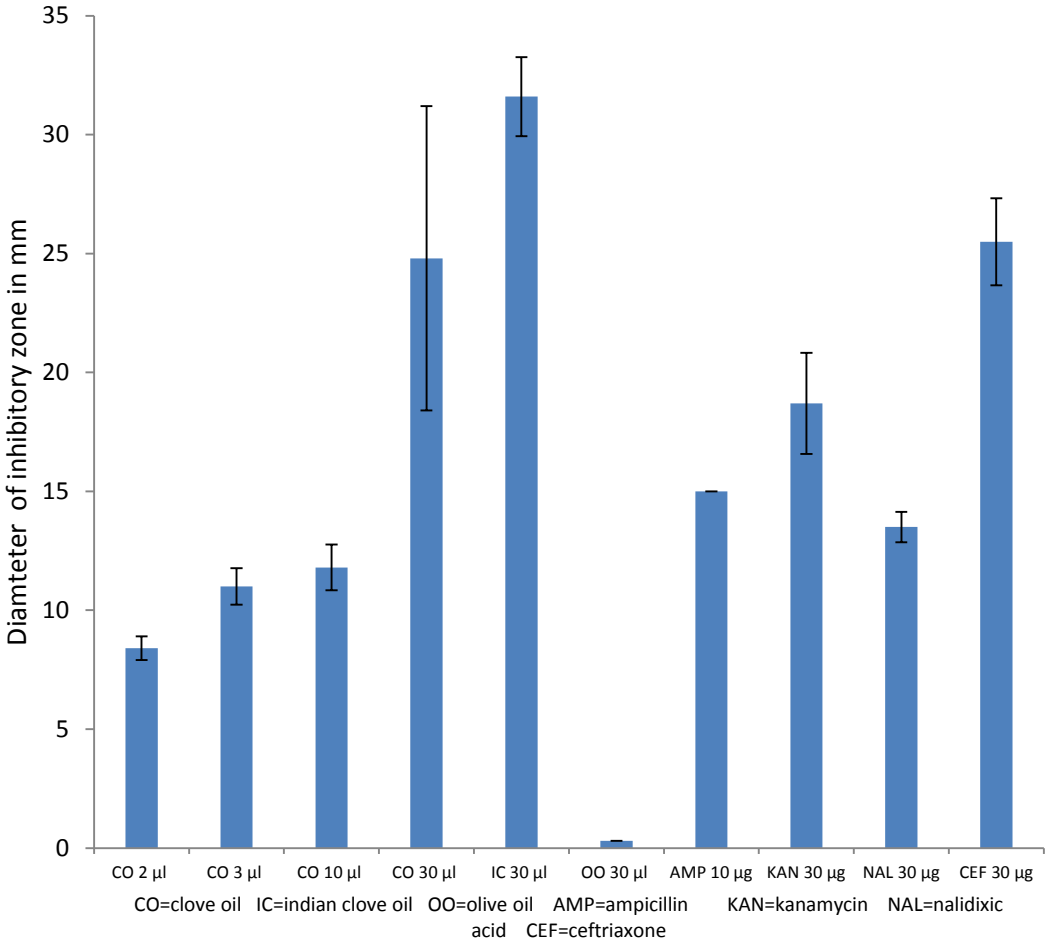
TABLE 6

ANTIBACTERIAL ACTIVITY OF CLOVE OIL AGAINST E. COLI

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/ well	5	8.4 ±0.50
	3 µl/well	5	11 ±0.77
	10 µl/well	5	11.8 ±0.96
	30 µl/well	5	24.8 ±6.40
Indian clove oil	30 µl/well	3	31.6 ±1.66
Olive oil (vehicle)	30 µl/well	5	0
Ceftriaxone	30 µg/disc	6	25.5 ±1.83
Kanamycin	30 µg/disc	4	18.7 ±2.13
Nalidixic acid	30 µg/disc	4	13.5 ±0.64
Ampicillin	10 µg/disc	3	15

FIGURE 20

HISTOGRAM SHOWING THE EFFECT OF CLOVE OIL AGAINST E. COLI



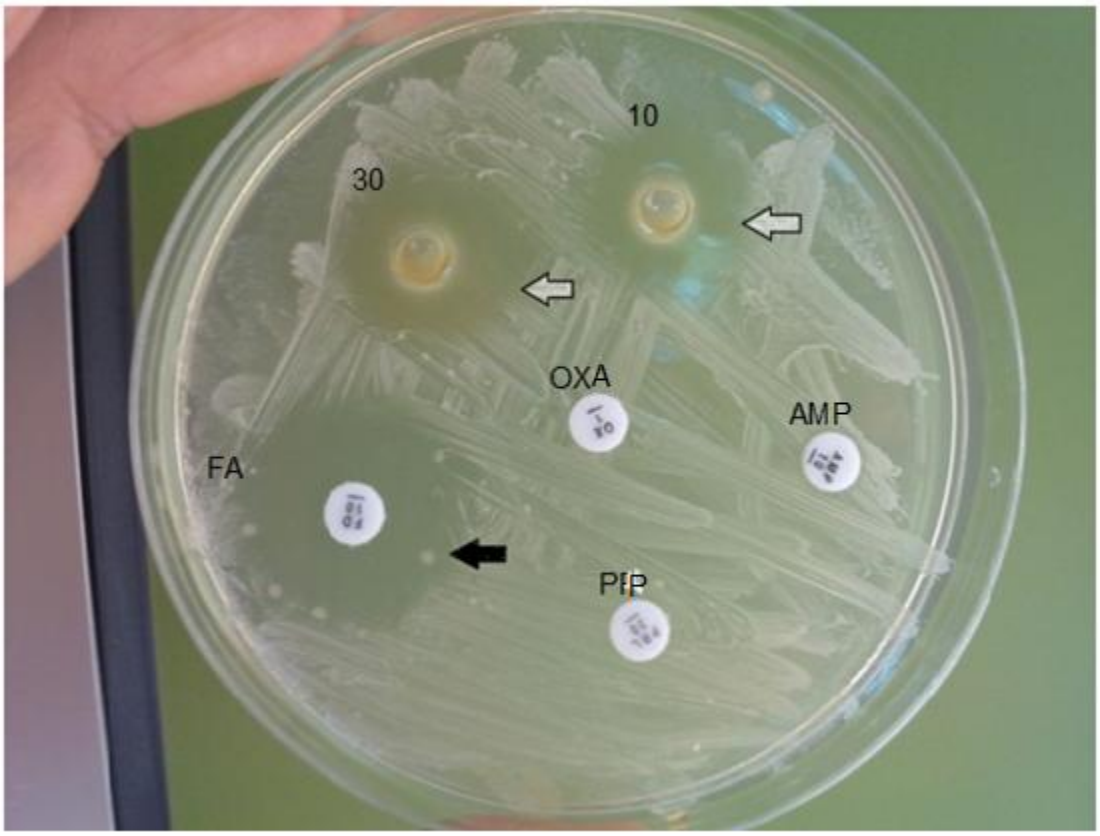
iii. Effect of different doses of clove oil on growth of staphylococcus aureus on nutrient agar plate :

Results are presented in (Table 7), (Figure 21 and 22). Lower doses (2 and 3 µl) did not show any inhibitory effect. However, higher doses (10 and 30 µl) showed inhibitory zone average of 14 and 15.8 mm respectively. Olive oil (30 µl) used as vehicle was completely ineffective. Fucidic acid (10 µg) used as positive control was significantly more effective in inhibiting growth of staphylococcus aureus than 10 µl of clove oil. Ampicillin (10 µg), oxacillin (1 µg), pipercillin (30 µg), kanamycin (30 µg), nalidixic acid (30 µg) and ceftriaxone (30 µg) did not show any inhibitory zone (Table 7).

Indian clove oil (30 µl) showed more inhibitory zone compared with 30 µl of clove oil which was extracted in our laboratory.

FIGURE 21

EFFECT OF CLOVE OIL ON GROWTH OF STAPHYLOCOCCUS AUREUS



White arrows represent wells filled with 10 and 30 µl of clove oil which had an effect. The solid arrows showed (FA) fucidic acid effect. Other discs (AMP) ampicillin,(OXA) oxacillin, and (PIP) pipercillin had no effect.

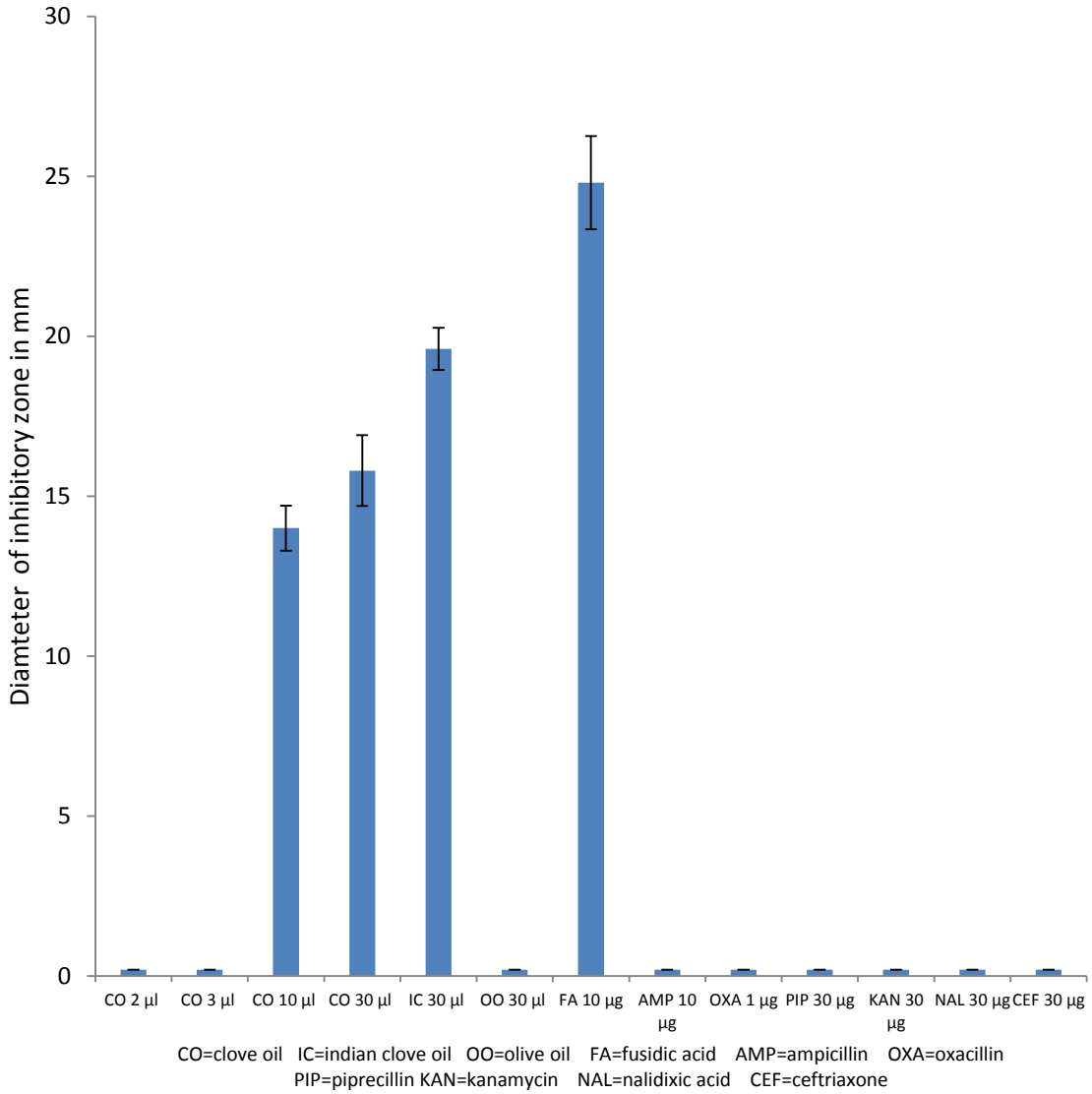
TABLE 7

ANTIBACTERIAL ACTIVITY OF CLOVE OIL AGAINST STAPHYLOCOCCUS AUREUS

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/well	3	0
	3 µl/well	3	0
	10 µl/well	5	14 ±0.70
	30 µl/well	5	15.8 ±1.11
Indian clove oil	30 µl/well	3	19.6 ±0.66
Olive oil (vehicle)	30 µl/well	5	0
Fusidic acid	10 µg/well	5	24.8 ±1.46
Ampicillin	10 µg/well	4	0
Oxacillin	1 µg/well	4	0
Pipercillin	30 µg/well	4	0
Kanamycin	30 µg/well	4	0
Nalidixic acid	30 µg/well	4	0
Ceftriaxone	30 µg/well	4	0

FIGURE 22

HISTOGRAM SHOWING ANTIBACTERIAL EFFECT OF DIFFERENT DOSES OF CLOVE OIL AGAINST STAPHYLOCOCCUS AUREUS



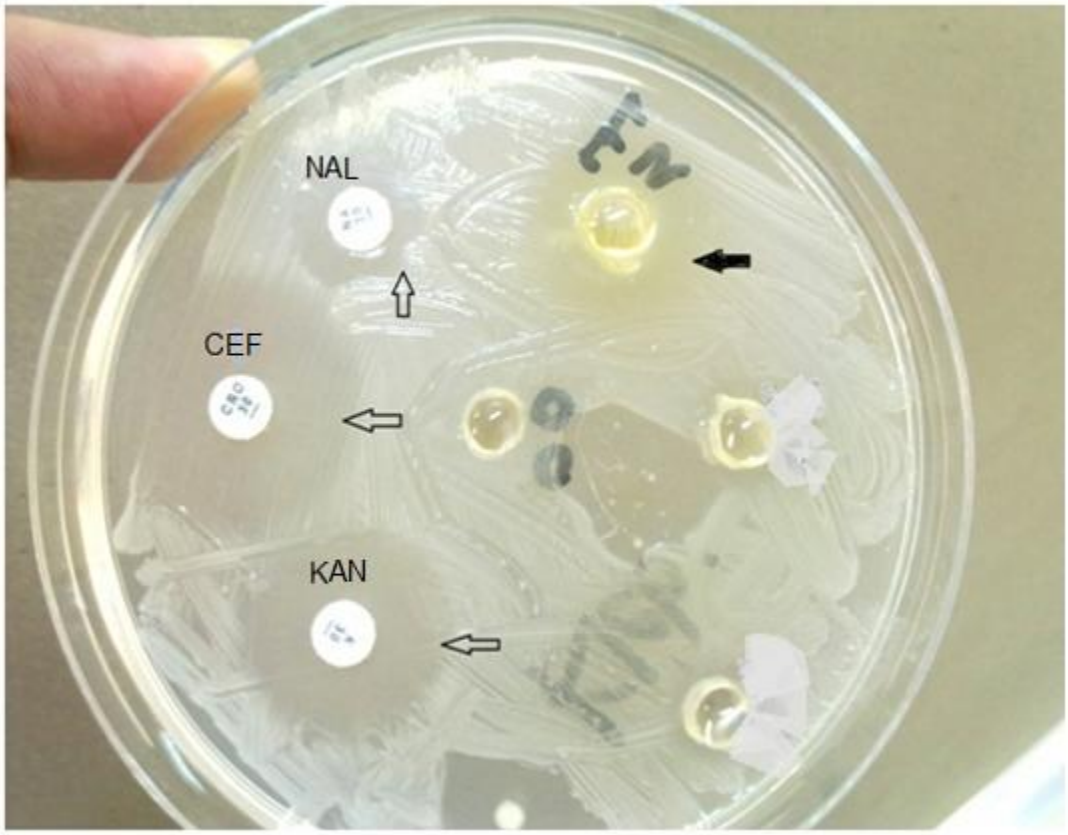
iv. Effect of different doses of clove oil on growth of klebsiella on nutrient agar plate :

Results are presented in (Table 8) (Figure 23 and 24). Clove oil in doses of 10 and 30 µl showed average of 10.4, and 17.6 mm inhibitory zones respectively which were dose-dependent. Olive oil (30 µl) used as vehicle control did not show any inhibitory zone.

Ceftriaxone (30 µg), kanamycin (30 µg), and nalidixic acid (30 µg) showed inhibitory zone of 27.6, 18.8, and 14.0 mm respectively. The inhibitory zone produced by 30 µg of ceftriaxone was more than the 30 µl dose of clove oil. The inhibitory effect of 30 µg doses of both kanamycin and nalidixic acid were comparable to the inhibitory effect produced by 30 µl of clove oil.

FIGURE 23

EFFECT OF CLOVE OIL ON GROWTH OF KLEBSIELLA



Solid arrow showed the clove oil 30 µl had an inhibitory effect while the other two dilution 2 and 3 µl had no effect. Empty arrows indicated to (CEF) ceftriaxone, (NAL) nalidixic acid, and (KAN) kanamycin discs which also had an inhibitory effect. Note in the center olive oil containing well.

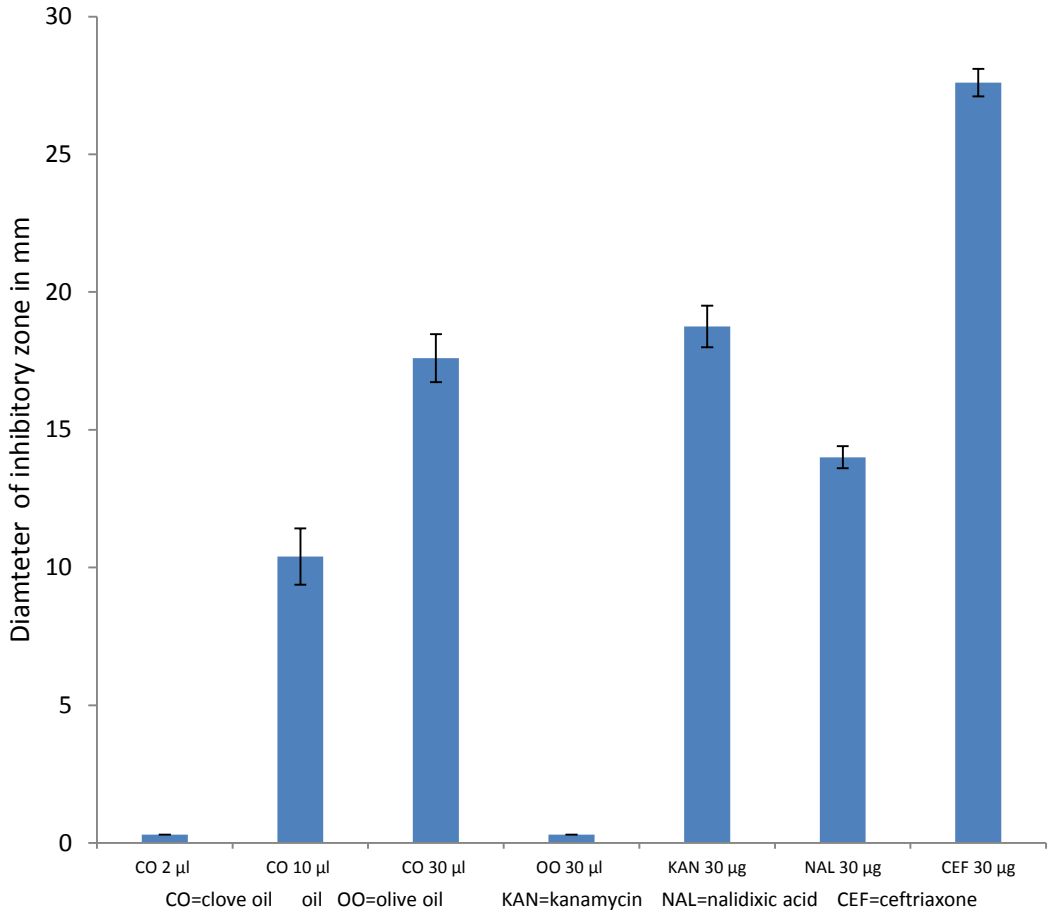
TABLE 8

ANTIBACTERIAL EFFECT OF CLOVE OIL AGAINST KLEBSIELLA

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/well	5	0
	10 µl/well	5	10.4 ±1.02
	30 µl/well	5	17.6 ±0.87
Olive oil (vehicle)	30 µl/well	5	0
Ceftriaxone	30 µg/well	5	27.6 ±0.50
Kanamycin	30 µg/well	4	18.75 ±0.75
Nalidixic acid	30 µg/well	4	14 ±0.40

FIGURE 24

HISRTOGRAM SHOWING THE EFFECT OF CLOVE OIL AGAINST KLEBSIELLA



v. Effect of different doses of clove oil on growth of proteus on nutrient agar plate :

Results are presented in (Table 9) and (Figure 25). Lower doses (2 and 3 µl) did not show any inhibitory effect. However, higher dose (30 µl) produced 19.2 mm of inhibitory zone. Olive oil (30 µl), the vehicle did not show any inhibitory effect. Ceftriaxone (30 µg) and kanamycin (30 µg) showed more inhibition of growth of proteus; the average of inhibitory zone were 32.8 and 24.0 mm respectively. Nalidixic acid (30 µg) was completely ineffective.

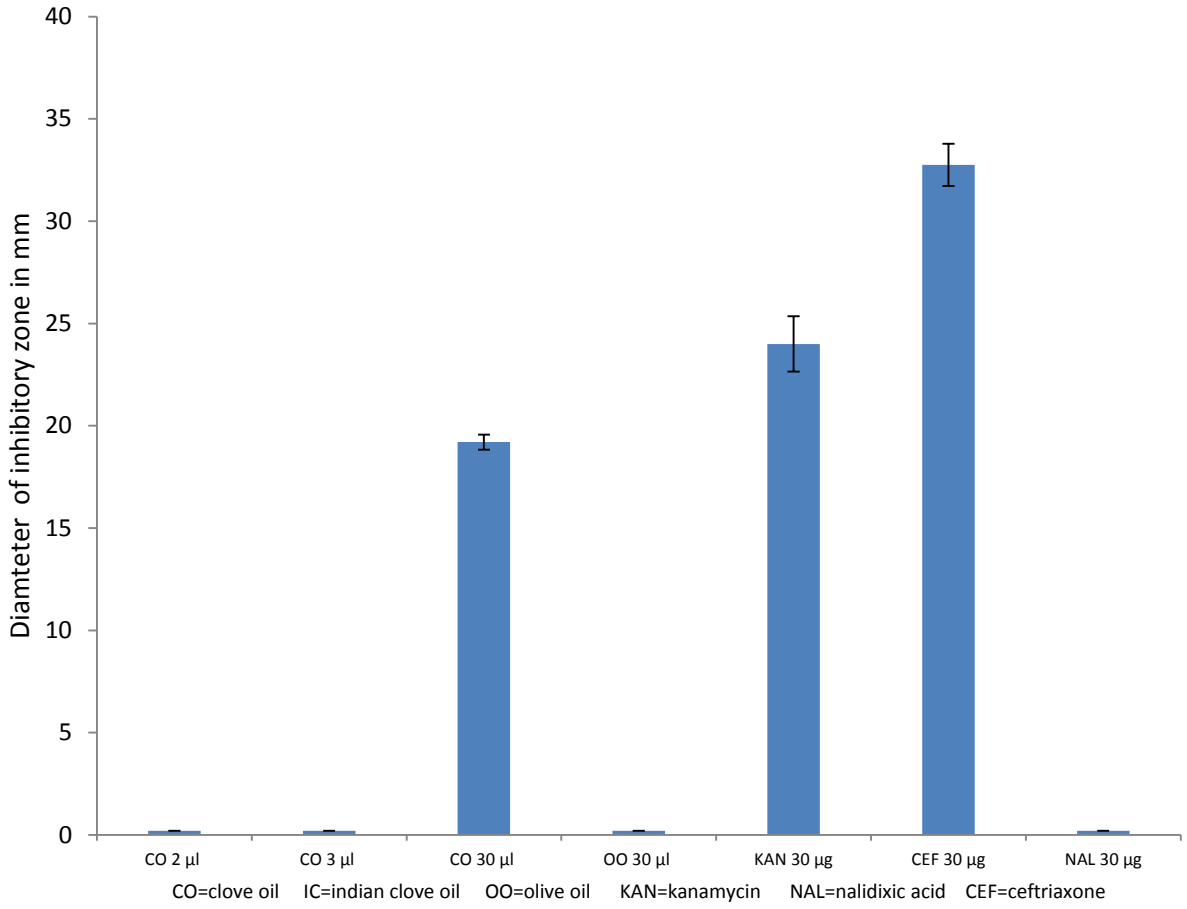
TABLE 9

ANTIBACTERIAL EFFECT OF CLOVE OIL AGAINST PROTEUS

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/well	5	0
	3 µl/well	5	0
	30 µl/well	5	19.2 ±0.37
Olive oil (vehicle)	30 µl/well	5	0
Ceftriaxone	30 µg/well	4	32.75 ±1.03
Kanamycin	30 µg/well	4	24 ±1.35
Nalidixic acid	30 µg/well	4	0

FIGURE 25

HISTOGRAM SHOWING ANTIBACTERIAL EFFECTS OF DIFFERENT DOSES OF CLOVE OIL AGAINST PROTEUS

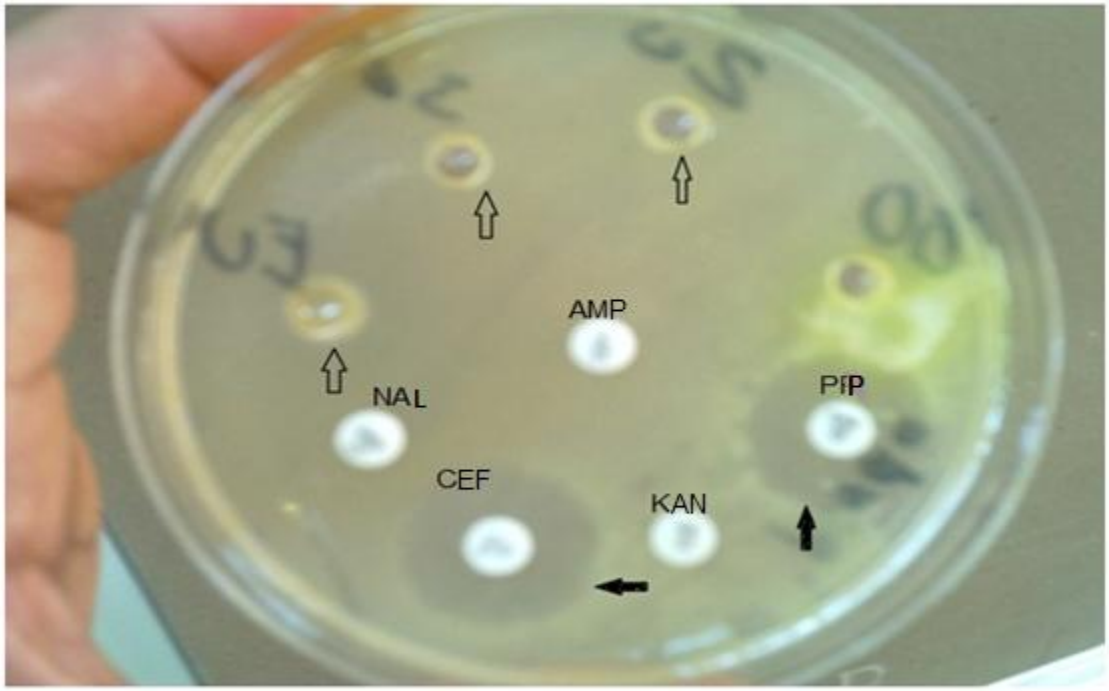


vi. Effect of different doses of clove oil on growth of pseudomonas aeruginosa on nutrient agar plate :

Results are presented in (Table 10) (Figure 26 and 27). Clove oil in all doses used (2, 3, and 30) did not show any inhibitory effect against pseudomonas aeruginosa. Pipercillin (30 µg) and ceftriaxone (30 µg) used as positive control showed 19.3 and 17.7 mm of average inhibitory zone respectively. Kanamycin (30 µg), Nalidixic acid (30 µg) and ampicillin (30 µg) were also ineffective in inhibiting the growth of pseudomonas aeruginosa.

FIGURE 26

EFFECT OF CLOVE OIL ON GROWTH OF PSEUDOMONAS AERUGINOSA



Empty arrows showed 2, 3, and 30 µl clove oil did not have any effect. Solid arrows showed (PIP) pipercillin and (CEF) ceftriaxone discs exhibited antibacterial effect, while (KAN) kanamycin, (AMP) ampicillin, and (NAL) nalidixic acid discs were ineffective.

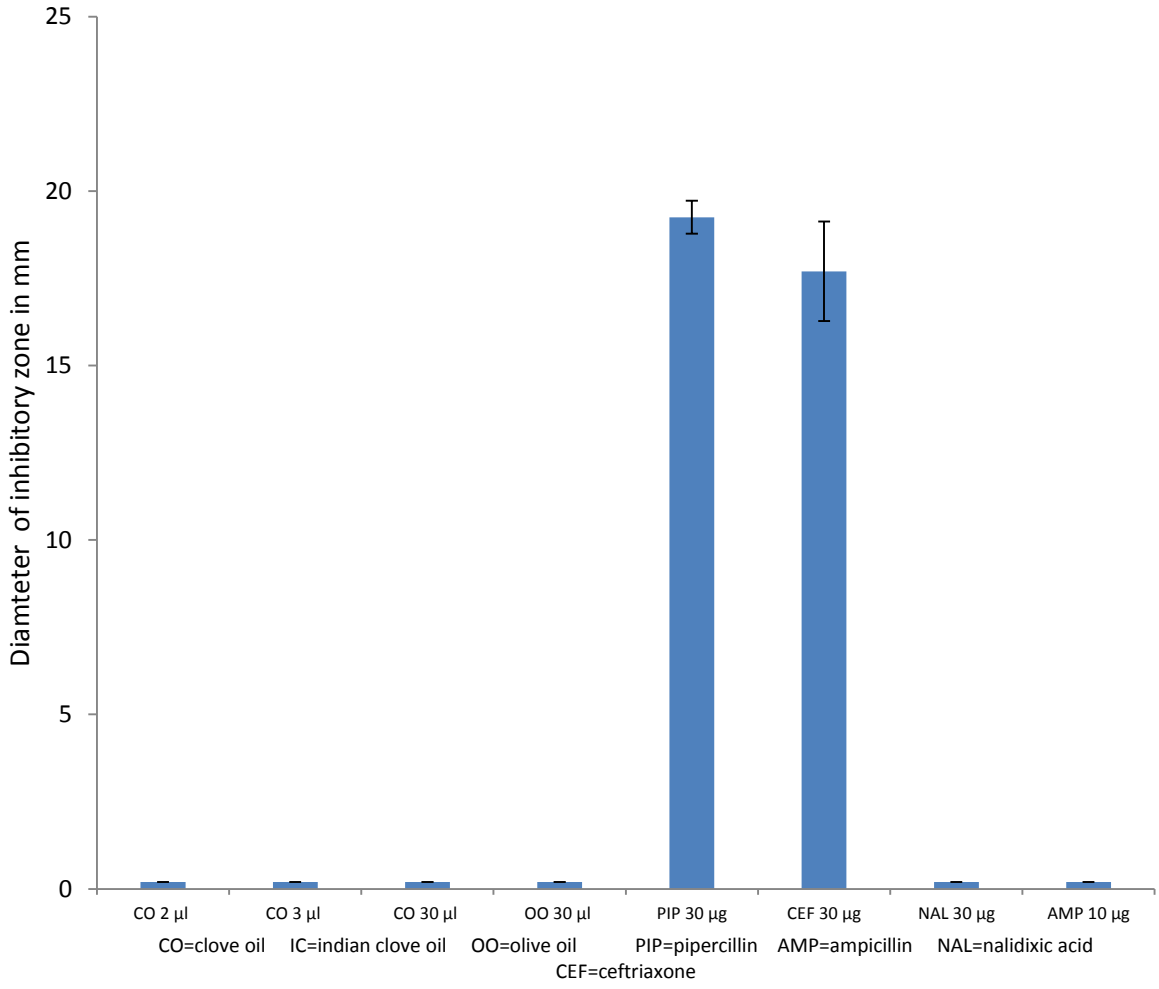
TABLE 10

ANTIBACTERIAL EFFECT OF CLOVE OIL AGAINST PSEUDOMONAS AERUGINOSA

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/well	5	0
	3 µl/well	5	0
	30 µl/well	5	0
Olive oil (vehicle)	30 µl/well	5	0
Pipercillin	30 µl/well	4	19.25 ±0.47
Ceftriaxone	30 µg/well	4	17.7 ±1.43
Nalidixic acid	30 µg/well	4	0
Ampicillin	10 µg/well	4	0

FIGURE 27

HISTOGRAM SHOWS ANTIBACTERIAL EFFECT OF DIFFERENT DOSES OF CLOVE OIL AGAINST PSEUDOMONAS AERUGINOSA



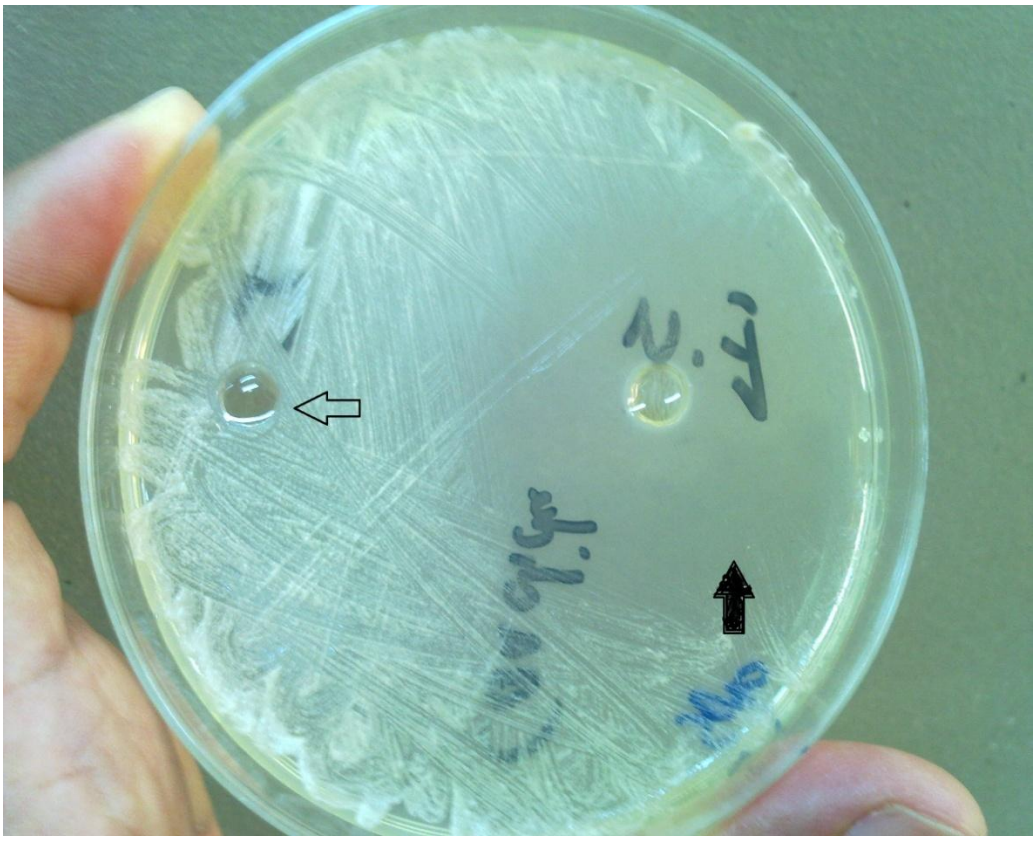
3-9-B ANTIFUNGAL STUDIES :

EFFECT OF DIFFERENT DOSES OF CLOVE OIL ON GROWTH OF CANDIDA ALBICANS GROWN ON SABOURAUD'S DEXTROSE AGAR PLATE :

Results are presented in (Table 11) and (Figure 28). Clove oil very effectively inhibited the growth of candida albicans on sabouraud's dextrose media. Tow µl of clove oil produced 17 mm inhibitory zone whereas higher doses (3 µl and 30 µl) showed 48 mm of inhibitory zone. Olive oil used as vehicle was completely ineffective in inhibiting the growth of candida. Indian clove oil (30 µl) also showed almost same degree of inhibitory zone (44.5 mm) as shown by 3 and 30 µl of clove oil extracted in our laboratory.

FIGURE 28

EFFECT OF CLOVE OIL AGAINST CANDIDA ALBICANS



Solid arrow represent 30 µl of clove oil. Empty arrows represents olive oil.

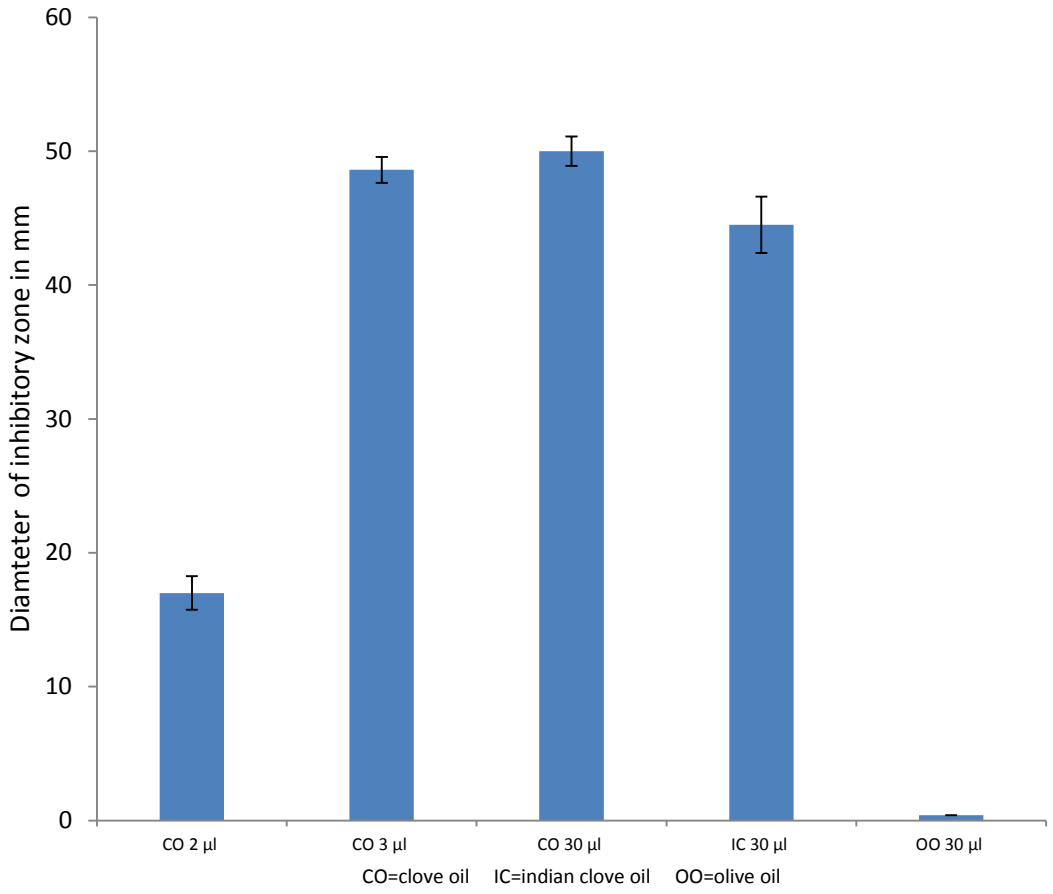
TABLE 11

ANTIFUNGAL EFFECT OF CLOVE AGAINST CANDIDA ALBICANS

Drug	Dose	No. of experiments	Diameter of inhibitory zone (mm)
Clove oil	2 µl/well	5	17 ±1.26
	3 µl/well	5	48.6 ±0.97
	30 µl/well	5	50 ±1.09
Indian clove oil	30 µl/well	4	44.5 ±2.10
Olive oil (vehicle)	30 µl/well	5	0

FIGURE 29

HISTOGRAM SHOWS ANTIFUNGAL EFFECT OF DIFFERENT DOSES OF CLOVE OIL AGAINST CANDIDA ALBICANS



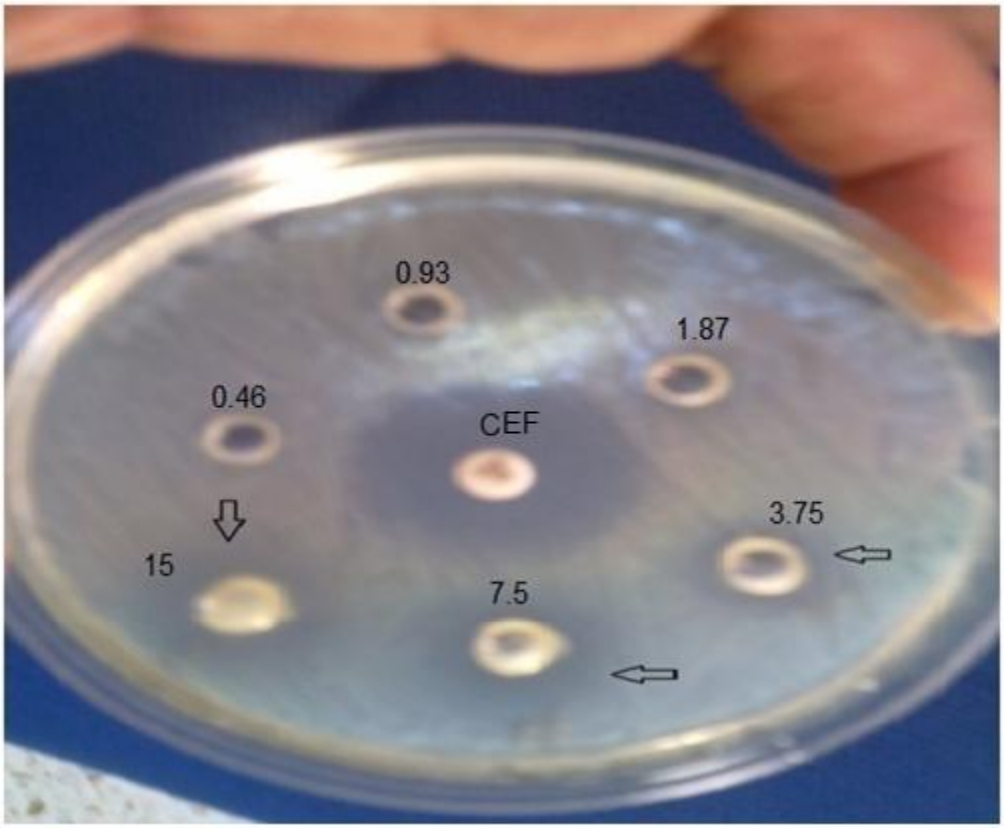
3-9-C DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF CLOVE OIL :

i. Minimum inhibitory concentration (MIC) of clove oil against E. coli:

(Table 12) and (Figure 30) showed that 30 µl of 100% clove oil produced an inhibitory zone of 24.8 mm in diameter. Serial dilution of clove oil with olive oil showed reduction in the diameter of inhibitory zone in that 15 µl, 7.5 µl , and 3.75 µl clove oil produced 18.0, 14.3, and 10 mm of inhibitory zone respectively. Further dilution of clove oil did not show inhibitory zone indicating that the MIC of clove oil against E. coli is 3.75 µl (Figure 30). Olive oil (30 µl of 100%) used as vehicle for diluting clove oil did not show any inhibitory effect against E. coli (Table 12).

FIGURE 30

MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST E. COLI



Arrows showed 3 different concentration of clove oil (15, 7.5, 3.75 µl) i.e MIC= 3.75. Further dilution of clove oil (the other 3 wells) had no effect. (CEF) ceftriaxone disc in the center.

TABLE 12

THE MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST E. COLI

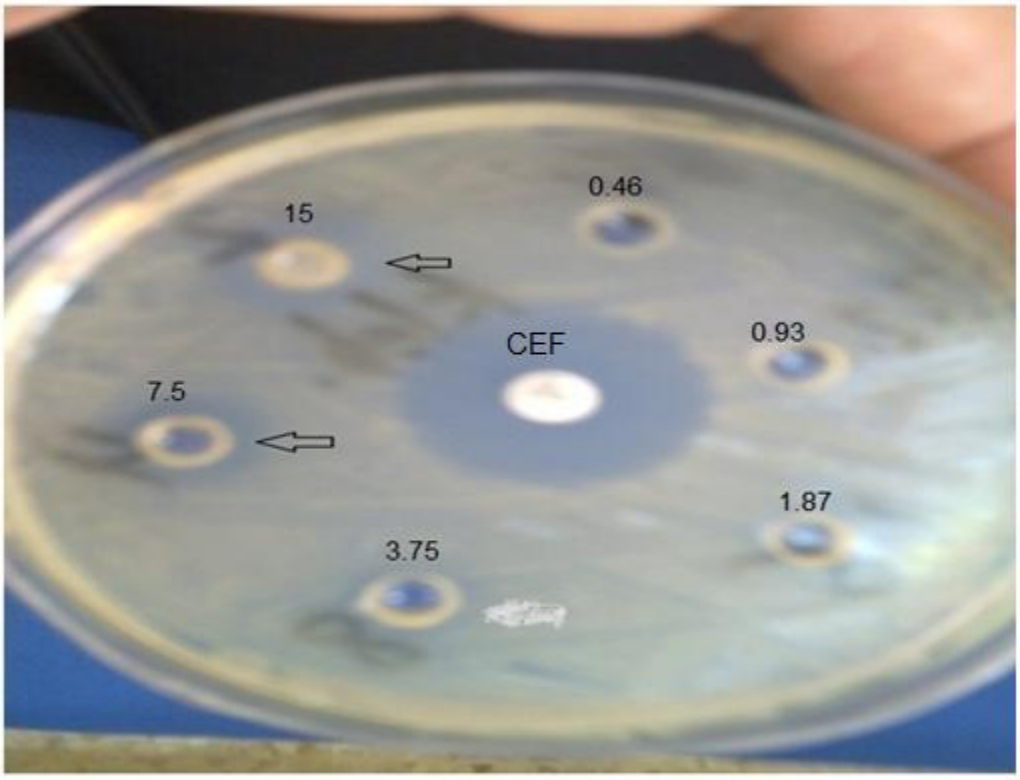
DRUG	DILUTION	CLOVE OIL CONCENTRATION / 30 µl	DIAMETER OF INHIBITION ZONE (mm)
CLOVE OIL	0%	30 µl	24.8 ±6.40
	50%	15.0 µl	18.0 ±1.15
	25%	7.50 µl	14.3 ±0.33
	12.5%	3.75 µl	10.6 ±1.33
	6.25%	1.87 µl	0
	3.12%	0.93 µl	0
	1.56%	0.46 µl	0

ii. Minimum inhibitory concentration (MIC) of clove oil against klebsiella:

Results are presented in (Table 13) and (Figure 31). Clove oil in doses of 30 µl, 15 µl, 7.5 µl produced inhibitory zone of 17.5, 15, and 13 mm in diameter. Lower doses did not show inhibitory zone, therefore 7.5 is the minimum inhibitory concentration of clove oil against klebsiella. Olive oil (30 µl), the vehicle used for diluting clove oil did not show any inhibitory zone.

FIGURE 31

MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST KLEBSIELLA



Arrows showed 2 doses of clove oil (15, 7.5 µl) which had an inhibitory effect. Other 4 dilution showed no effect. MIC = 7.5 µl. (CEF) ceftraixone disc in the center showing inhibitory zone.

TABLE 13

THE MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST KLEBSIELLA

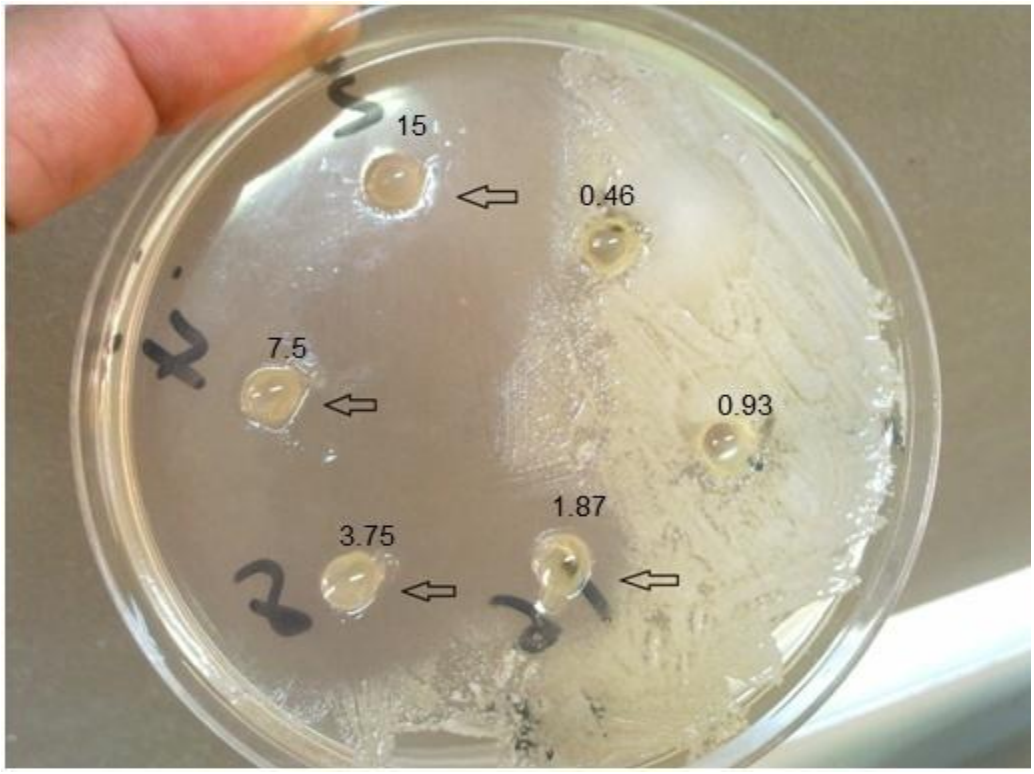
DRUG	DILUTION	CLOVE OIL CONCENTRATION / 30 µl	DIAMETER OF INHIBITION ZONE (mm)
CLOVE OIL	0%	30 µl	17.6
	50%	15.0 µl	15
	25%	7.50 µl	13
	12.5%	3.75 µl	0
	6.25%	1.87 µl	0
	3.12%	0.93 µl	0
	1.56%	0.46 µl	0

iii. Minimum inhibitory concentration of clove oil against candida albicans

Results are presented in (Table 14) and (Figure 32). Clove oil of 1.87 µl (16 times dilution) showed an average inhibitory zone of 15.66. Further dilution 32 times and more did not show any inhibitory zone. Therefore the minimum inhibitory concentration for clove oil against candida albicans is 1.87 µl.

FIGURE 32

MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST CANDIDA ALBICANS



Arrows showed 4 doses of clove oil(15, 7.5, 3.75, and 1.87 µl) showed inhibitory effect, while further two dilution had no effect.
MIC = 1.87 µl.

TABLE 14

THE MINIMUM INHIBITORY CONCENTRATION OF CLOVE OIL AGAINST CANDIDA ALBICANS

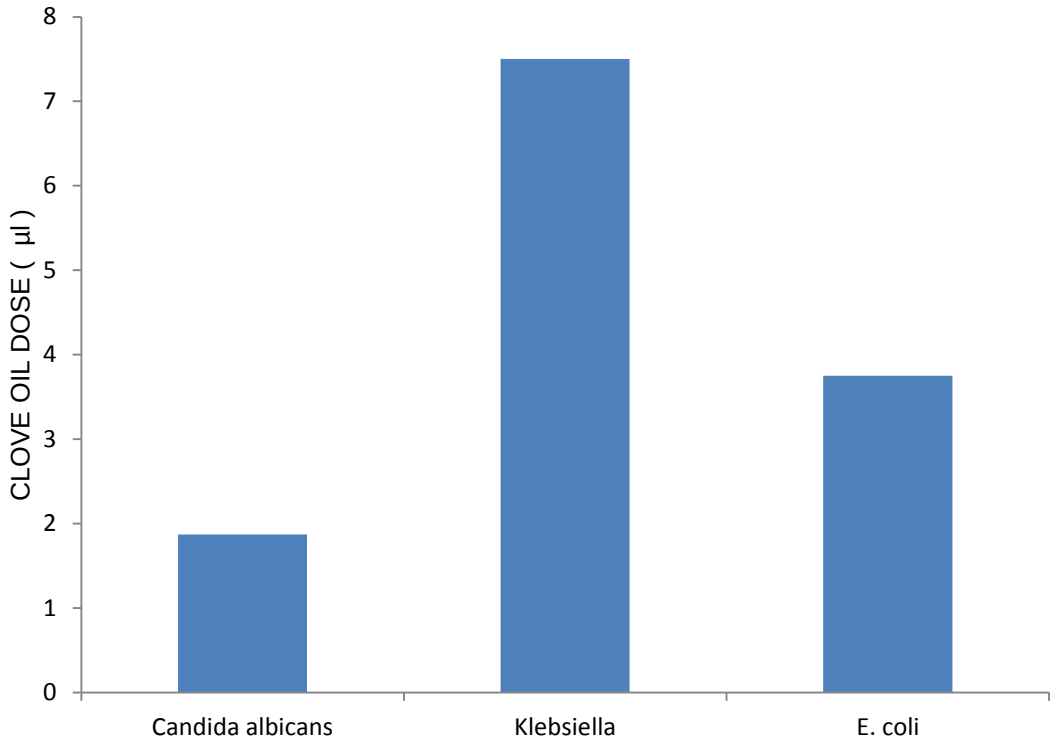
DRUG	DILUTION	CLOVE OIL CONCENTRATION / 30 µl	DIAMETER OF INHIBITION ZONE (mm)
CLOVE OIL	0%	30 µl	50 ±2.10
	50%	15.0 µl	50
	25%	7.50 µl	50
	12.5%	3.75 µl	50
	6.25%	1.87 µl	15.66 ±0.33
	3.12%	0.93 µl	0
	1.56%	0.46 µl	0

iv. COMPARISON OF (MIC) OF CLOVE OIL AGAINST E.COLI, KLEBSIELLA AND CANDIDA ALBICANS

(Figure 33) showed that candida albicans was the most sensitive to clove oil followed by E. coli and klebsiella was the least sensitive.

FIGURE 33

HISTOGRAM SHOWING MINIMUM INHIBITORY CONCENTRATIONS OF CLOVE OIL AGAINST E. COLI, KLEBSIELLA, AND CANDIDA ALBICANS



3-10 CLINICAL STUDIES OF CLOVE OIL AGAINST SUPERFICAIL SKIN FUNGAL INFECTION (DERMATOPHYTOSIS)

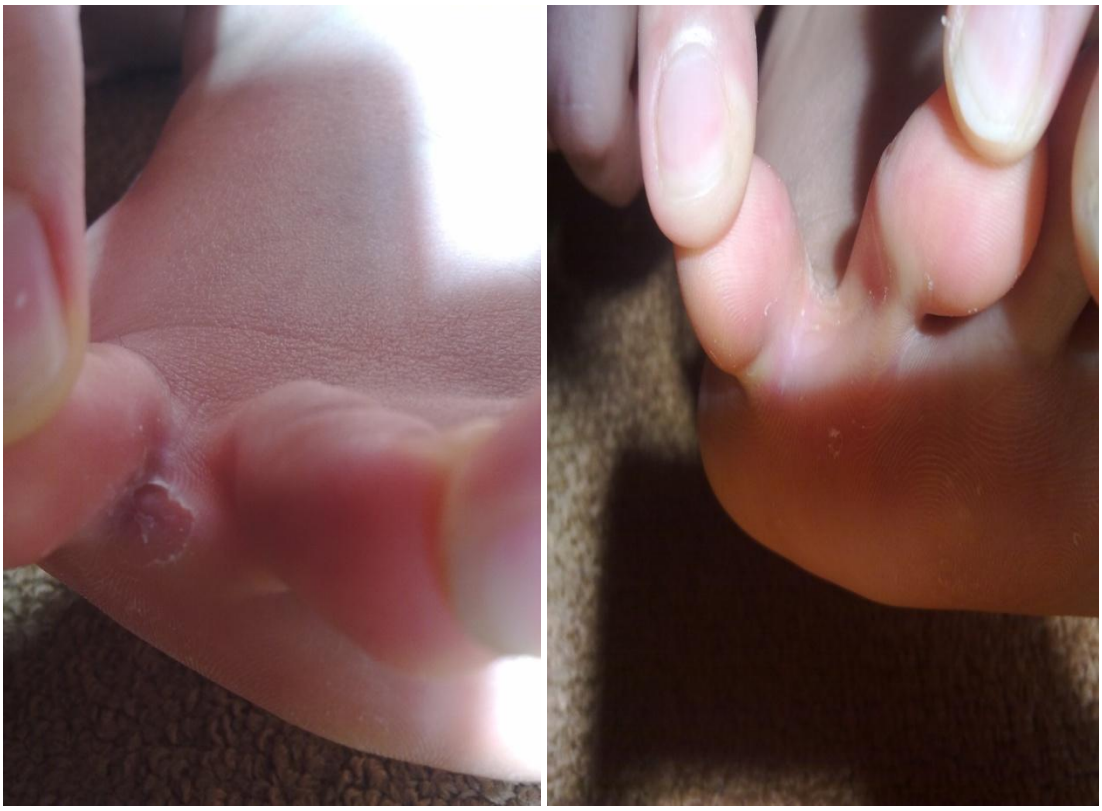
A- Case 1 : (Tinea pedis, or athlete's foot)

Twenty five years old Libyan housewife was having tinea pedis (athlete's foot) which was presented as erythema and maceration with silvery scales in the fourth digital interspace on her right foot. She was having this problem for about 10 days before seeking treatment. She claimed her athlete's foot comes frequently and was resolved with common over the counter antifungal preparations. Although, the clinical diagnosis was clear, a potassium hydroxide (KOH) examination was conducted. Few scales from the infected area were gently scraped with a sterile blade. Those scales of skin were mounted on microscopic slide with one drop of 10% KOH. That slide was covered with a slip and left for 20-30 minutes then it was examined under a microscope. Septate and branching hyphae were seen. She was treated with diluted clove oil 15% twice daily for 3 weeks. After 3 weeks period of treatment a complete resolution was noticed (Figure 34).

FIGURE 34

TINEA PEDIS: RIGHT FOOT SHOWS ERYTHEMA AND DESQUAMATION BEFORE APPLYING CLOVE OIL. COMPLETE HEALING AFTER APPLICATION OF CLOVE OIL
3 WEEKS LATER.

BEFORE **AFTER**



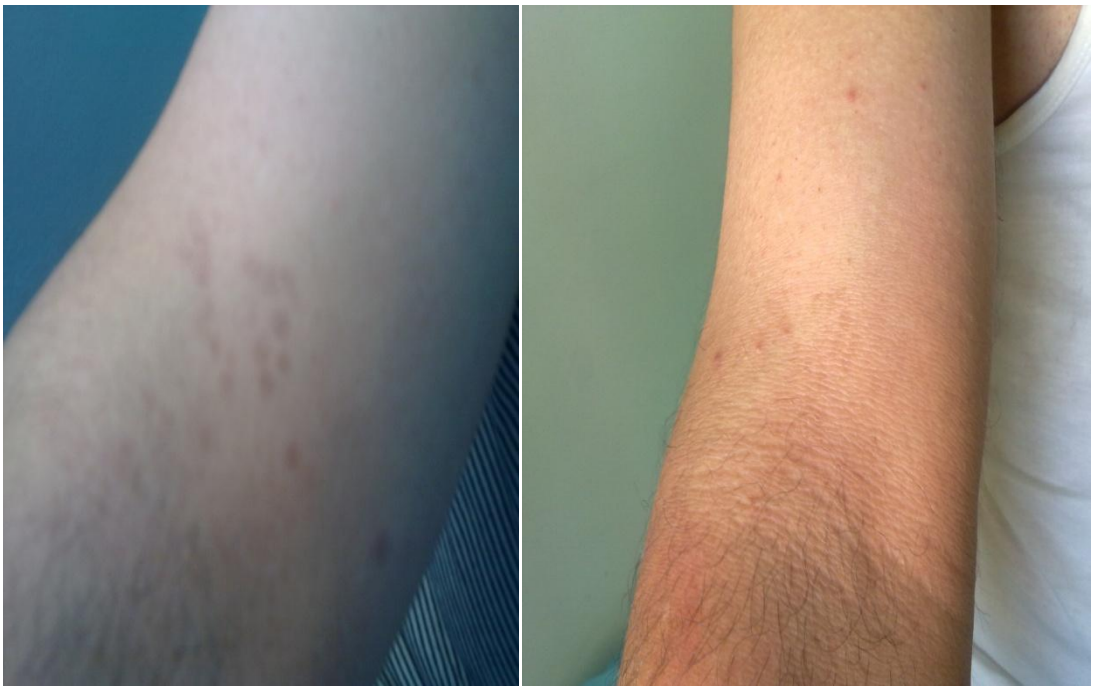
B- Case 2 : (Tinea versicolor)

Forty one years old male Libyan school teacher came with a few days history of brown and white discoloration of his skin. Since the problem is recurrent every summer, the patient was informed that he had a fungal infection (pityriasis or tinea versicolor). On inspection, it was clear that he had hyperpigmented macules with fine scales most noticed on both arms. He also had hypopigmented macules on both shoulders and upper back. Malassezia (M. furfur), a dimorphic fungus of normal skin flora is responsible for the disease when it transforms from yeast to hyphal (mycelial) form. This conversion may be triggered by hot and humid weather, and excessive sweating (hyperhidrosis). With a sterile blade few scales were scraped from the infected area mounted on microscopic slide with one drop of 10% potassium hydroxide (KOH), then covered with a slip left for 20-30 minutes, then examined under microscope. The diagnosis was made by seeing the hyphae with yeast cells (spaghetti and meatballs). The patient was treated with clove oil 15% twice daily for one month, but he applied only for 2 weeks.

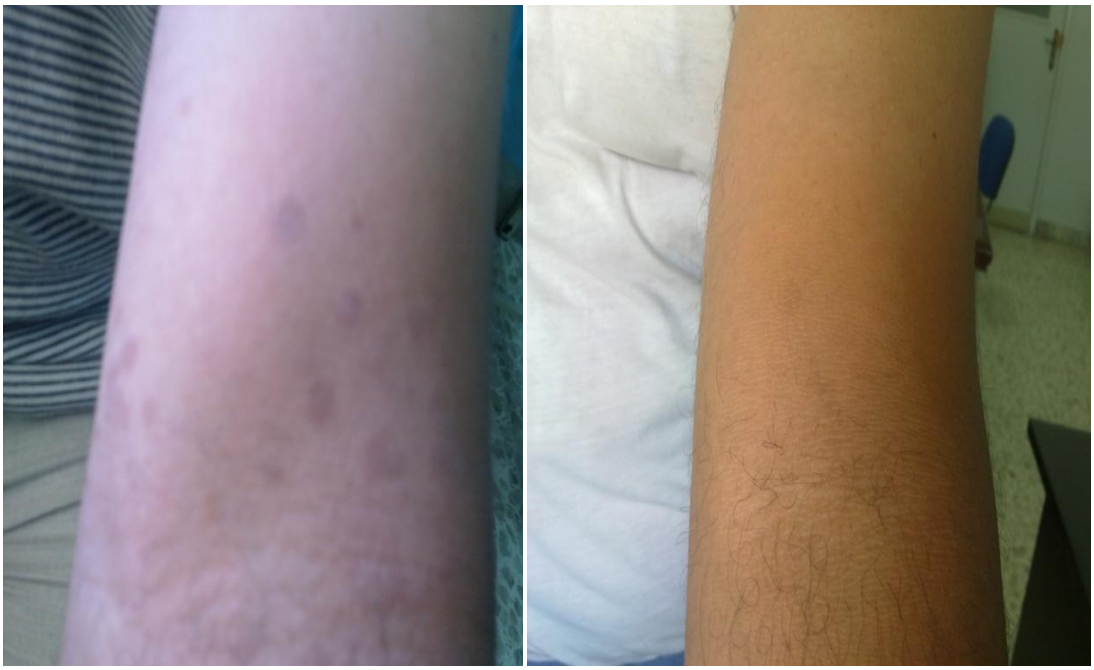
FIGURE 35

PITYRIASIS VERSICOLOR: BEFORE PATIENT WITH HYPERPIGMENTED MACULES ON BOTH ARMS. AFTER APPLICATION OF CLOVE OIL 2 WEEKS LATER.

BEFORE (right arm) **AFTER (right arm)**



BEFORE (left arm) **AFTER (left arm)**



C- Case 3 : (Tinea pedis)

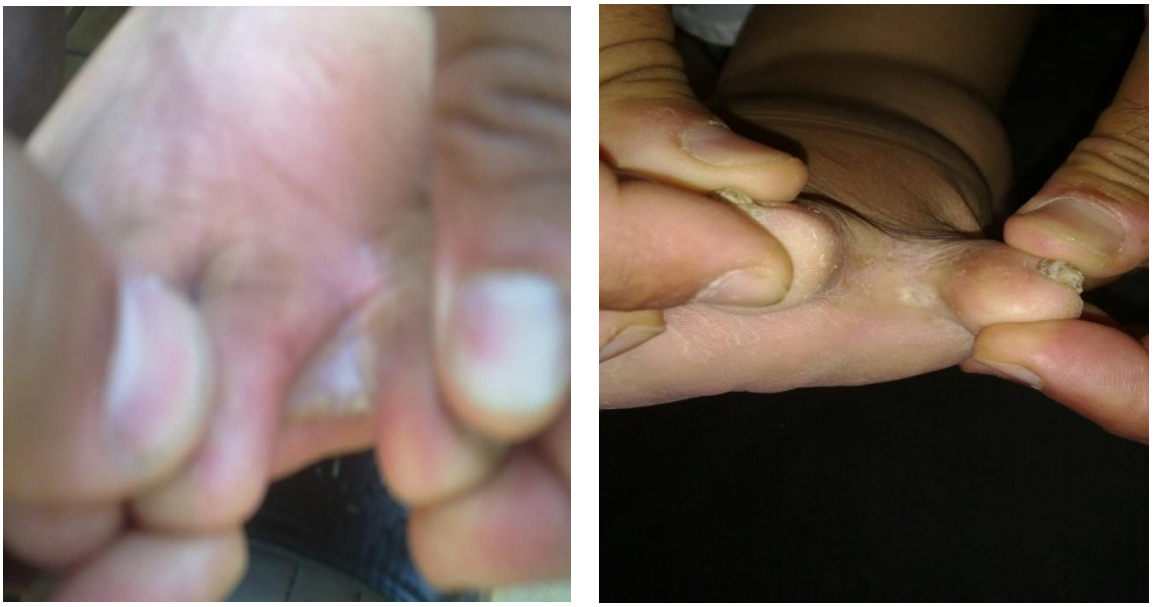
Sixty seven years old Libyan lady known case of hypertension, breast cancer (operated), and renal impairment came with history of recurrent tinea pedis of both feet. The third and fourth interdigital spaces of both feet were showing maceration, erythema, and silvery scales. Oedema of both feet were obvious. The diagnosis was made by scraping few scales from the infected area were mounted on microscopic slide with 10% KOH and covered with a slip and left over for 30 minutes then examined under microscope. It showed septate and branching hyphae. She was prescribed clove oil 15% twice daily, but she managed to put only for one week.

FIGURE 36

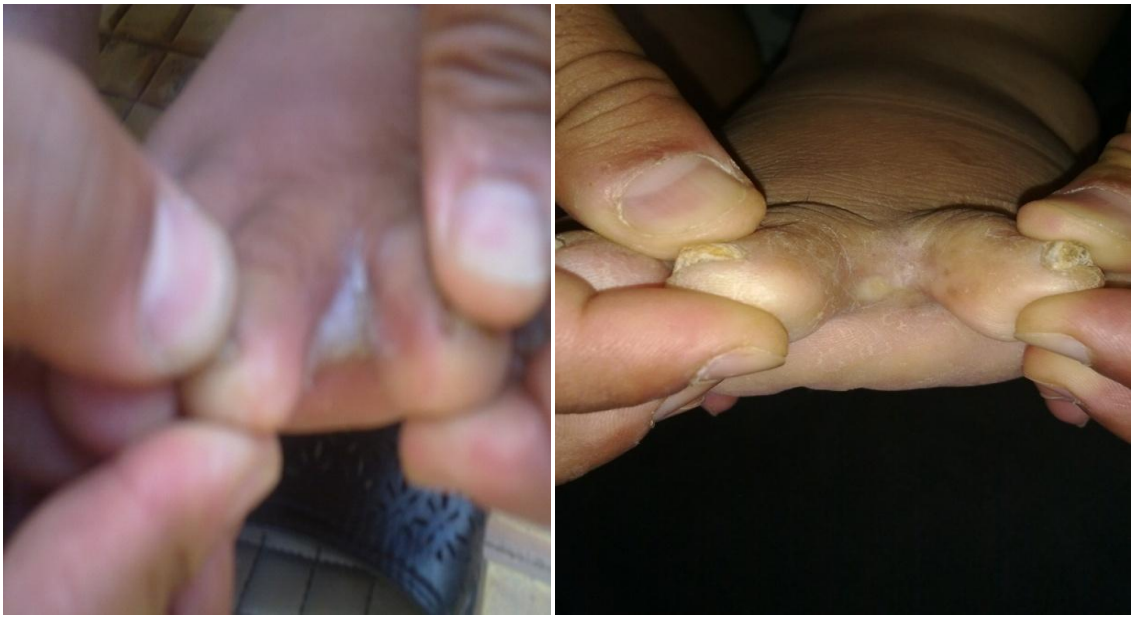
TINEA PEDIS :

A: 4TH INTERDIGITAL SPACE BEFORE: SHOWING WHITE SCALES THE AFTER: CLOVE OIL TREATMENT (RIGHT FOOT)
B: 3RD INTERDIGITAL SPACE BEFORE AND AFTER CLOVE TREATMENT (RIGHT FOOT).

A **BEFORE** **AFTER**



B **BEFORE** **AFTER**



Ch. 4 DISCUSSION

In recent years traditional medicine especially herbal medicine has become very popular for treating different diseases. it has been reported that between 50,000 and 80,000 flowering plants are used in traditional medicine worldwide (IUCN, 2007). In fact, medicinal plants are regarded as the main source of health care system in many societies especially in Asia and Africa. In Africa, up to 80% of the population uses traditional medicine to help meet their health care demands. In China, traditional medicine accounts for around 40% of all health care delivered. In India a huge percentage of the population (65%) are dependent on traditional medicine which is the only source of health care available as reported by the government of India (WHO, 2002). In recent years, traditional medicine has become more and more popular in many developed countries. The percentage of population used at least once traditional medicine is 48% in Australia, 70% in Canada, 42% in USA, 38% in Belgium, and 75% in France (WHO, 2002).

In developing countries broad use of traditional medicine is due to easy accessibility and affordability. For example, in Uganda the ratio of traditional medicine practitioners to population is between 1:200 to 1:400. This is in contrast with the availability of allopathic practitioners for which the ratio is 1:20,000. Moreover, the allopathic practitioners are in cities and urban areas, therefore, the population of rural areas are left with the only available source of health care i.e. traditional medicine (WHO, 2002).

There are other additional reasons for traditional medicine to become popular are their low cost and relative safety. Therefore, in the present scenario, research on

medicinal plants are becoming more and more popular and the present work is the outcome of this scenario.

The present study is an attempt to explore the possibility of finding some medicinal use of the clove flower buds obtained from *Syzygium aromaticum*.

The essential oil obtained from the clove flower buds contains eugenol as the main constituent which has been claimed to be analgesic and has been used in toothache by topical application (Yu and Hungju, 1981).

There are some sporadic reports about some other folk-medicinal uses of this plant but it appears from the literature that there is no systematic scientific research conducted on clove oil as the folk uses of herbal medicines are growing very fast. It was thought to do systematic scientific research on clove oil to establish its efficacy and safety.

The present work was on clove due to several reasons. Clove has easy accessibility. It is found in almost every grocery shop and in almost every kitchen to be used as flavoring agent in food preparation. Clove is reasonably cheap and commonly consumed item. It was reported that clove oil is approved by FDA (Food and Drug Administration) as flavoring agent because clove oil comes under the heading of Generally Recognized As Safe (GRAS) (FDA. 2011). Therefore, even for clinical uses, clove oil has less ethical problem.

In *in vitro* experiments conducted in this study, both clove aqueous extract and clove oil have blocked the spontaneous rhythmic contractions of isolated rabbit intestine. Clove aqueous extract also blocked Ach-induced contraction of isolated rabbit intestine. In *in vivo* experiments, clove inhibited the movement of charcoal meal in mice small intestine experiments. These findings indicated that clove aqueous extract as well as clove oil have spasmolytic action. The spasmolytic effect of clove aqueous extract might be due to presence of clove oil in aqueous extract as it is partially soluble in water (Toxnet, 2003). Indeed, the spasmolytic effect of clove aqueous extract is comparatively less than clove oil.

The clove oil caused inhibition when tested on spontaneously contracting rabbit intestine preparation, thus showing spasmolytic action. The contraction of smooth muscle preparation including rabbit jejunum is dependent upon an increase in cytoplasmic free calcium which activates the contractile element (Karaki and Weiss, 1983).

The inhibitory effect of clove oil may be due to interference either with the calcium releases from sarcoplasmic reticulum or most likely due to influx of calcium through calcium channels (Lima et al., 2011).

There have been several reports regarding antinociceptive effect of clove (Diaz and Sembrano, 1985; Oztürk and Ozbek, 2005; Kurian et al., 2006; Guénette et al., 2007; Daniel et al., 2009) which makes clove oil to be used in dentistry for alleviating toothache for several decades (Kozam, 1977; Ohkubo and Shibata, 1997). However, most of reports advocate the topical use of clove oil in

toothache. There are some reports which indicated that clove oil has analgesic action when administered orally (Daniel et al., 2009; Guénette et al., 2007). The present study shows that the clove aqueous extract as well as clove oil produced analgesic action in acetic acid-induced writhing model in mice. The clove oil was more effective as analgesic than clove aqueous extract. Clove aqueous extract at (0.1 and 0.2 ml) oral doses significantly decreased (44% and 65%, respectively) the number of acetic acid-induced writhes in mice. In comparison, clove oil administered orally in the same doses (0.1 and 0.2 ml) produced (83% and 87% decrease in the number of writhes induced by acetic acid in mice. Collier et al. (1968) postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate nociceptive neurons. Indeed, traditional NSAIDs can inhibit synthesis of prostaglandins (PG_s) by inhibiting cyclooxygenase (COX) in peripheral tissues and interfere with the mechanism of transduction of primary afferent nociceptors. This reduction of chemically-induced pain (acetic acid-induced writhing) in mice by clove aqueous extract and clove oil suggests that clove extracts predominantly inhibits peripheral pain mechanism by inhibiting PG_s synthesis (Daniel et al., 2009).

The present work also shows that the analgesic action of clove oil can not be reversed by naloxone (an opioid antagonist) which was in contrast to the analgesic action of morphine that was reversed by naloxone (Table 5). All these observations indicate that clove extracts produce their analgesic action most probably by peripheral mechanism by inhibiting PG_s synthesis and not through opioid receptors.

Yang et al. (2003) postulated that eugenol antinociceptive action is due to stimulation of vanilloid receptors by eugenol. Eugenol activated inward currents while capsazepine, a competitive vanilloid receptor antagonist, completely blocked eugenol induced inward currents. This experiment supports the *in vivo* studies carried out by Ohkubo and Shibata (1997) who demonstrated the inhibitory effect of capsazepine on eugenol induced antinociceptive activity in mice. These studies provide a strong evidence that eugenol produces its antinociceptive effects through different mediators and, at least in part, via vanilloid receptors.

There appears to be a revival in the use of traditional approaches to protect livestock and food from disease, pests and spoilage in industrial countries. This is especially true in regard to plant volatile oils and their antimicrobial evaluation, as can be seen from the comprehensive range of organisms against which volatile oils have been tested. These have included food spoiling organisms, food poisoning organisms, fungi, and animal plant viruses (Dorman and Deans, 2000).

Clove oil in the present study has antibacterial action against both Gram positive and Gram negative bacteria and remarkable antifungal action against candida albicans (Table 6,7,8,9,11) and (Figure 19,21,23,28). These results confirm the earlier reports of antimicrobial action of eugenol and clove oil (Taguchi et al., 2005; Devi et al., 2010; Pandey and Singh, 2011).

Clove oil antibacterial action was against staphylococcus aureus but the effect was weaker than fusidic acid. Clove oil was more effective antibacterial against E. coli, klebsiella, and proteus but was not effective against pseudomonas aeruginosa. The antibacterial effect against E. coli was comparable to ampicillin and ceftriaxone but more effective than kanamycin and nalidixic acid. The effect of clove oil against klebsiella was comparable to kanamycin and nalidixic acid but less effective than ceftriaxone. The inhibitory effect against proteus was seen in higher dose (30 µl) and found less effective than ceftriaxone and kanamycin. Clove oil was very effective against candida albicans. Market clove oil (the clove oil manufactured in India and supplied for topical application in toothache) was used in some experiments and was found to be equally effective as our clove oil in both antibacterial and antifungal tests. From all these results it appears that clove oil has a potential to be used as antibacterial and antifungal drug.

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. The increasing failure of chemotherapeutic agents and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of medicinal plants for their potential antimicrobial activity (Recio and Rios, 1989). Infectious diseases are the leading cause of death worldwide. Development of antibiotic resistance become global concern (Silver and Bostian. 1993; Westh et al., 2004). The efficacy of many existing antibiotic is threatened by the emergence of multidrug-resistant microbes (Bandow et al., 2003). Natural products, either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug leads.

There is constant and urgent need to find out new antimicrobial drugs with diverse chemical structure and novel mechanisms of action for the new and reemerging infectious diseases (Rojas et al., 2003). Therefore, researchers are nowadays turning their attention towards folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Plant-based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Therefore, further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

In view of increasing demand for antimicrobial of plant origin, the present work on clove oil is an effort to establish its efficacy as antimicrobial. The result showed that it is effective against both Gram positive and Gram negative bacteria. However, it was less effective against Gram positive bacteria (staphylococcus aureus) as compared to Gram negative (E. coli, klebsiella, and proteus). The difference in sensitivity has been supported by other works (Pandey and Singh, 2011; Oyedemi et al., 2009). It is not known exactly why Gram negative bacteria should be more susceptible to clove oil. However, it may be related to the outer membrane composition (Pelczar et al.,1988; Oyedemi et al., 2009). The mechanism of action of the antimicrobial activity of plant biopreservatives is not fully understood (Draughon, 2004), however, The antimicrobial activity of clove has been attributed to eugenol, which is the main active constituent of its essential oil (Pruthi, 1980). The mechanism of action of eugenol has been

studied by Oyedemi et al. (2009) who reported that it acts as antibacterial by damaging cell wall and cell membrane of both Gram positive and Gram negative bacteria or according to Wendakoon and Sakaguchi (1993) by inhibition of enzymes and genetic materials. From the results, it appears that clove oil has demonstrated more remarkable antifungal effect against candida albicans.

Because of presence of large population with immunocompromised state, fungal infection becomes a serious problem. Candida albicans is the most frequently isolated fungal pathogen of nosocomial infections (White et al., 1998), so there is a need for discovering a new natural antifungal drug to control candidiasis (Chami et al., 2005; Kauffman and Carver., 1997). The essential oil of clove has been described as having a potent antifungal activity (Tampieri et al., 2005).

Encouraged by *in vitro* very potent antifungal action of clove oil and the fact that clove oil is generally regarded as safe, it was thought to do clinical trial on human patients suffering from dermatophytic infection. Although, 14 patients were selected but, due to poor compliance, the study was completed only on three patients. Two patients with tinea pedis, and one patient with tinea versicolor. Clove oil was found to cure these fungal infections almost completely after 2-3 weeks of topical application. It is difficult to make any conclusion on such a small number of patients but it gives an idea that it can be an effective against dermatophytosis. However, more clinical tials are needed to establish its efficacy and safety.

Ch. 5 REFERENCES

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جامعة بنغازي

كلية الطب

قسم علم الأدوية

دراسة على استخدام القرنفل كمسكن للألم وكمضاد للإسهال و كمضاد للجراثيم

دراسة بحثية ضمن المتطلبات لنيل درجة الماجستير في العلوم الطبية الأساسية (علم الأدوية)

مقدمة من : عبدالحميد محمد السنوسي

تحت إشراف

أ.د محمد حسين أختر مشرف أول

أ.د عبدالقادر الدبياني مشرف ثان

ملخص الدراسة

تمت دراسة تأثير كل من المستخلص المائي و زيت القرنفل كمضادات للإسهال وكمضادات للجراثيم. المستخلص المائي و زيت القرنفل لهما تأثير مثبط لحركة نسيج أمعاء الأرنب المفصولة و كان تأثير الزيت أكثر وضوحا. كما أن لهما تأثير مثبط لحركة للأمعاء عندما استخدم الاسيتايلكولين الذي يحدث انقباض للأمعاء وايضا كان تأثير الزيت أكبر. ولكن ليس لهما تأثير على انقباض الأمعاء عندما استخدم كلوريد الباريوم. كما أن زيت القرنفل انقص حركة مرور الفحم في امعاء الفئران وكان 0.3 مل من الزيت يعادل 10 ملغرام لكل كيلوجرام من الاتروبين. عندما أعطي زيت القرنفل بالفم للفئران التي أصابها الاسهال بزيت الخروع لوحظ ان الاسهال قد قل.

المستخلص المائي وزيت القرنفل لديهما تأثير مسكن للألم المحدث في الفئران بحقنها بمادة حامض الأستيك و أيضا تأثير الزيت كان أكبر

المستخلص المائي لم يحدث تأثير على البكتيريا ولكن زيت القرنفل أظهر تأثير مثبط لنمو البكتيريا و فطر الكانديدا

أظهر زيت القرنفل تأثير مضاد للفطريات الجلدية في عدد قليل من المرضى الذين استخدموا الزيت.