Sudan University of Science and Technology

College of Graduate Studies

Characterization and Biological Activity Study of Ziziphus spina-Christi seed Oil

دراسة الخصائص و النشاط البيولوجي لزيت بذرة النبق

A thesis submitted in partial fulfillment of the Requirments of the Degree of M.Sc in chemistry

By

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الآية

قال تعالى :

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلاَّ قَلِيلاً

صدق الله العظيم

سورة الإسراء(85)

DEDICATION

To my parents,

my sisters, brothers

and my friends

Acknowledgments

At First, my respectfull thanks should be to Allah, almight who gave me the strength, Patience to complete this work.

I would like to express my deep gratitude to Professor Mohamed El Mukhtar Abdelaziz

my thanks also extend to Dr. Amal Ziyada of the industrial research & consulting centre and all the staff in the department of chemistry, Sudan university of science and technology.

thanks to all those who helped me.

Abstract

The aim of this study is to identify the physicochemical properties of Ziziphus Spina Christi seed oil and to evaluate its anti-microbial activity

The seeds were crushed and the nutritional values ,which in clude oil content , moisture content , total ash , fiber content , protein content and carbohydrate were identified. The results obtained were 27.8 %, 4% , 3.5% , 21.3% , 31.9%, 11.5% , respectively,

The oil was extracted by petroleum ether and the physical characteristics of the oil, refractive index , density and viscosity were studied, and the results were 1.467 , 0.928 g\ cm^3 , 37.31 Poise, respectively .

The chemical characteristics of the oil, saponification value, acid value and peroxide value were studied, and the results were 159.6 mg \g , 4.7 mg \g , 0.8 meq\kg , respectively .

The oil was analysed by gas chromatograph, and 29 chemical compounds were idendtified of which linoleic , oleic , palmitic and stearic acid have the highest percentages of 35.86% , 19.83% , 15.41% , 8.75% respectively

In addition, antimicrobial activity was evaluated for two types of fungi and the results were Aspergillus niger(11.5) and Candida albicans (18).It was also evaluated for four types of bacteria E coli(13.5), Pseudomonas aeruginosa(12), Staphylococcus aureus(11.5) and Bacillus subtilis (0). The results of ziziphus spina Christi seed oil antimicrobial activity have shown that it works as good antimicrobe against all the microorganism mentioned except the bacteria Bacillus subtilis .

المستخلص

الهدف من هذه الدراسة التعرف علي الخصائص الفيزيوكيميائية لزيت بذرة النبق وتقييم النشاط المضاد للميكروبات.

تم كسر البذور والتعرف علي القيمة الغذائية التي تتضمن محتوي الزيت , محتوي الرطوبة , الرماد الكلي , محتوي الألياف , محتوي البروتين والكاربوهيدريد . النتائج المتحصله عليها كانت كالأتي 27.8 %, 4 %, 3.5% ,21.3 %, 31.9 %, 11.5 %علي التوالي .

تم استخلاص الزيت عن طريق ايثر البترول,ودرست الخواص الفيزيائية للزيت, معامل الانكسار, الكثافة, اللزوجة, وكانت النتيجة 1.467 $0.928g\backslash cm^3$, 37.31 Poise علي التوالي. دُرست الخواص الكيميائية للزيت قيمة التصبن, القيمة الحمضية و قيمة البيروكسد وكانت النتيجةg\159.6mg, g\4.7mg, kg\0.8meq علي التوالي.

تم تحليل الزيت بواسطة كروماتوغرافيا الغاز,وتم التعرف علي29 مركب كيميائي واعلاها نسبه حمض اللينوليك, الاوليك, البالمتيك والاستياريك 35.86% , 19.83%, 15.41%, 8.75% علي التوالي.

إضافة لذلك تم تقييم النشاط المضاد للميكروبات علي نوعين من الفطريات وكانت النتيجة Aspergillus niger(11.5mm) Candida albicans, (18mm). وقيمت كذلك علي أربعة أنواع من البكتريا Ecoli (13.5mm), Pseudomonasaeruginosa(12mm) Staphylococcusaureus (11.5mm) و Bacillus subtilis(0mm), لقد أظهرت نتائج نشاطية مضادات الميكروبات لزيت بذرة النبق انه يعمل كمضاد جيد لكل الميكروبات السابق ذكرها ماعدا باكتيريا Bacillus subtilis.

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1.1 Introduction

Wild food plants are those plants with edibles parts that are found growing naturally without having been purposely cultivated (katende et al ., 1999). Several types of food can be obtained from wild plants (Elaine and cherukat , 2009) . A number of important wild edible plants have been identified among them is ziziphusspinachristi which has served as source of food and medicine for thousands of years (Baytop , 1984 ; Gultekin , 2007) . Collection of wild foods is a tradition that rural communities in the developing countries practice to secure their food supply especially during times of food shortage (Loghrust, 1986; Lockett and Grivetti, 2000; Msuya*et al.*, 2010). The physical and economic accessibility in addition to high diversity of wild foods are crucial factors for survival of communities suffering, periodically or seasonally, from food scarcity and poverty (Falconer and Arnold, 1989). Also the long accumulating knowledge and experience that local population develops over generations, concerning uses, gathering, processing and storage of wild foods, make these foods of considerable importance (Somnasang and Moreno- Black, 2000) . Furthermore, wild foods play a major role in diet of rural children and have a nutritional value in some cases superior to the quality of domesticated foods (Ogle and Grivetti, 1985; Legwaila*et al.*, 2011) . In addition, sale of wild foods of high marketability makes an indirect contribution to enhancing household's food security by generating alternative income opportunities. The socioeconomic potential of wild food in developing world was discussed (Agea, 2010; Lulekal*et al.*, 2011).

In rural areas of the Sudan, where a wide genetic diversity of plants exist, edible wild fruits play a vital role in securing food and enhancing

household's economy (Gebauer*et al.*, 2002; El Tahir and Gebauer, 2004; Goenster*et al.*, 2011) . Most of these fruits are consumed in different forms and have a wide use in folk medicine in everyday life of local population (El Gazali*et al.*, 1987; Abdelmuti, 1991). Despite their significant role, wild edible fruits are under-estimated and only a little attention has been paid to explore their various potentials.

Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems (Dahiru et al., 2005).

Medicinal plants are plants that have recognized medicinal uses. They range from plant which is used in the production of mainstream pharmaceutical product to plant used in herbal medicine preparation (Lothtipour et al., 2008).

Medicinal plant is defined as any plant which in one or more of its parts contains substance that can be used for therapeutic purpose or as precursors for the synthesis of useful drugs (Sofowora, 2008).

Many of the edible wild plants have both therapeutic and dietary function .such medicinal foods have been part of Eastern medicinal theories since ancient time and have recently received attention within the fields of functional foods, neutraceuticals and phytonutrients . So many of the present day drugs are known to have been isolated from such natural sources and their isolations were based on the information about the uses of the agents in traditional medicine (Abalaka et al., 2010) .

Herbal medicine is one of the oldest forms of medicinal treatment in human history, and could be considered one of the forerunners of the modern pharmaceutical trade. Medicinal plants can be found growing in numerous setting all over the world. Some medicinal plants are wild crafted, meaning that they are harvested wild by people who are skilled

at plant identification. Sometimes plant cannot be cultivated, making wild crafting the only way to obtain them and some people believe that wild plant have more medicinal properties (Serrentino, 1991).

Other medicinal plants may be cultivated; one of the advantages of cultivation is that it allows for greater control over growing conditions. Cultivation also allows for mass production, which makes production of the medicinal plant(s) commercially feasible as they can be processed in large numbers and priced low enough that people will be able to afford them (WHO,1991).

Chemists are always interested in studying medicinal plants which have not been researched before, to identify which ingredient(s) in the plants that are active, and to see their mode of action. Usually, the goal is to develop a synthetic version of the active ingredient which can be easily produced in a laboratory and packaged in pharmaceutical preparations (De Silva, 2005). The use of medicinal plants as a source for relief from illness can be traced to five millennia in written document of the early civilization in China, India and the near east (Doughari, 2006; Lothtipour et al., 2008) .

The basic aimto carryout analytical study ofZiziphusspina-christi seed oil which is the few natural oils ofinterst and is one of the natural oil neglected that maybe a promsingsourcefortheproduction of vegetable oil in Sudan and may be used in the medical field.

1.2 Literature Review

1.2.1 The genus Ziziphus

The genus Ziziphus belong to the family Rhamnaceae. It is a genus ofabout 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world (Dweck , 2005)

The genus Ziziphus is known for its medicinal properties as hypoglycemic, hypotensive anti-inflammatory, antimicrobial, antioxidant, antitumour, and liver protective agent and as an immune system stimulant (Said *et al.*, 2006).

1.2.2 Botanical classification

Kingdom	Plantae
Division	Mangoliophyta
Class	Mangoliphta
Order	Rosales
Family	Rhamnaceae
Genus	Ziziphus
Species	Z .Spina- Christi
Botanical name	ZizyphusSpina- Christi (L) Desf
Synonms	RhamnusSpina-Christi L .

Common Synonym (S)

English	Christi thorn
Arabic	Sider (tree) ,Nabak fruit

1.2.3 ZiziphusSpina-Christi (L.) wild

ZiziphusSpina Christi (L.) , locally known as sider , is a multipurpose tree species belonging to the botanical family Rhamnaceae (Abeer and Reem , 2009) **.**

For a long time, in folklore medicine, sidr has been used for the treatment of some diseases, such as digestive disorders, weakness, liver complaints, obesity,urinary troubles, diabetes, skin infections, loss of appetite, fever, phyaryngitis, bronchitis, anemia, diarrhea, and insomnia (Han and park , 1986 ; Kirtikar and Basu , 1984) **.** Previous studies suggest that Zizyphusspina-christican be very usefulin the control of hepatic and nephroticabnormalities . Undertook a study to provide scientific validity for the folkloric use of Zizyphusspina-christias an antimicrobialagent (Adzu et al .(2003) andMaydell 1990) .

The plant used for medicinal purposes and as natural remedy in many ancient cultures such as those of Egypt, Greece and Rome (Al –Haider and Aqeel, 1993). Also it has been used for treating fever, malaria, common cold headaches and asthma . The plant is already used in many parts of the world for the care of skin, the chemical composition and phytochemicals present in the plant would suggest the ethno botanical pattern of this plant, their antimicrobial and antifungal properties are important in cosmetic application (Hosseinzedn et al., 2007). It has shown to have antimicrobial activity against bacteria , fungi and other phathogens that are normally guite resistant (Eldridge ,1997) .

This plant has been extensively studied and its chemical composition determined (Younes et al ., 1996) . The main constituent were betulic and ceanothic acid (Abdelaaty et al., 2001) , and three cyclopeptide alkaloids as well as four saponin glycosides (Mehran et al., 1996) . Several flavonoides have been isolated from leaves of ziziphusspina Christi (Amos et al., 2001) . The oilof ziziphusspina Christi has been used for washing the hair and the body (Amine , 1991) .

Application of the powdered leaves is said to darken and lengthen women's hair (Irvine , 1961) , and they are also used in the folk medicine as an antiseptic ,antifungal and anti-inflammatory agent , and for healing skin diseases such as atopic dermatitis (Nafisy , 1989) .

Fruits of ziziphusspina Christi taste like a mixture of apples and dates which can be eaten as fresh as well as in dried form (NAS , 1980) .

The dried plup- flour and water are also mixed with sesame and formed into small balls for immediate use later . In Sudan the fruit of ziziphusspina Christi is known by the colloquial name nabag(Abdelazim et al., 1987) . The fruits are applied on cut and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery (Abalaka et al., 2010) .

The flowers are important source for honey bee. The winter honey (i.enabag honey) collectedfrom the flowers of the sidr is in high demand bycitizens for its medicinal qualities in addition to its excellent taste and fragrant smell (Adzu and Haruna ,2007) .

*Z. spina-christi*seed has been used for nutritional and medicinal purpose in many middle-east countries and other parts of the world (Al- Ghamdi, 2001; El Dakhakhny et al., 2000) and it has beencosidred as potentially a good source of antimicrobial compound. The use of these compounds for application in crop production was not as many as in medicinal field. (Hussaini et al., 2010).

The seeds are sedative and are taken sometimes with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Maydell , 1990) .

According to information collected from Attareen and places of beauty in the markets that ZSC seed oil used by female skin care and hair , mostly imported from outside Sudan .

1.2.4 Botanical describtion

Ziziphusspina-christi is a shrub, sometimes a tall tree, reaching a height of 20 m and a diameter of 60 cm; bark light-grey, very cracked, scaly; trunk twisted; very branched, crown thick; shoots whitish, flexible, drooping; thorns in pairs, one straight, the other curved.

Leavesglabrousonuppersurface,finely pubescent below, ovatelanceolate or ellipsoid, apex acute or obtuse, margins almost entire, lateral veins conspicuous .

Flowers in cymes, subsessile, peduncle 1-3 mm.

Fruit about 1 cm in diameter (Zargari , 1988) .

1.2.5 Antioxidant activity

Several studies have indicated that medicinal plants contain a wide variety of natural antioxidants such as phenolic acid flavonoids and tannins which possess more potent antioxidant activity (Thirumalai et al., 2011) . Flavonoids which are most potent nutritional antioxidant (Anderson , 1980) . Antioxidants are chemical substances that reduce or prevent oxidation. They have the ability to counteract the damaging effects of free radicals in tissuesand thus are believed to protect against cancer,arteriosclerosis, heart disease and several other diseases (Bandyopodhyay et al., 2007) . Epidemiological evidence suggests that antioxidant contained in fruit and vegetables can help to prevent or affect the development of disease . In addition antioxidants have several industrial uses , such as preservatives in foods to prolong their shelf life . The deterioration of some foods has been linked to the oxidation of lipids which leads to the formation of undesirable secondary lipid peroxidation products causing thus a decrease in the nutritional value of lipid foods , their safety and sensory attribuates .

Therefore , synthetic commercial antioxidants have been widely used in food industry to retard the oxidation process (Huang and Wang , 2004 ; Wang et al., 2011) . A number of related compounds called tocopherols showed vitamine E activity , the main one being alpha-tocopherols . Vitamine E is found in many foods , but wheat germ , vegetables oils , nuts , margarine and egg yolk are particularly good sources . Vitamin E is a natural antioxidant in vegetables oils it has to reduce rancidity by preventing the oxidation of unsaturated fatty acids . It may also play a part in producting ascorbic acid against oxidation in fruits and vegetables .

In the body , vitamin E has an important role as antioxidant . Substrate known as free radicals, which are produced as a result of normal chemical reaction in the body , can damage the lipids (fatty compounds) found in cell membrane . The free radicals oxidize the lipids , forming peroxides . The lipid peroxidation can cause damage to the cell membrane and leaking of the cell contents which in turn is through to increase the risk of inflammatory disease . Vitamin E protects lipids , especially polyunsaturated fatty acid , against free radical damage molecules inside the cell such as DNA and protein ; cell with damaged DNA are prone to cancer . Vitamin E is therefore through to give some protection against some forms of cancer . Other vitamin , especially beta- carotene and vitamin c , also have antioxidant properties .

The amount of vitamin E required in the diet depends on the amount of polyunsaturated fatty acid in the body’s tissues.

1.2.6 Socioeconomic impact of ziziphusspina- Christi

Socio- economic attributes , such as size of landholding , livestock numbers , education level and farmers age , were found to influence positively the income from farming activities which it could be represented by the genes ziziphusspina Christi on the things such as products and usages :-

1.2.6.1 Product

Food : The fruit is edible and occasionally sweet , but the flavour and texture are inferior to other ziziphusspp, which has been domesticated in Africa and especially northern India . The fruits contain 14.16 % vitamin C .

Fodder : The leaves provide valuable animal forage and fodder under open grazing conditions , but the nutritional value is apparently not high for most domestic livestock .

The fruits are eaten by sheep and goats and foliage by camels .

Fuel : Its wood yields an excellent charcoal , but given the current status of the species , and its slow growth rate , this usage is certainly to be discouraged .

Timber : The termite resistant red or dark brown wood is hard and heavy , used for spear shafts , posts , roofing beams , utensils and cabinet making .

Alcohol : An alcoholic drink is made from the fruits .

Medicine : The leaves contain various alkaloids , including ziziphine , jubanine and amphibine , alpha terpinol , linalool and diverse saponins.

In the sahel region , the roots are used to treat headaches , while the spines or ashes of this species are applied to snake bites . Boilded leaves are applied to various surface wounds , and also

have antihelminthic and antidiarrhetic properties . In Egypt and the southern sahara , a narcotic beverage is made from the fruits and which is considered to be a tranquilliser and sedative . In Mocrocco , the fruits are used as an emollient and a stringent agent. It also is reputed to reduce abscesses and boils while a cataplasm of young leaves is also used to reduce eye inflammation.

Poison : It has been reported that applying Christi thorn bark in larger doses reduce nematode activity in cereal fields and leads to significant increase in the yield of sunflower (Ismail , 1998) .

1.2.6.2 Services

Erosion control : Because it develops a very deep taproot and spreadinglateral roots , it is used for stabilizing sand dunes and other unstable soils.

Shade or shelter : Christ thorn is planted around towns and villages for shade . It makes useful windbreaks and shelterbelts .

Soil improver : The tree improves soil quality by increasing available phosphorus .

Boundary or barrier or support : The thorn branches are used for fencing It con also be grown to form a stok-proof living fence .

Intercropping : z. Spina Christi is intercropped with millet in west Africa (Orwa et al., 2009) .

1.2.7 Extraction of fixed oil :-

Over the centuries , four basic methods of extracting vegetable oil from the various seeds were developed . Those four methods are : Tthe first was boiling in water leading to a partial separation of oil , which was skimmed off the top of the vessel . The second

was the cage-type press in which pressure was put on stationary mass by levers , screw jacks or hydrolic cylinders and the vegetable oil flowed from the compressed mass to collecting rings bellow . Both these methods are more or less obsolete .

The third method is the mechanical screw press , and fourth is solvent extraction (Bredeson , 1978) .

Objectives :-

Determination of physicochemical properties of ZSC seed oil .

Determination of fatty acid composition of ZSC seed oil by GC – MS .

Evaluation of antimicrobial activity of ZSC seed oil .

Chapter Two

Materials and Methods

2.1 Chemicals

All the chemicals are of A.R grade and were supplied by Riedel-dehaen , Germany .

Petroleum ether

Potassium hydroxide

Hydrochloric acid

Phenolphthalein

Ethanol

Glacial acetic acid

Chloroform

Sodium thiosulphate

Starch indicator

Sodium hydroxide

Methanol

Sulfuric acid conc

Sodium chloride

Normal hexane

Diethyl ether

Sodium sulphate

Potassium iodide

Acetone

Boric acid

Methyl red indicator

2.2 Apparatus:

Hammer

Density bottle
Conical flask
Measuring cylinder
Desiccators
Porcelain dish
Beakers
Burette
Round buttom flask
Rotary evaporator
Test tubes
Syringe filter
Soxhlet

Capillary tube viscometer

Balance

Kijeldahl tube

Refractometer – belling ham &stanley – made in England

GC-MS-Shimadzu- GC.MS-QP 2010 ultra – Japan .

2.3Methods

2.3.1 sample collection and preparation

The fruits of ziziphusspinaChristi were obtained from local market from in Sinnar State .

Fruits pulp were removed; Then the internal seeds were obtained by breaking down of the external seedsmanually using hammer and milled to paste

2.3.2 ziziphusspinachristi chemical analysis

2.3.2.1 Oil extraction and determination of oil content

The oil content of sample was determined according to AOAC (1997) using soxhlet apparatus as follows:

Five grams of sample were taken and placed in a thimble.

The thimble was covered using cotton wool. An empty, dry and clean round flask with a known weight was connected to siphoning apparatus and 200 cm^3 of petroleum ether (with a boiling point of 40 to 60^oC) were added. Extraction was carried out for 8hrs during which the solvent was distilled off. The round flask containing the extracted oil was weighed. The extracted oil was calculated and expressed as percentage according to the equation:

$$oil\ content\ 0/0 = \frac{w_2 - w_1}{s} \times 100$$

$w_1 \equiv weight\ of\ empty\ round\ flask$

$w_2 \equiv weight\ of\ the\ round\ flask\ containing\ the\ extracted\ oil$

$s \equiv original\ weight\ of\ the\ sample$

2.3.2.2 Moisture content

The dry seed (5g) was weighed into a clean dry aluminum dish of a known weight. The sample was dried in vacuum oven at a temperature of 105°Cfor2 hours, cooled in a desiccator and weighed, to constant weight. The moisture content was calculated following the method of AOAC, (1997).

$$Moisture\ content\ \% = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_s}}\%$$

Where:

W1= weight of wet sample .

W2= weight of dry sample .

Ws= weight of sample.

2.3.2.3 Total ash

The dry seed sample (2g) was placed in a dry clean porcelain dish and heated progressively for 6 hours at 550°C until, grey -reddish ash was obtained according to (AOAC**,** 1997). The sample was cooled in a desecrator, weighed and total ash calculated using the following formula:

$$Total\ Ash\% = \frac{(weight\ of\ dish\ +\ ash) - (weight\ of\ empty\ dish)}{weight\ of\ sample}\%$$

2.3.2.4 Crude fiber

About 5g of seed paste was transferred into a 200 cm^3Labeled beaker after which 1.25 % sulphuric acid 50cm^3 and150 cm^3 distilled water were added. The sample mixture was then boiled for 30 minutes under reflux flask and later treated with 1.33% potassium hydroxide 50 cm^3 and 150cm^3 water the solution was re-boiled again for 30 minutes and filtered using vacuum crucible filter on system. The sample in the crucible was rinsed with water followed by acetone. The samples was put into a pre-weighed crucible and transferred to the oven at 105 °C to dry for 4 hours, cooled in a desiccator and weighed .The weighed sample was used in the furnace set at 660°C for 5 hours until it became grey ash which was

cooled in the dedicator and weighed (AOAC**,** 1997). The weight of ash was then calculated as follows:

$$\boldsymbol{Fiber\ content}\ \% = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_s}}\%$$

Where:

W1= weight of crucible with sample before ashing.

W2= weight of crucible with sample after ashing.

Ws= weight of sample.

2.3.2.5 Crude protein

Protein can be determined through the following stages:-

a - Digestion stage:

The dry seed powder (0.2g) was placed in kijeldahl tube and a 1g mixture (catalyst; sodium sulphate and copper sulphate) was added the mixture was digested with concentrated sulphuric acid (25cm^3) for 2 hours in fume hood until the solution became clear.

b - Distillation stage:

Distilled water (1cm^3) was added to the solution and allowed to cool. Sodium hydroxide (45%) was also added without agitation. The flask was then connected to the distillation bulb with the tip of the condenser immersed in a standard acid solution (boric acid 2%)

Containing 5 drops of the methyl redindicator. The flask was then heated to release ammonia into the indicator solution.

c - Titration stage

The excess acid in the distillate was titrated with 0.1M standard NaOH The conversion factor of 6.25 was used (AOAC**,** 1997) and percentage of Nitrogen calculated as below:

$$Nitrogen\ \% = \frac{(\mathrm{cm}^3\ of\ acid \times M\ of\ acid)\ \times 14 \times 100}{weight\ of\ sample \times 1000}$$

$$Protein\ \% = N\% \times protein\ factor\ (6.25)$$

2.3.2.6Total carbohydrates

Total carbohydrates were determined by difference using the method in **(**AOAC**,** 1997).

$$\mathbf{(100 - (}\boldsymbol{Moisture\ content\ +\ Oil\ content\ +\ Protein\ content\ +\ Fiber\ content\ +\ total\ Ash}\mathbf{)}.$$

2.3.3 Physical characteristic of ziziphus – spina Christi seed oil:

2.3.3.1 Refractive index

The refractive index of oil sample was determined using refractometer.

The sample chamber containing the lens was opened and cleaned with acetone then plugged to source of light.

The equipment was calibrated with a drop of water, after which a drop of oil sample was added into the sample chamber and closed. The adjustment was turned crossed the cross bar then reading was taken. The reading was taken at 40°C.

The method used for determination of refractive index was adapted from A.O.A.C (2000).

2.3.3.2 Density

The density bottle was cleaned with acetone, ether and dried in oven. The weight of empty bottle was taken, after which the bottle was filled with the distilled water and properly covered. The weight was then recoded using a sensitive balance, after which the distilled water was removed from the bottle. The bottle was properly washed and filled with oil sample, after which the weight was taken and finally, the density was computedat 25°C using the relationship below (Akapan et al. 2005).

$$Density = \frac{w_0 - w}{w_1 - w}$$

Where:

$w \equiv weight\ of\ empty\ rbottle\ (g)$

$w_0 \equiv weight\ of\ the\ bottle\ and\ oil\ content\ (g)$

$w_1 \equiv weight\ of\ bottle\ and\ water\ content\ (g)$

2.3.3.3 Viscosity

Capillary tube viscometer test method

The method of determining viscosity (Kinematic Viscosity) utilized the capillary tube viscometer. The oil sample was placed into a glass capillary U-tube and the sample was drawn through the tube using suction until it reached the start position indicated on the tube side. The was then released, allowing the sample to flow back through the tube under gravity. The narrow capillary section of the tube controls the oil's flow rate.

Once the oil meniscus touched the highest line, time was started and suction continued until the oil meniscus touched the lower line. The reading was taken at 25C°.

The method used for determination of viscosity was adapted from A.O.A.C (2000).The viscosity was calculated as follows:

$$\frac{viscosity\ of\ oil}{viscosity\ of\ water} = \frac{density\ of\ oil\ \times flow\ time}{density\ of\ water\ \times flow\ time}$$

2.3.4 Chemical characteristic of ziziphus-spina Christi seed oil:

2.3.4.1 Saponification value (SV)

The determination of saponification value was carried out according to the AOAC methods (1990).

Two grams of oil sample were introduced into a 200cm^3 conical flask – 25cm^{3}of 0.5M alcoholic KOH solution was added, and the contents of the flask were boiled under reflux for 1 hour with frequent rotation. 1cm^3 of phenolphthalein indicator was added. While the solution was still hot,

and the excess alkali was titrated with 0.5MHCl (a) the volum was recorded. A blank was determined at the same time and conditionand therequired volume of the acid (b) was also recorded:

$$SV = \frac{b - a \times 28.05}{S}$$

Where:

$a \equiv volume\ of\ acid\ used\ for\ the\ first\ titration\ with\ oil\ sample$

$b \equiv volume\ of\ acid\ used\ for\ the\ second\ titration\ blank\ solution$

$S \equiv weight\ of\ oil\ sample$

2.3.4.2 Acid value (AV)

Acidity determination was carried out according to the British Standard Institution (1958).

Two grams of oil were dissolved into solvent mixture, 25 cm^3 of ethanol and 25 cm^3 of diethyl-ether, 1 cm^3 of phenolphthalein was added and the

solution was thereafter, titrated with0.1M potassium hydroxide until a pink end point was reached.

The formula used to calculate acidity value was follow:

$$AV = \frac{(a-b) \times M(KOH) \times M_{wt}(KOH)}{S}$$

Where:

$a \equiv$ *reading of oil (cm^3)*

b ≡ reading of blank (cm^3)

$S \equiv weight\ of\ sample$

$M \equiv molarity\ of\ KOH$

$\mu_{wt} \equiv$ *molecular weight*

2.3.4.3 Peroxide value (PV)

The peroxide value of the oil sample was determined according to the AOAC method (1990).

Three grams of the oil was weighed into 250 cm^{3}conical flask. 30 cm^3 of glacial acetic acid and chloroform (3:2) were added and the solution was swirled to dissolve the oil. 0.5 cm^3 of saturated potassium iodide was added to the flask, and the content of the flask were shaken for one minute before adding 30 cm^3 of distilled water. The content were titrated with 0.01M sodium thiosulphate until the yellow colour almost disappeared. The number of cm^3 0.01M sodium thiosulphate required(a) were recorded. The same process was repeated for blank. The number of cm^3 of 0.01 sodium thiosulphate required by the blank (b) was recorded. The peroxide value was calculated using the relationship below:

$$PV = \frac{(a-b) \times M \times 1000}{S}$$

Where:

$a \equiv titration\ of\ sample$

$b \equiv titration\ of\ blank$

$M \equiv molarity\ of\ sodium\ thiouslphate$

$S \equiv weight\ of\ oil$

2.3.5 Determination of fatty acids composition

2.3.5.1 Sample preparation (Methylation)

Two ml of the ziziphusspina Christi seed oil were taken in to test tube, then 7cm^{3}of methanolic sodium hydroxide and 7 cm^3 of methanolicsulfuric acid was added (methanolic sodium hydroxide prepared by taking 2 grams from the sodium hydroxide dissolvedand transferred to 100 cm^3 volumetric flask and completed to the mark by methanol, methanolicsulfuric acid prepared by taking1 cm^3 from concentrated sulfuric acid and transferred to volumetric flask and completed to volume to 99 cm^3 by methanol), after that gentle shaking was made for three minutes, then the contents was leftin overnight, after that 2 cm^3 from supersaturated sodium chloride solution was added and 2 cm^3 from normal hexane was added then the mixture was well shaken.After three minutes two layer separated, the upper layer is the organic layer which contains the fatty acid and the lower layer is the aqueous layer. 5 µdm^3 from hexane collected was taken and diluted in 5 cm^{3}diethyether. One gram from sodium sulphate was added as drying agent and filtered through syringe filter (0.45 µm), the filtered was transferred directly to the GC-Ms vial and 1 µl injected directly to GC-MS.

2.3.5.2 GC-MS Analysis

GC-MS was carried out on sample of oil using GC-MS QP 2010 Ultra instrument, operating with following parameter:

Column: Rtx – 5MS …. Length (30m) …. Diameter (0.25mm) …. Thickness (0.25 µm), carrier gas:Helium, column oven temp:60.0^{o}C, injection temp: 280.0C$^{\circ}$, injection mode:split,flow control mode: linear velocity, pressure: 93.1 Kpa, total flow: 50 cm^{3}/min, column flow:1.50 cm^{3}/min, linear velocity: 44.7 cm/sec, purge flow: 30cm^{3}/min, split ratio: - 1.0, and oven temperature program: rate: 10.00, temperature (C^{o}): 60 – 300, hold time (min): 0.00

2.3.6 Antimicrobial Activity

2.3.6 .1 Preparation of the oil extract

The oil extract of zizphusspinachristi seed was prepared as in section (2.2.2).

2.3.6.2 Testing of antimicrobial susceptibility (Discdiffusion method)

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999).

Bacterial suspension was diluted with sterile physiological solution to 10^{8}cfu/ml (turbidity = Mc far land standard 0.5). onehundred microdecimeters of bacterial suspension were uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter

paper discs (Whatman No1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20 µdm^3 of solution of each plant extracts.

The inoculated plates were incubated at 37C°for 24h in the inverted position. The diameters (mm) of the inhibition zones were measured.

The results were expressed in terms of the diameter of the inhibition zone:<9mm, inactive; 9-12mm, partial active; 13-18mm, active; > 18mm, very active.

Chapter Three

Results and Discussion

3.1 Results

3.1.1 Chemical composition of ZSC seed

Table (3.1) shows the Chemical composition of ZSC seed

Composition	Mean ± SD %
Oil content	27.8 ± 1.69
Moisture	4 ± 0.76
Ash	3.5 ± 0.01
Fiber	21.3 ± 1.8
Protein	31.9 ± 0.61
Carbohydrate	11.5 ± 2.65

Values in the table were expressed as means ± SD

3.1.2 Physicochemical properties

Physicochemical properties of the oil extract from ziziphusspina-Christi seed was determined by various methods described in sections (2.2.3) and (2.2.4) and values of these properties were recorded in table (3.2)

Table (3.2) physicochemical properties of ZSC seed oil

Properties	Values ± SD
Refractive index	1.467 ± 0.00
Density g/cm^3	0.928 ± 0.00
Viscosity Poise	37.31 ± 0.02
Saponification value mg KOH/g	159.6 ± 0.99
Acid value mg KOH/g	4.7 ± 1.15
Peroxide valuemeg/kg	0.8 ± 0.28

Values in the table were expressed as means ± SD

3.1.3 Fatty acid profile using GC/MS

GC-MS is technique widely used for analysis of the oil. In this study the oilextracted from the seed of Z-spina-Christi was analyzedby shimadzu,QP2010ultra,Gaschromatography–Massspectrometer,described in section (2.3.5). The result total ion chromatogram was in fig (3 – 1).

By this technique 29 compounds were identified in table below:

Table (3 – 3) Determination of fatty acid composition in oil extract from ZSC seed:

Peak	Name	R.Time	Area%
1	Heptanoic acid, methyl ester	4.698	0.02
2	Octanoic acid, methyl ester	6.070	0.06
3	Butylated Hydroxytoluene	11.369	0.10
4	Dodecannoic acid, methyl ester	11.404	0.02
5	5-Octadecenoic acid, methyl ester	13.449	0.02
6	Methyl tetradecanoate	13.722	0.36
7	6-Octadecenoic acid, methyl ester, (Z)-	14.534	0.02
8	Cis-5-Dodecenoic acid, methyl ester	14.639	0.04
9	Pentadecanoic acid, methyl ester	14.798	0.09
10	Methyl hexadec-9-enoate	15.590	0.14
11	9-Hexadecenoic acid, methyl ester, (Z)-	15.635	0.30
12	Hexadecanoic acid, methyl ester	15.863	15.41
13	Cis-10-Heptadecenoic acid (Z,Z), methyl ester	16.599	0.20
14	Heptadecanoic acid, methyl ester	16.808	0.46
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.595	35.86
16	9-Octadecenoic acid, (Z)-, methyl ester	17.661	19.83
17	Methyl stearate	17.796	8.75
18	Methyl 10- trans, 12-cis-Octadecadienaoate	18.320	0.09
19	Methyl 5,13- docosadienoate	19.104	0.34
20	11,14-Eicosadienoic acid, methyl ester	19.140	0.36
21	Oxiraneoctanoic acid, 3-octyl-, methyl ester	19.272	1.92
22	11-Eicosenioc acid, methyl ester	19.319	3.61
23	Methyl 18-methylnonadecanoate	19.514	4.56
24	Heneicosanoic acid, methyl ester	20.327	0.13
25	13-Docosenoic acid, methyl ester	20.943	0.51
26	Methyl 20-methyl-heneicosanoate	21.130	3.76

27	Tricosanoic acid, methyl ester	21.885	0.32
28	Tetracosanoic acid, methyl ester	22.624	1.87
29	Squalene	23.369	0.84
			100.00

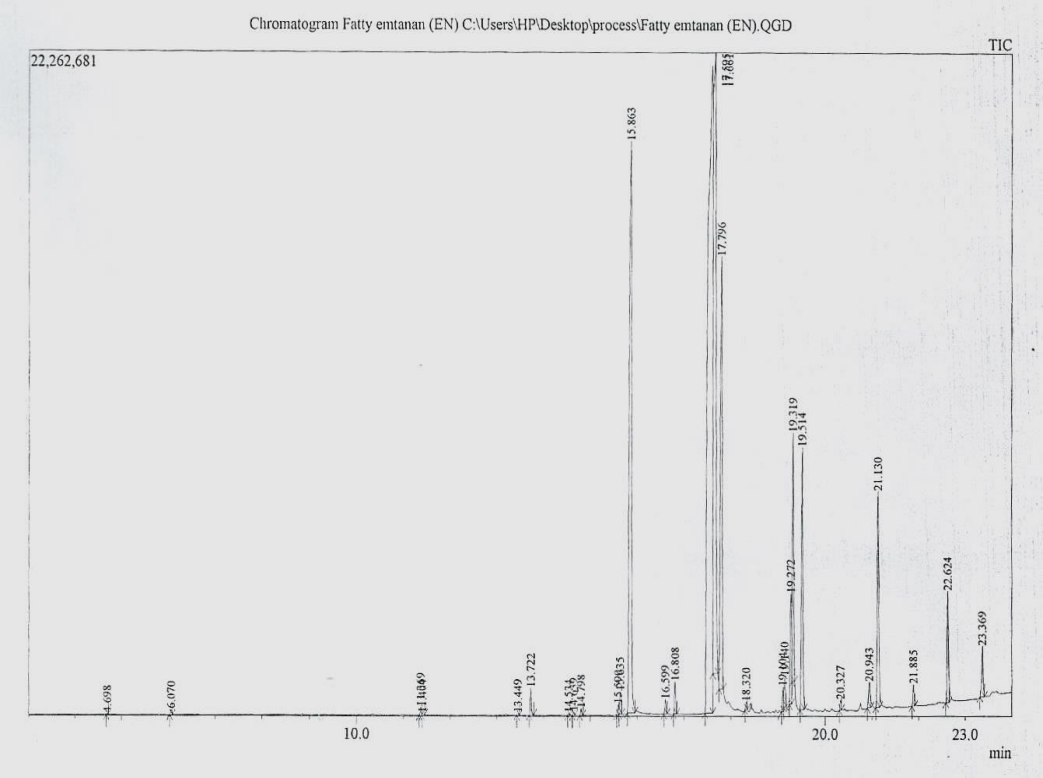

Figure (3-1): Chromatogram of Fatty Acid

3 .1 .4 Antimicrobial Activity

The oil extract of zizphusspina-christi seed were tested for antimicrobial activity against four bacterial and two fungi by method described in section (2.2.6.2) After the incubation the diameter of the resulted growth inhibition zones was measured, averaged and the results were summarized in table (3.4)

Table (3.4) Diameter zone of inhibition (mm) produced by petroleum ether extracts of the seed oil of ZSC:

Bacteria and Fungi	**Average zone of inhibition (diameter, mm) oil extract**
Escherichia Coli	13.5
Pseudomonas aeruginosa	12
Staphylococcus aureus	11.5
Bacillus subtilis	0
Aspergillusniger	11.5
Candida albicans	18

3.2 Discussion

The seed of ZSC was rich in protein (31.9%) , therefore it could be utilized as feed or food stocks for animals . While the ash content was lowest (3.5%) , the moisture content was 4% ; low moisture decrease the probability of microbial growth . Also , the seeds contained great amount of oil 27.8% ; the percentage of oil makes this seed adistinct potential for the oil industry .

Saponification value is number of milligrams of potassium hydroxide required to neutralize the fatty acids from the complete hydrolysis (Link , 1980) .The SV of the ZSC seed oil was found to be 159.6 mg/g.

Acid value is number of milligrams of potassium hydroxide necessary to neutralize , acid value was determined by titration of the sample with potassium hydroxide (Link, 1980) , AV was found to be 4.7 mg/g.

Peroxide value (PV) is the number that expresses in milliequivalents of active oxygen in the quantity of peroxide contained in 1000 g (Link, 1980) . It is one of the most widely used tests for oxidative rancidity in oil and fat , PV is measure of the concentration of peroxide and hydroperoxide formed in the initial stage of lipid oxidation . In general , the lower proxide value , the better the guality of the oil , the more stable the oil (Borchani et al ., 2010) . The PV was found to be 0.8 meq/kg.

GC-MS is a technigue widely used for analysis of the volatile oils . In this study , oil extracted from ZSC seed consedred fixed oil , it was removed from fixed oil to volatile oil by methylation and analyzed by GC-MS , 29 compounds were determined andthe percentages of fatty acidswere calculated . Higher percentage from free fatty acids were found to be in the oil linoleic acid , oleic acid , palmitic acid and stearic acid (35% , 19% , 15% , 8%) , respectively .

The results shown in table (3-3) indicate that the oil extract from ZSC seed had good antimicrobial activity aganist some micro-organisms as follows :

Oil active against Escherichia coli and Candida albicans .

Partial active against Staphylococcus aureus , Pseudomonas aeruginosa and Aspergillusniger . Inactive against Bacillus subtilis .

Conclusion

The seeds of ZiziphusSpina-Christi contain relatively high concentration of oil content which is rich in free unsaturated fatty acid , make it a potential source of natural antioxidant value for their sensory , nutritional and health attributes. The previous result of oil extract from ZiziphusSpina-Christi seed possesses potential antimicrobial activity against some microorganisms and indicate of the importance of all parts of ZiziphusSpina-Christi tree as medicinal plant.

Recommendation

- To use seed of Z.S.C as supplement food source in the tropical and subtropical region .

- To use seed oil of Z.S.C as natural antioxidant.

- More studies should be paid in Z.S.C seed oil to recognize the shelf life.

-More attention and care should be taken for growth and maintenance of Z.S.C tree.

- Sudan is considered potentially rich source for different types of oil seed crops and lowering it is cost.

- Pharmaceutical scientists recommend to develop new treatments to fight diseases resulting from microbes. it is known that excessive misuse of antibiotics may result in the emergence of new strains of bacteria that is resistant to antibiotics and this issue is expected to become more serious problem in the future .

It is also one of the big challenges that pharmaceutical scientists are facing and to address this challenge scientists are now studying the antimicrobialproperties in many of the natural oils.

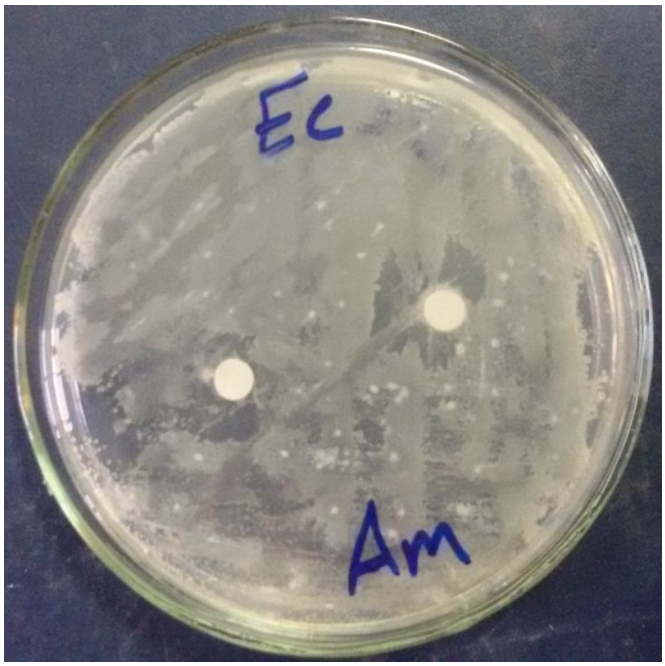

Figure (1): Inhibition zone of oil extract of the seed of ZSC against Escherichia coli

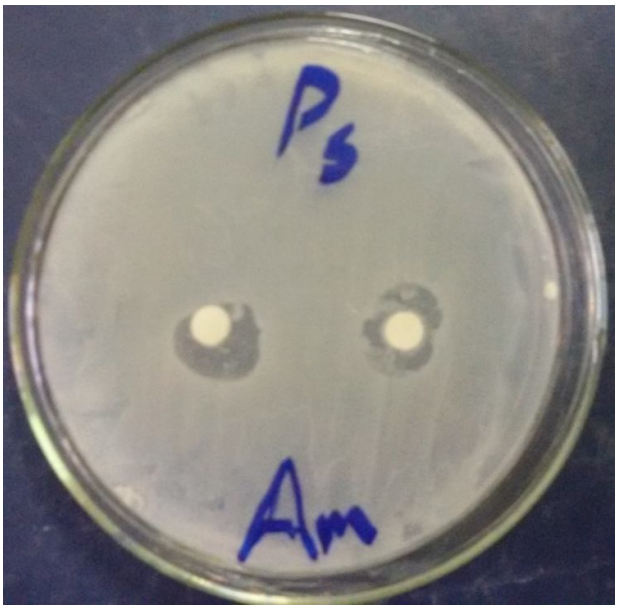

Figure (2): Inhibition zone of oil extract of the seed of ZSC against Pseudomonas aeruginosal

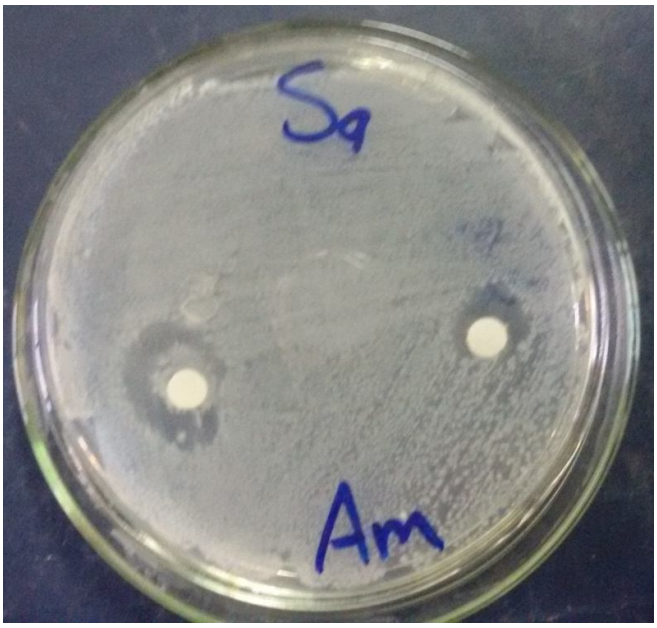

Figure (3): Inhibition zone of oil extract of the seed of ZSC against Stahpylococcus aureus

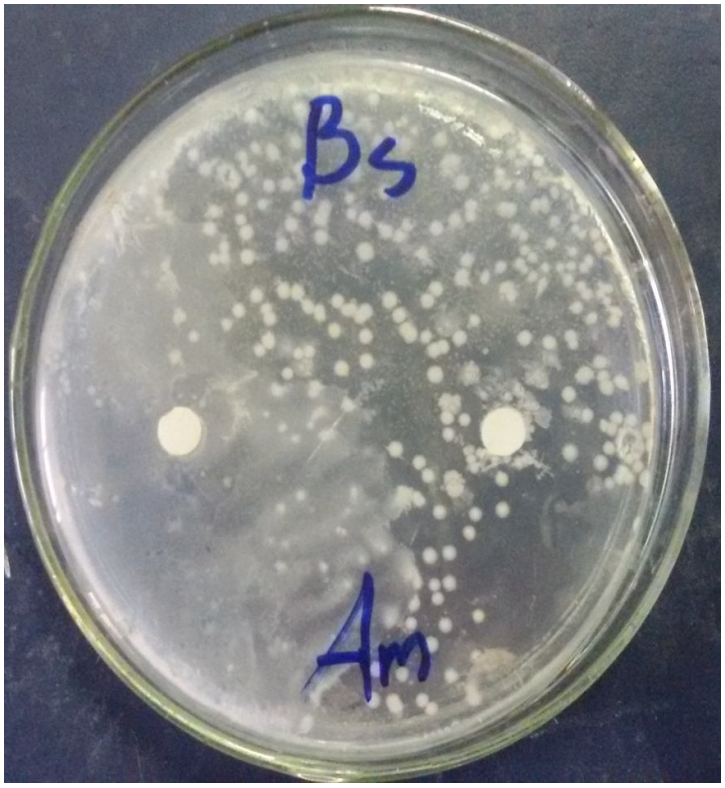

Figure (4): Inhibition zone of oil extract of the seed of ZSC against Bacillus subtilis

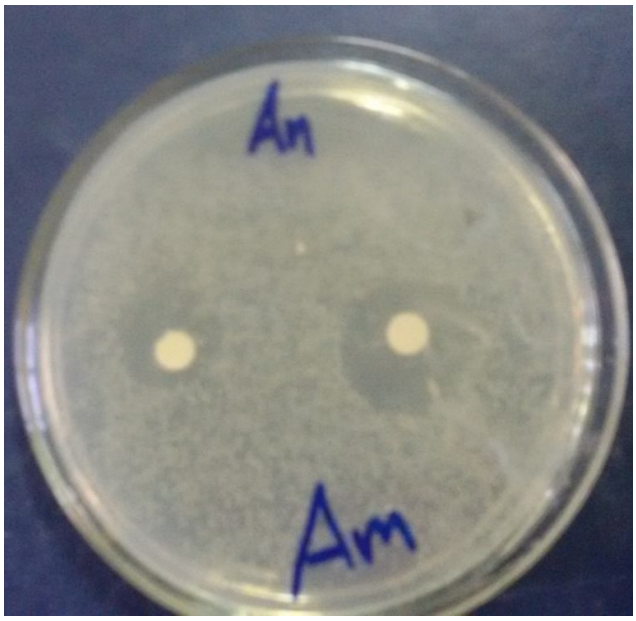

Figure (5): Inhibition zone of oil extract of the seed of ZSC against Aspergillusniger

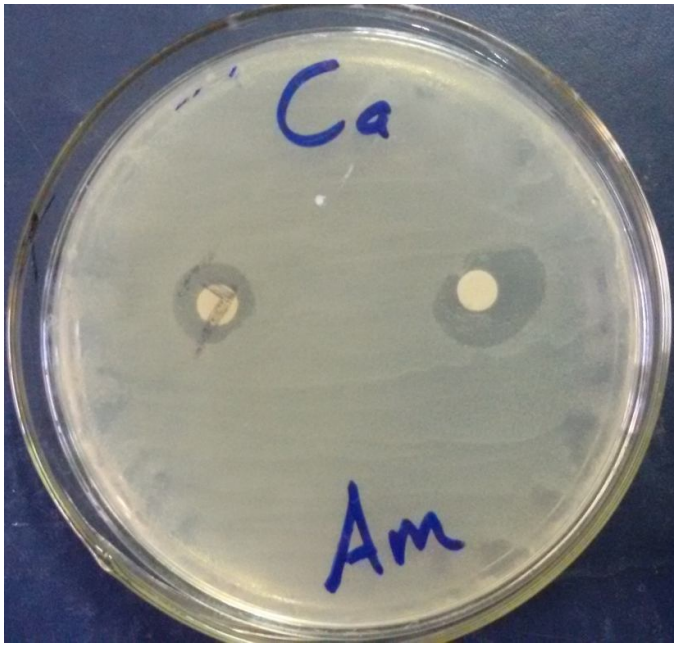

Figure (6): Inhibition zone of oil extract of the seed of ZSC against Candida albicans

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