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EXTRACTION AND IDENTIFICATION OF OIL EXTRACT FROM ANISE (*PIMPINELLA ANISUM L.*) SEEDS AND STUDY OF ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT: *Oil extract from Anise (Pimpinella anisum L.) was isolated and identified using several techniques such as thin layer chromatography, infrared spectrum, UV-spectrum and boiling point. The antibacterial activity of oil extract was determined against two different pathogenic bacteria such as Escherichia coli NCTC 5933and Staphylococcus aureus NCTC6571, the results reflect promising bacteria employed. The minimum inhibition concentration (MIC) of oil extracted was estimated against types of gram positive and gram negative were 10 and 2.5 µg/ml respectively.*

KEYWORDS: Anise, Oil extract, Antibacterial activity, Chromatography, Infrared spectrum.

INTRODUCTION

Essential oils are odorous products obtaind from natural raw materials such as leaves fruits, roots and wood .Essentials oils of plants and their other products from secondary metabolism have had a great usage in folk medicine feel flavoring, fragrance and pharmaceutical industries (1).

Anise(*Pimpinella anisum L.)* is a flowering plant in the family Apiaceac, Anise contains B vitamins and choline .It also contains :calcium ,iron, potassium and magnesium. Anise is used for loss of appetite, digestive problems excessive mucus in coughs and has been used as a stimulant for the vital orangs such as heart, liver, lugs and brain, Anise is known for being one of the best herbs for relieving pains of colic (2).

Anise is generally used as a appetite stimulant ,breath sweetener ,coughs ,colic ,gas intestinal purifier ,mucus obstructions ,nausea , nervousenerga and pneumonia(3).

MATERIALS AND METHODS

Plant materials

The seeds of Anise (*Pimpinella anisum L.*) were cleaned and blended by use (Electrical mill blender), the seeds powder were kept until required.

All of the chemicals were purchased from Sigma- Aldrich Co. (St. Louis, MO,USA), and the solvents were obtained from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

The Cultures of the bacteria used *Escherichia coli* and *Staphylococcus aureus* were procured from Department of Biology. Cultures were maintained on the medium suggested by the respective laboratory and sub-culturing was done fortnightly.

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Extraction method

34.000gm of seeds powder were extracted with 250ml of n-hexane ,using soxhlet continuous extraction method for 24 hrs .The extract was filtrated ,evaporated by rotary evaporator (Rota vapor RE,Buch) and taken up in maximum of n-hexane (4), the weight of oil was 4.300gm.

Identification

The chemical identification:

The chemical identification of function groups of the oil extract was implemented using several tests such as Liberman test ,double bond test and Ester test, table (1).(5)

Thin layer chromatography:

(TLC) were carried out on the oil extract by using (Ethyl acetate: methanol(19:1)) ,the plates were dried and the spots which appeared were developed with Uv-Lump at 366nm and Iodine vapor (4).

Determine of boiling point

Boiling point of oil compound was determination by use n-hexane as soluble.

Sepectroscopy

Ultra violet and visible spectra ,Uv-visible spectrum of the oil which was extract from the dried seeds of Anise (*Pimpinella anisum L.*) by using the n-hexane as the solvent ,and the spectrum recorded with a computerized thermos spectronic model LR 115161(England).

Infrared spectrum FT-IR spectrum was recorded with FT-IR 8400SSHIMADZU–Japan.

The determination of the antimicrobial activity of the oil extract:-

A filter disk assay was used to determine the antimicrobial activity of the oil extract (2mg /ml) agains types of reference strains of gram positive and gram negative bacteria which are (*Escherichia coli*. Atcc25922 and *staphylococcus aureus* ATCC25923 (6) ,(7) .

Which were tested using plates of muller Hinton agar .The antimicrobial activity was defined as the clear zone of growth inhibition.

The minimum inhibition concentration of the oil extract (MIC),was estimated against deferent types of reference strains of bacteria with deferent concentration of the oil extract ranging from (2.5-10)µg/ml (8).

RESULTS AND DISCUSSION

Table (1) indicate the preliminary phytochemical analysis for oil extract of Anise (*Pimpinella anisum L.*) consists one compound that have conjugated double bonds ,which appeared as a flourescent only one spot under the UV lamp (366nm) these compounds may be esterified with ether group ,also some spot were appeared as developer with I_2 –vapor detected to its organic

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nature. The chemical identification TLC procedure was run for this compounds and the results were shown in table (2).

Table (1): Preliminary qualitative test for oil extract

Phytochemical	Result
Lieberman Burchard test	+
Double test	+
Ester test	+

Absence = - ; Presence = +

Table (2): TLC for preliminary qualitative test for oil extract

Reagent / Sample	UV366	I_2-vapor
Oil extract	0.86	0.86

Table (3) and fig(1) show the full scan of IR spectrum of the oil extract. The UV-Visible spectrum fig(5) ,have shown two peaks at ʎ max (300) and (320) nm, due to($\pi \rightarrow \pi^*$) transition which is characteristic of conjugated double bonds of C=C and one peak at ʎ max (420)nm due to transition type ($n \rightarrow \pi^*$) which is characteristic of pairs of electrons on O atom (9),(10).

Table (3) Infrared absorption peaks and their related function groups for the oil extract of Anise (*pimpinella anisum L.*):-

Bond frequency (cm^{-1})	Band	Mod of vibration	Functional groups
3004.89	=CH	Str.	Aromatic -H
2923.88	OCH_3	Str.	Ether
2856.36	-CH	Str.	-CHof CH_3group
1745.4	C=O	Str.	Ester
1608.52	C=C	Str.	Aryl C=C
1371.29	C-O-C	Str.	Ether

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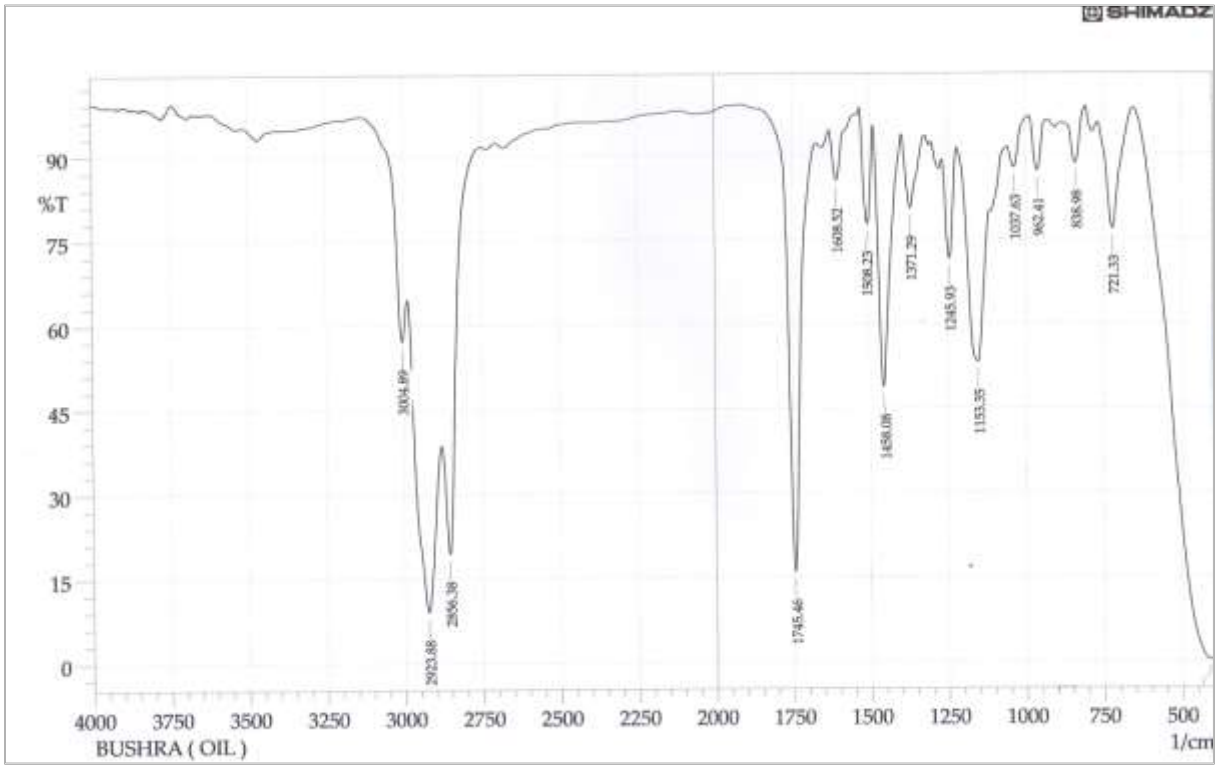
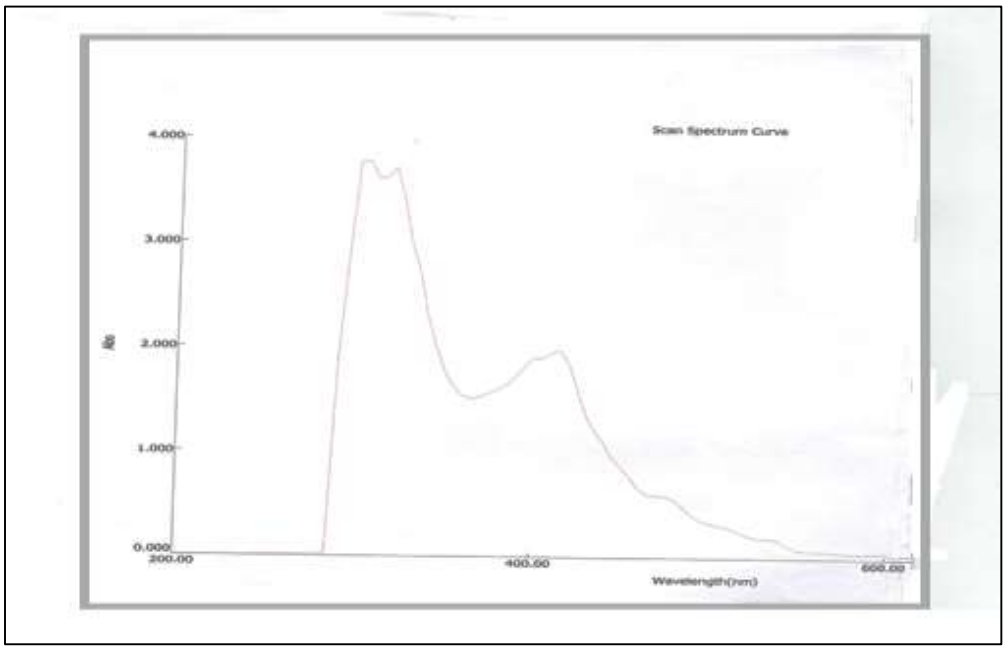


Figure(1):The full scan of IR spectrum for the oil extract of Anise (*pimpinella anisum L.*).



Figure(2): Uv spectrum for the oil extract of Anise (*pimpinella anisum L.)*

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The biological activity of the oil extract table (4) ,is due to the presence of the ether group in the structure of this oil extract ,so the ether and other organic solvents causes inhibition to the microbial cell growth through the precipated the cell wall microbial protein, and also to presence to the aromatic cycle in the structure of the oil extract ,so some of the microbial organism influence with the aromatic compound because it cannot to broken of these aromatic cycle (11) while the minimum inhibitory concentration (MIC) of the oil extract table(5), was equal 10 µg/ml against (*Staphylococcus aureus* NCTC6571) and 2.5 µg/ml against *Escherichia Coli* NCTC 5933.The oil extract appeared to have high antibacterial activity , this may be justified due to the destroy the cell membrane and then led to inhibit the microbial growth and may change the cell protein nature (Denaturation) and increase the permeability of the cell membranes(12), as many type of antibacterial compounds(13).

Table (4): Antibacterial activity of oil extracts against pathogenic (G+) and (G-) bacterial strains.

Bacteria / Extracts	S. aureus (Pathogenic)	E. coli (Pathogenic)
Oil	11mm	12mm

Table (5): Minimum Inhibitory Concentration (MIC)of oil extracts of Anise (*pimpinella anisum L.*):-

MIC(µg/ml)	Bacterial strains
10	*Staphylococcus aureus* NCTC6571
2.5	*Escherichia Coli* NCTC 5933

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