Extraction and Identification of Oil Extract from *Capsicum annuum* L. Fruits and Study of its Antimicrobial activity

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Abstract:-

An oil extract from fruits of red pepper *Capsicum annuum* L. was isolated and identified using several techniques such as: a thin layer chromatography, ultraviolet, visible, Fourier Transform Infrared Spectroscopy (FT. IR.) and some of colour reagents . The antimicrobial activity of the oil extract was determined against types of reference strains of bacteria : which are (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 5933, *Pseudomonas aeruginosa* NTCC 6750, and *Klebsiella pneumoniae*). The results indicate that the oil extract from the fruits of red pepper *Capsicum annuum* has varying degrees of inhibiting the test organisms.

The minimum inhibition concentration (MIC) of the oil extract was estimated by using filter disk assay against several types of gram positive and gram negative bacteria. The (MIC) of the oil extract against gram positive bacteria was (5) µg/ml. and was (2.5) µg/ml. against gram negative bacteria.

Key words: *Capsicum annuum***,** Fruits**,** Oleoresin**,** Extraction, Antimicrobial activity

Introduction :-

Paprika, *Capsicum annuum*L. (Solanaceae) is one of the oldest, most important and widely used carotenoid food colorants. The composition of the carotenoid pigments produced by paprika has been investigated for a long time **(1).**

Capsicum is rich with Vitamin C (ascorbic acid) and Zinc, two nutrients which are vital for a strong and healthy immune system. It is also rich with vitamins, A, C, rutin (a bioflavonoid), beta carotene, iron, calcium and potassium.

Capsicum also contains magnesium, phosphorus, sulphur, B-complex vitamins, sodium and selenium. The nutritional breakdown of Capsicum is as follows**, (2):**

- Fats: 9-17% .
- Proteins: 12-15% .
- Vitamin A and red carotenoids (capsanthin, carotene, lutein).
- Ascorbic Acid (Vitamin C).
- B-Complex vitamins.
- Potassium: 2014 mg per 100 edible grams.
- Rutin (flavonoid).

As a medical plant, the *Capsicum* species has been used as a carminative, digestive irritant, stimulant, and tonic. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache. *Capsicum annuum*L. has been reported to have hypoglycemic properties. A prolonged contact with the skin may cause dermatitis and blisters, while excessive consumption can cause gastroenteritis and kidney damage **(3).** The British Herbal Pharmacopoeia reported vasostimulant action of paprika **(4).** The Merck Index reported the therapeutic category of capsaicin, a pungent principle isolated

from cayenne or paprika, as topical analgesic. Cayenne has been shown to have counterirritant, antiseptic, diaphoretic, and gastric stimulating properties **(5 ; 6)**,also red paprika (*Capsicum annuum*) showed potent in vitro anti-tumor-promoting activity with inhibitory effects on Epstein-Barr virus early antigen (EBV-EA)activation induced by the tumor promoter 12-O- tetradecanoylphorbol-13-acetate (TPA) **(7)**. The pepper extracts have more than one antimutagenic compound and those functional nutrients apparently have a synergistic effect (**8).**The active Compounds in *Capsicum annuum* are alkaloids (capsaicin), fatty acids, flavonoids, volatile oil, carotene pigments (**1 ؛ 9).** Oleoresin of paprika is the oil extract of paprika, *Capsicum annuum*. This oleoresin contains about **37 to 54 pigments** depending on the mode of preparation (extract of unbleached or bleached paprika), of which only few could be completely or even tentatively identified. The main pigments are in general esters of **capsanthin and capsorubin.**

The aim of this study was to obtain on the oil extract (Oleoresin), from the local medical plant *(Capsicum annuum* L.), and study of the physiochemical properties and the antimicrobial activity of this medical herb.

Experimental Procedures:

1.Materials: Fruits of *Capsicum annuum* L. were used in their fully ripe stage.

2.Extraction: 10 grams of dried ground fruits of red peppers in their fully ripe stage were extracted with 100 mL of n-hexane, using a Soxhlet continuous extraction method for 24 hrs. The extract was filtrated, evaporated by a rotary evaporator (Rota vapor RE, Buchi) and taken up in maximum 5 ml. of n-hexane(**10**).

3. Identification:

TLC: To determine the composition of oil extract, a thin-layer chromatography was carried out for 1 hr. on silica gel plates (5 x 20) cm in a presaturated chamber of the mixture of (Chloroform: methanol: acetic acid : water)(170:15: 15: 12), the plates were dried and the spots which appeared were developed with UV- lamp at 254 nm. and Iodine vapour (**11**) .

Spectroscopy:

Ultra violet and visible spectra: UV-visible spectrum of the oil which was extracted from the dried fruits of *Capsicum annuum* was carried out in the College of Science, Department of Physics, by using the acetone as the solvent, and the spectrum recorded with a computerized thermo spectronic model LR 115161(England).

Infra red spectrum : FT-IR spectrum was recorded with FT – IR 8400S SHIMADZU-Japan in the College of Science, the Chemistry department.

The Chemical identification (12):

The chemical identification of the functional groups of the oil extract was implemented using several tests such as:-

1. **Lieberman-Burchard test**: 1 ml. of the Lieberman-Burchard reagent was add to1 ml. of the oil extract and mix. the appearance of green – deep green blue solution indicate the presence of the oil which extracted from *Capsicum annuum* fruits.
2. **Alcohol test** : Alcohol group test was performed using CrO_3 reagent, the appearance of green – deep green blue solution indicate the presence of OH group .

3. **Double bond test** : Two tests were using to investigate the presence of C=C groups, the first test was the adding of the $KMnO_4$ 1% reagent, were the brown solution formed. The second test, was the using of the Br_2 in CCl_4 reagent, the purple colour of the reagent disappeared indicate the presence of the C=C groups .

4. **Ester test** : This test was performed using ethanol, alcoholic KOH reagent and drops of phenonaphaline indicator and heat, the purple colour of the reagent disappeared indicate the presence of the ester bond .

4.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 ,(50 mg / ml.), against types of reference strains of gram positive and gram negative bacteria: which are (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 5933, *Pseudomonas aeruginosa* NTCC 6750, and *Klebsiella pneumoniae*) which were tested using plates of Muller- Hinton agar .The antimicrobial activity was defined as the clear zone of growth inhibition**(13**).

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 - 50000) µg/ml **(13).**

Results and Discussion:

Extraction: The oil extract was isolated in a good yield , 8.5 % , from the dried ground fruits of red peppers *Capsicum annuum*.

TLC: The chromatography procedure was run in 1 hr., and the results were analyzed under UV light and I_2 - vapour, table (1).The results illustrated that the oil extract of *Capsicum annuum* fruits consists of compounds that have conjugated double bonds , which appeared as a fluorescent spot under the UV lamp (254 nm). These compounds may be capsanthin and capsorubin which were esterified with fatty acids within the oil extract; also some spots were appeared as developer with I_2-vapour detected to its organic nature.

Table (1): A thin layer chromatography, R_f values for the oil extract of *Capsicum annuum* fruits

Solvent systems	Developers	R_f Values
(Chloroform: methanol : acetic acid : water) (170: 15: 15: 12).	**UV_{254} nm (1-spot)**	**0.97**
	Iodine vapour(4- spots)	**0.97** **0.8** **0.29** **0.19**

Table (2): Infrared absorption peaks and their related functional groups for the oil extract of *Capsicum annuum* fruits

Band frequency (cm^{-1})	Band	Mode of Vibration	Functional group
3440	-OH	Stretch	Alcohol
3006	=CH	Stretch	Unsaturated(=CH)
2925(A sym.)	-CH	Stretch	-CH of CH_3 groups
2854 (sym.)	-CH	Stretch	-CH of CH_3 groups
1743	C=O	Stretch	Ester

Spectroscopy:

Ultra violet and visible spectrum: The UV-visible spectrum , fig (1), has shown two peaks at λ max 230 and 280 nm. due to presence of pairs of electrons type ($n \longrightarrow \pi^*$) on O atom, and one peak at λ max 485nm. due to presence of conjugated double bonds of C=C type ($\pi \longrightarrow \pi^*$) (**14**) ; (**15)** within the structure of compounds found in the oleoresin of *Capsicum annuum* such as capsanthin , capsorubin and another carotenoid compounds, fig **(** 2**), (1)** ; **(6) .**

Infrared spectrum : FT-IR spectrum for the oil extract of *Capsicum annuum* is shown in fig (3), the appearance of a single peak at 3440 cm^{-1} related to the vibration stretching for OH bond indicated the presence of alcohol group. The band at 3006 is related to the vibration stretching for =CH bond of unsaturated hydrocarbons within the structure of compounds found in the oleoresin of *Capsicum annuum* . The bands at 2925 and 2854cm^{-1} are related to the vibration stretching for C-H bond of methyl group asymmetric and symmetric respectively . The band at 1743 cm^{-1} is related to the vibration stretching for C=O bond of ester group due to presence of carotenoid compounds esterified with the fatty acids of the oil extract**(15).**

The Chemical identification : The chemical identification of the functional groups of the oil extract was implemented and found to be very useful for qualitative determination of alcohol group ,C=C bonds and ester bond within the structure of compounds found in the oil extract of *Capsicum annuum* ,table (3) .

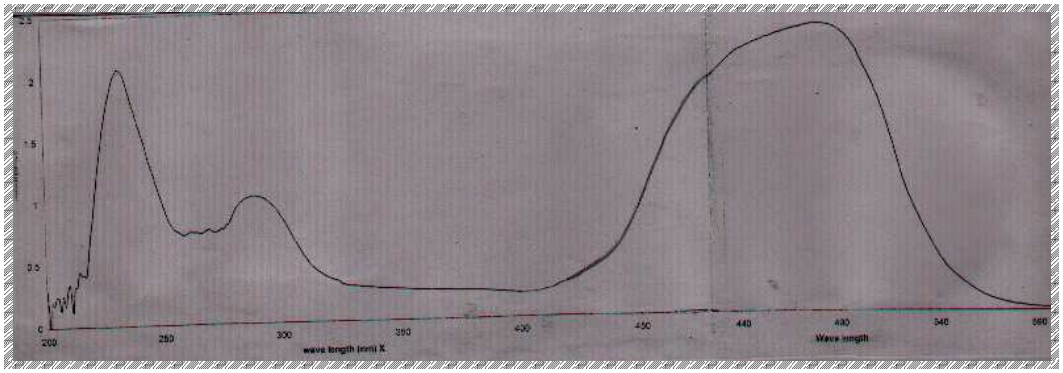

Figure (1) : Ultraviolet and visible spectrum for the oil extract of *Capsicum annuum* fruits

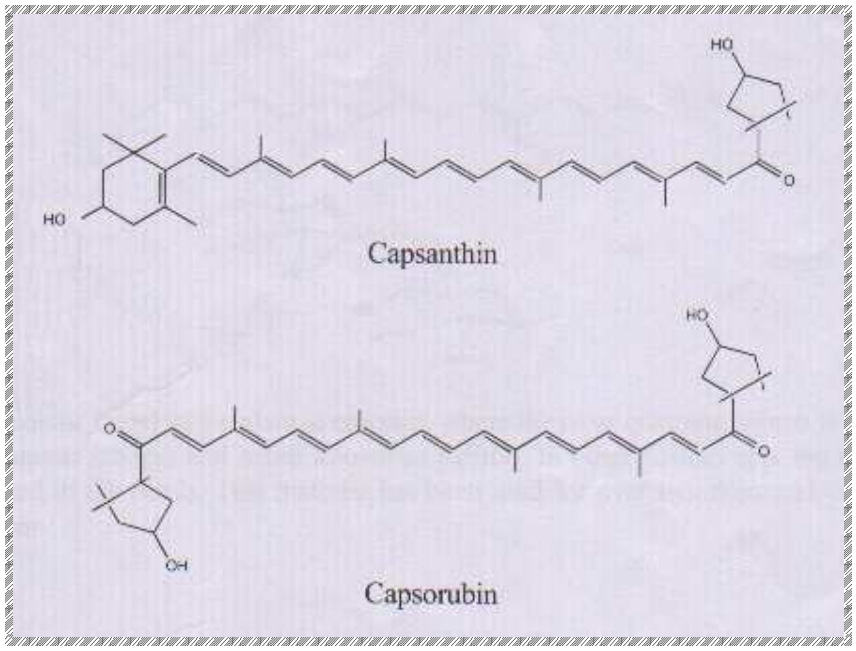

Figure (2): The chemical structures of capsanthin and capsorubin compounds

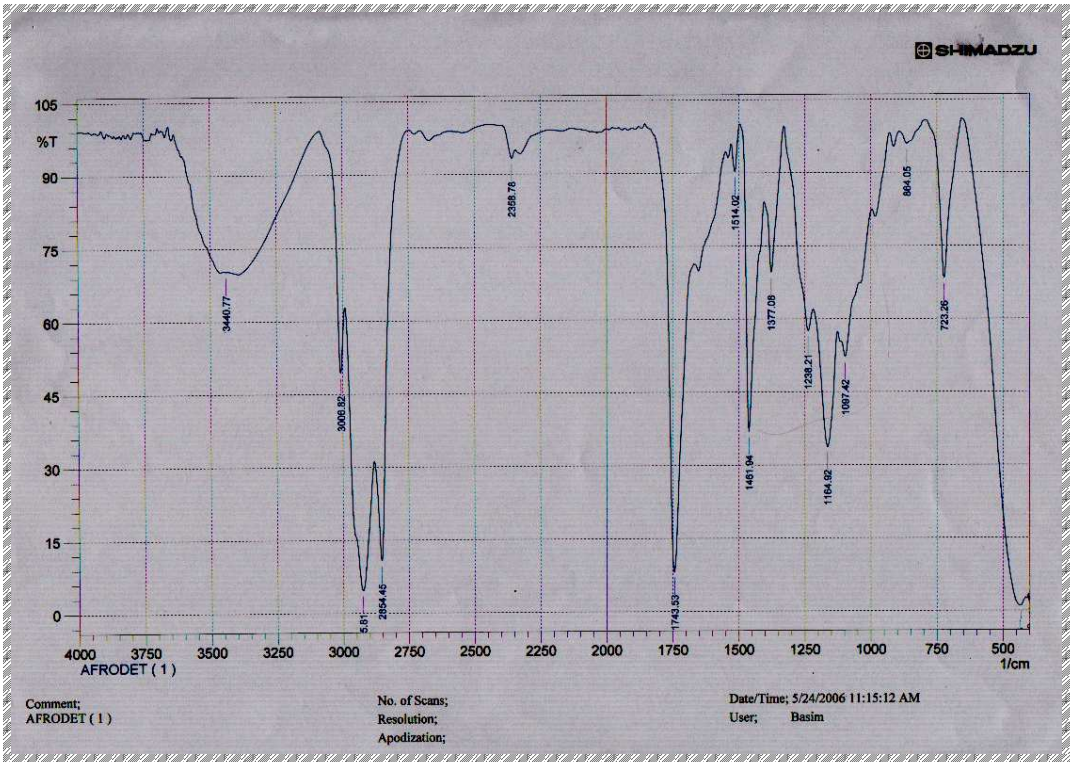

Figure (3): Infrared spectrum for the oil extract of *Capsicum annuum* fruits

Table (3): The Chemical identification of the oil extract of *Capsicum annuum* fruits

Colour reagent	Result
Lieberman-Burchard test	**+ ve**
Alcohol test	**+ ve**
Double bond test	**+ ve**
Ester test	**+ ve**

The antimicrobial activity of the oil extract:-

The antimicrobial activity of oil which was extracted from the fruits of *Capsicum annuum*, was determined using a filter disk assay. The results , table (4), show that the oil extract has the a good antimicrobial activity against gram positive and gram negative bacteria, this may be justified due to the presence of capsanthin compound, so this capsanthin which is considered as one of the major carotenoids of red pepper fruits, occurs esterified with fatty acids in ripe fruits, (account for 70-80% of total capsanthins) **(16)** ; this compound was known as the active compound in the red pepper **(1),** which has antimicrobial effects **(8)**, antioxidant effects **(16)** ,anti-tumor and antimutagenic effects **(17).** The results , table (5)**,** show that the MIC values of the oil extract were (5) µg/ml. against gram positive bacteria *Staphylococcus aureus* NCTC 6571and (2.5) µg/ml. against gram negative bacteria which are *Escherichia coli* NCTC 5933, *Pseudomonas aeruginosa* NTCC 6750 and *Klebsiella pneumoniae* .The presence of alcoholic groups (-OH) in the structure of the studied oil extract increase the activity of the plant extracts to inhibit the microbial growth. So the alcoholic compounds and their derivatives are considered as antiseptic agents **(18),** which are changing the cell protein nature (Denaturation) and increase the permeability of cell membranes **(19).**

Table (4): The antimicrobial activity of the oil extract

Bacterial strains	Inhibition zone(mm)
Staphylococcus aureus NCTC 6571	4
Escherichia coli NCTC 5933	15
Pseudomonas aeruginosa NTCC 6750	19
Klebsiella pneumoniae	8

Table (5): The minimum inhibition concentration of the oil extract

Bacterial strains	MIC (µg/ml)
Escherichia coli NCTC 5933	2.5
Staphylococcus aureus NCTC 6571	5
Pseudomonas aeruginosa NTCC 6750	2.5
Klebsiella pneumoniae	2.5

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عزل وتشخيص المستخلص ألدهني من ثمار نبات الفلفل الأحمر
ودراسة فعاليته الضد ميكروبية

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كلية العلوم / قسم الكيمياء.

الخلاصة:

عزل مستخلص زيتي من ثمرة نبات الفلفل الأحمر ، درست خواص المستخلص الفيزيوكيميائية مثل طيف الأشعة تحت الحمراء(Fourier Transform Infrared spectrum(FT. IR. و طيف الأشعة فوق البنفسجية والأشعة المرئية ultraviolet and visible spectrum، وحددت قيم معدل جريانه Rf بتقنية كروماتوغرافيا الطبقة الرقيقة (TLC) Thin layer chromatography وضمن أنظمة تظهير مختلفة، فضلاً عن إجراء بعض الكشوفات اللونية النوعية له. اختبرت قابلية المستخلص الزيتي المعزول على تثبيط نمو أنواع من الجراثيم القياسية الموجبة والسالبة لصبغة كرام وهي *Staphylococcus aureus* NCTC 6571 و *Escherichia coli* NCTC 5933 و *Pseudomonas aeruginosa* NTCC 6750 و *Klebsiella pneumoniae* وقد اظهر المستخلص القدرة على تثبيط جميع الأنواع الجرثومية المستخدمة إلا إن كفاءته في تثبيط نمو الأنواع السالبة لصبغة كرام كانت أعلى منها في الموجبة لصبغة كرام.كما حدد أدنى تركيز مثبط MIC من المستخلص الزيتي تجاه نمو عدد من الجراثيم القياسية الموجبة والسالبة لصبغة كرام، وقد كان اقل تركيز مثبط له تجاه الجراثيم الموجبة لصبغة كرام بحدود (5 ميكروغرام/ مل)،في حين كان اقل تركيز مثبط تجاه الجراثيم السالبة لصبغة كرام بحدود (2,5ميكروغرام/ مل).