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Preparation of *Mentha Crispata* Extract and Detection of its Biological Application.

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ABSTRACT
We have tested the anti-bacterial activity against pathogenic bacteria in current research. Such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella spp.* and *candida albecance* in the leaf extracts of Mentha crispate. The leaf extracts were to have strong antibacterial action against a variety of bacteria which are pathogenic as can be seen via in vitro agar well diffusion me. Advantageous groups of the extract were determined by FTIR. The DPPH assay was estimated the antioxidant action of extract and the effects was showed 86.22 % in 75µg/ml concentration. MTT assay was used for detecting the cytotoxicity of extract against stomach cancer, the outcomes showed the moral level of cytotoxic action with increasing concentration.

Keywords: Mentha crispata, Antimicrobial activity, Antioxidant activity, an activity.

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INTRODUCTION

The Lamiaceae family contains more than 4000 species in 200 genera. In the Lamiaceae family, mint is one of the most cultivated and aromatic plants. Mint is grown in temperate regions in many parts of the world (South America, Antarctica, Europe and Asia) (Seidemann, 2005). Mentha leaves have been traditionally used fresh and dried with various spices. Mentha species are comprised of biologically active components, which are used in traditional medicines. Additionally, mint species can be used in traditional medicine for common ailments, such as colds, coughs, sinusitis, fever, bronchitis, and nausea (Hussain, et al., 2010). Furthermore, that mint plant has also been reported to have insecticidal.
, antimicrobial, antispasmodic, antioxidant (Grant,.2010). It is also known that Mentha has antiviral and fungicidal activity. Against influenza, herpes and other viruses, menthol is virucidal. Influenza antiviral medicines is aqueous extracts of peppermint leaves (Huxley, 1992). Mentha is a vine with herbaceous plants. It is 30-100 cm (12-9 in) long, variably hairless with hairy leaves and stems, and a wide-spread underground fleshy rhizome through which it grows. The 5-9 cm (2-31/2) of serrated leaves are in length and 1.5-3 cm (1/2- 11/4 in) in thickness. For oral prevention, mint is also used. Powdered mint leaves were used for whitening teeth during the Middle Ages (Seidemann, 2005). For chewing, for mouth burns, new mint plants are used; for decoction, it is used as mouthwash to alleviate gingival pain (Hussain, et al.2010). Mint is used in the manufacture of oral dentifrices as it can provide general breathing cleanliness. Further, Studies have started performed about whether or not it contributes directly to the prevention of dental caries and plaque; nevertheless, it is confirmed that it produces an uncomplimentary. The aim of this research was to prepare Mentha extract and analyze the extract for antioxidant, antibacterial and anticancer activity.

MATERIAL AND METHOD

Mentha crispata

The Mentha leaves were composed from the popular market of Baghdad, after that were washed with distilled water, and dried with air drying.

Mentha crispata extract

Soxhlet apparatus method was used for extraction. Fifty gm. of Mentha leaves were extracted with ethanol 250 ml (70%) for seven-hours. The extract was dried and kept.

Strains of bacteria

The laboratory of microbiology was supplied the work with strains of bacteria these: *E. coli, Staph. aureus, P. aerugenosa, Klebsiella* and *C. albcances*.

Maintenance of cell cultures

The Iraq biotech. Cell Bank Unit was obtained to get a stomach cancer cell line. The cell line was kept in 1640-RPMI improved by 100 µg/mL from streptomycin, 100 units/mL from penicillin & bovine fetal 10%. EDTA-Trypsin was used for passaging the cells and reseeded at 50% confluence double in a week, then incubated at 37 °C.(Mueller & Wiese, 2004).

MTT assay

The cytotoxic effect was examined by MTT assay. 96-well plate was used to conduct the cell viability. The colon cell line was seeded at 1 × 104 cells/well. The joining monolayer was completed after 24 hrs. Then, Tested material was added to the cells. After 72 hrs, the cell activity was examined by taking out the medium, addition 28 µL of 2 mg/mL solution of MTT. The cells were incubated for 60 minutes at 37 ° C. Then MTT solution has been removed. Crystals residual in the well was solubilized with DMSO 130 µL of with shaking and incubation at 37 °C to 15 min (Hayon, et al. 2003). The microplate reader at 492 nm was used to examine the absorbency. The test was done in triplicate. The next equation was used to calculate the ratio of cytotoxicity:

$$\text{The cytotoxicity rate} = \frac{\text{Optical density of control }^{(A)} - \text{Optical density of test }^{(B)}}{\text{Optical density of control }^{(A)}} *00$$

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A study Antioxidant activity

DPPH Preparation

The DPPH is 1,1-Diphenyl-2-picryl- hydrazyl, dpph(2.3 mg) was thawed in ethanol (3.3) ml and the aluminum foil test tubes are shielded from light by covering them. Ascorbic acid (vitamin C), the concentration G+ve of usage (20µg / ml).

DPPH (Activity Radical Scavenging) .

Mentha crispata 's antioxidant activity was measured using stable radical DPPH .Different concentrations of Mentha crispata (25, 50 and 75 µg/ml) are used to test the activity of antioxidants .Each single concentration (10µl) was mixed with ethanol (490µl) and the quantity was then completed by adding (500µl) DPPH solution to one specific ml .

Then followed for 15 minutes with incubation at room temperatures. Based on the decrease in absorbance at 517 nm, the left-over amount of DPPH was determined . According to the equation formula, DPPH inhibition as a percentage was computed (Kareem et al. 2019).

$$\text{Scavenging \%} = \frac{(\text{OD control} - \text{OD sample}) * 100}{\text{OD control}}$$

Antibacterial Assay for Ethanolic extract

The antibacterial activities of alcoholic extract were evaluated against E.*coli* , *P. aeruginosa* and *Staphylococcus aureus, Klebsiella and C.albcances.* To access the activity, the overnight grown bacterial cultures were used with CFU 10^5ml. The antibacterial activity was carried out by the method of Kirby-Bauer disc diffusion. On Muller Hinton agar plates, the assay was performed. Sterile Hi-media cotton swab was used to spread the inoculum on agar plates. The diameter of the inhibition zone (mm) was measured after overnight incubated at $37c^0$ for 24 hrs. All samples were tested in triplicate. Controls included solvent without plant extract (Majid *et al.*,2019).

RESUIT AND DISCUSSION

IR analysis: - The analysis of infrared light interacting with a molecule is infrared (IR) spectroscopy. Chemists use it to evaluate functional groups in molecules. Figure1. represents the IR spectrum of menthe crispata display absorption points at 3402.54 cm^{-1} corresponding to O-H assembly and the bands 3447.83 cm^{-1} due to phenolic 3447.83 cm^{-1} peak at 2922.25 cm^{-1} related toward the aromatic group C-H and at 2850.88 cm^{-1} corresponding to the alphatic group C- also peaks at 1739.85 cm^{-1} corresponding to the group C= O alphatic ester and at 1701.27 cm^{-1} corresponding to C= O Lactame.as shown in fig.1 (Berthomieu, & Hienerwadel, 2009, Cisse, *et al*. 2009).

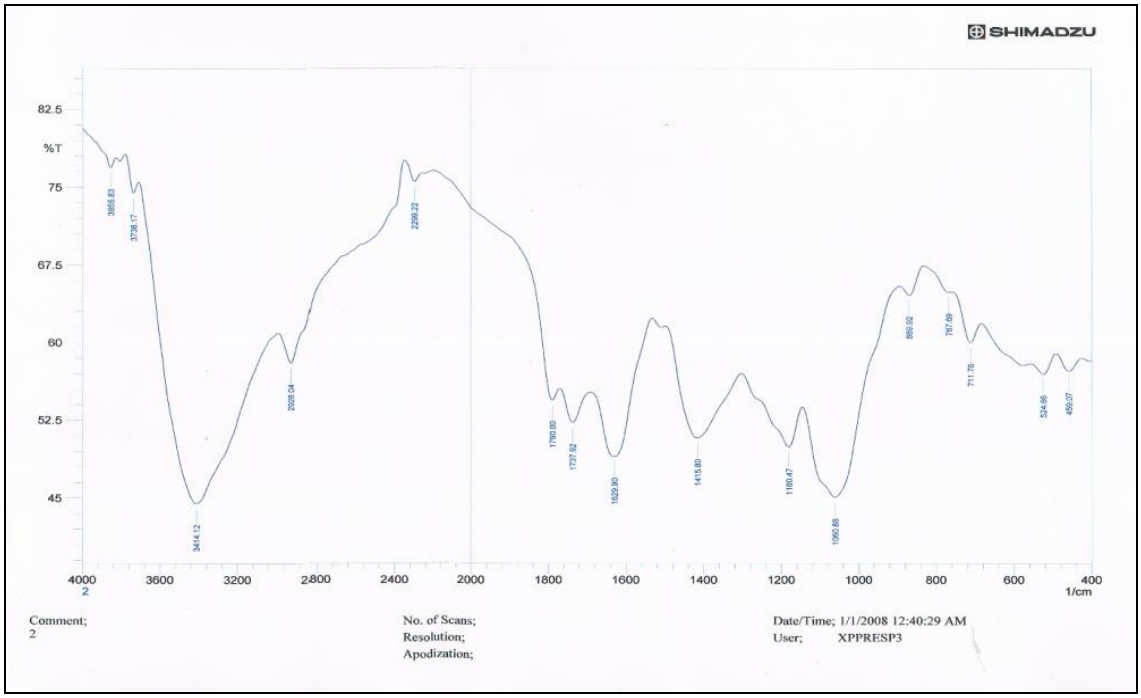

Fig.1. Spectrum peak pick report Ethanolic extract *of Mentha crispata*

Antioxidant activity.

The results from this study indicated the DPPH displacement was directly proportional to the increase in concen. The scavenged effect tests a free stable dark-purple tulip, is one of the most used tests. When natural antioxidants are returned by an electron and a proton (Keser, *et al* . 2012), the color changed to yellow. At 77.56, 81 and 86.22 respectively, the concen. of (25,50 and 75µg / ml)is certain to free radicals compared with *G+ve* as (ascorbic acid) as seen in figure (2). The increase in current free radical displacement can be due to the high content of particular phytochemical components of Mentha crispata, as presented by FTIR, for example, tannin, flavonoids, and phenols, were aimed at antioxidant compounds.

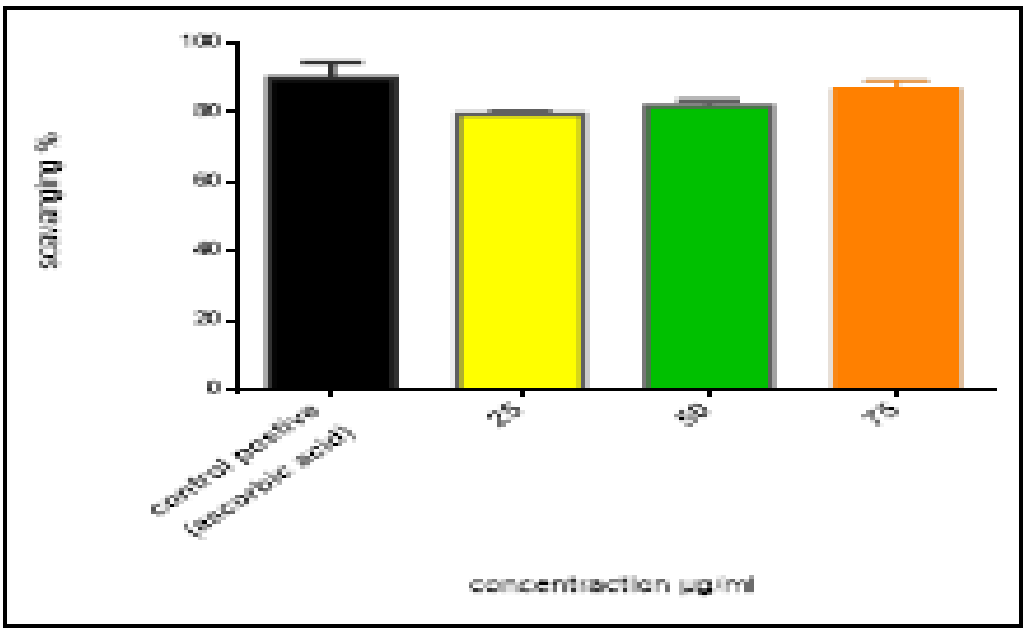

Fig. 2. DPPH scavenging activity of *Mentha crispata*

Antimicrobial activity

Table 1. & figure 3. shows the results of effect of ethanolic extract of Mentha crispata leaves has varying effects on the growth of bacteria in this study. The results were giving the highest effect on the growth of *S.aureus* bacteria via inhibition region (13.59mm), *P. aeruginosa* bacteria by inhibition zone reached to (12.43mm) *E.coli* by inhibition zone (12.55mm),*c. albacances* (14.22mm) and *Klebsiella* spp (16.6mm). Many strong composites suchas menthone, menthyl acetate, menthol, menthofuran, and limnone, Mentha are present in the leaf that shows significant activity (Fleming *et al* , 1998). In particular, In the action of dyspepsia, impaired digestion, eructions, epigastric bloating, and flatulence, which are tropically utility as tropical defensive agents (Alkofahi *et al*., 1990), these complexes have greater therapeutic utility to alleviate nasal mobbing in itch relief and common cold.

Table1. Antibacterial activity of *Mentha crispate* extract Concentration mg mL $^{-1}$

Microorganism	Inhibition zone *of Mentha crispate* extract		
	C	(1) % 50	(2) % 100
P. aeruginosa	-	10.33 mm	12.43 mm
S. aureus	-	12.56 mm	13.59 mm
E.coli	-	9.43 mm	12.55 mm
C. albacance	-	12.55 mm	14.22 mm
Klebsiella spp.	-	13.5 mm	16.6 mm

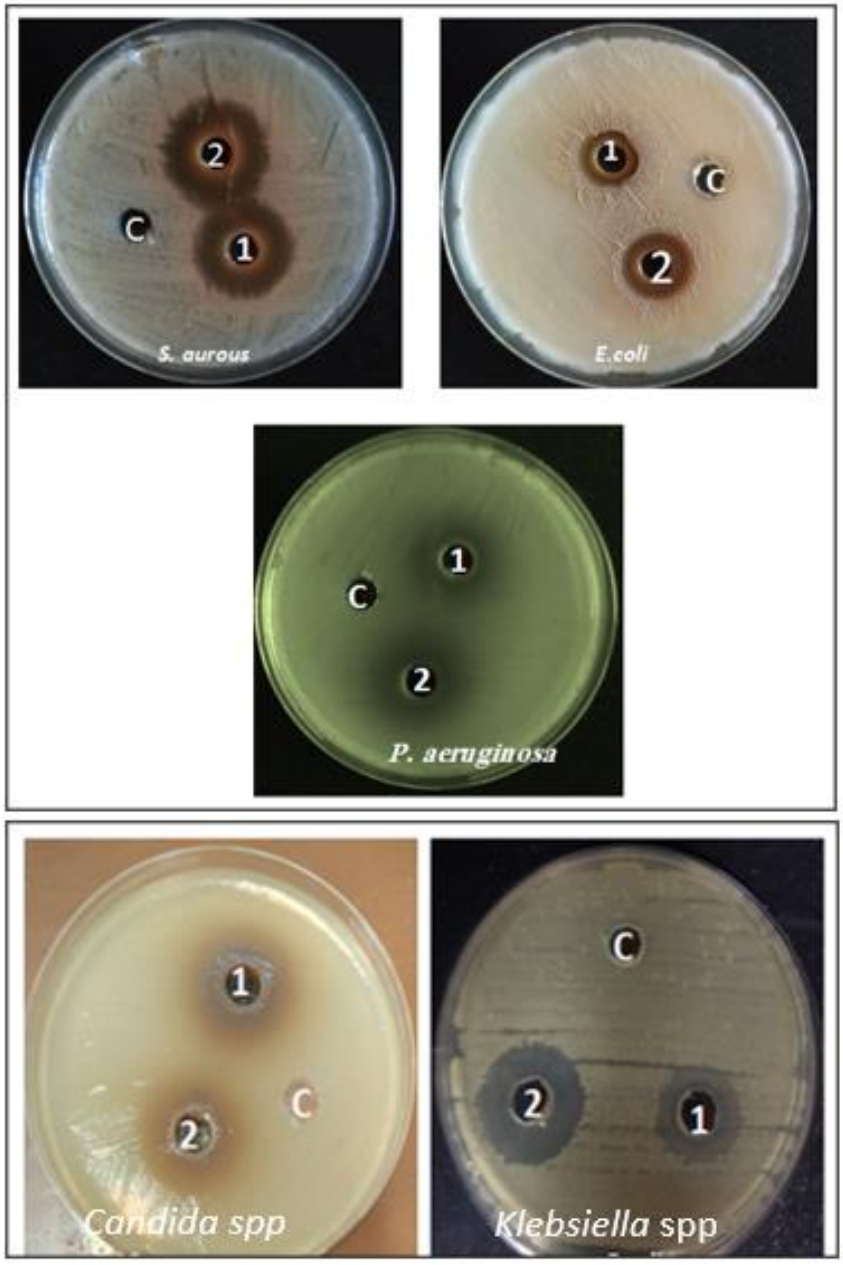

Fig. 3. Inhibition zone effects of *menthe crispate*. 50 and 100 mg per ml, concentration respectively. C= control.

Anticancer activity

Figures 4 and 5 was shown in role of mint extract against stomach cancer. A range of components have been believed to produce species of the genus Mentha, including cinnamic acids and aglycone, glycoside, and/or acylated flavonoids. Terpenoid carvone, which has been shown to assist in cancer cell inhibition, is the main chemical constituent of Mentha crispata. Perillyl alcohol, an extra terpenoid contained at lower concentrations in Mentha crispata, has a beneficial effect on the regulation of different cell substance present in cell growth and differentiation. (Pan,2010andCraig,1999 and Raghad et al .,2020)

Fig. 4. The shape of cells after treatment with Mentha crispata.

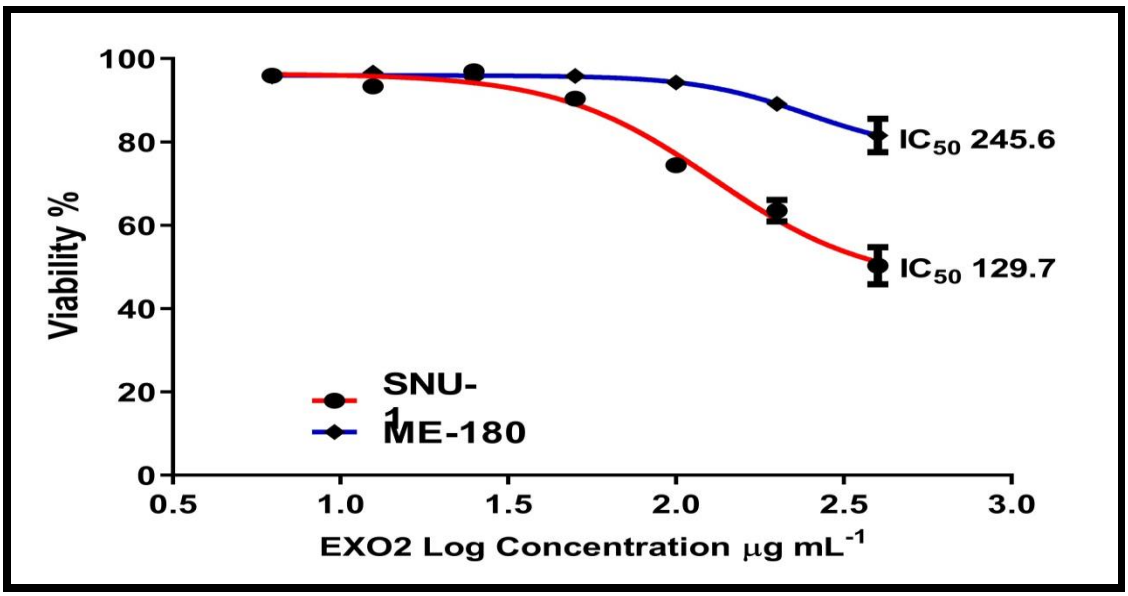

Fig 5: The role of *menthe crispata* against colon cancer.

CONCLUSION

Menthe crispata extract displayed a high level in antibacterial, antioxidant and anticancer action, There for *Menthe crispata* can be used successfully in the application of biology.

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