Al-Azhar Bulletin of Science

Volume 33 | Issue 1 Article 10

6-1-2022

Section: Botany, Microbiology and Zoology

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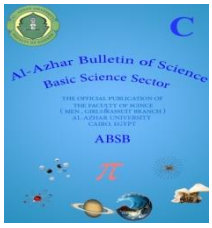
How to Cite This Article

Abdelmagied, Osama; Mourad, Mohammed; Ahmed, Ayman; and Younis, Ahmed (2022) "In vitro Prospecting and characterization of antifungal extracted from some plants," *Al-Azhar Bulletin of Science*: Vol. 33: Iss. 1, Article 10.
DOI: https://doi.org/10.21608/absb.2022.120597.1171

Al-Azhar Bulletin of Science, Section C, Vol. 33, No. 1 (June) 2022, pp. 117-131
http://doi.10.21608/absb.2022.120597.1171

Al-Azhar Bulletin of Science: Section C



IN VITRO PROSPECTING AND CHARACTERIZATION OF ANTIFUNGAL EXTRACTED FROM SOME PLANTS

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Received: 16 Feb 2022; Revised: 12 Apr 2022; Accepted: 12 Apr 2022; Published: 01 Jun 2022

ABSTRACT

Five extracting solvents (water, methanol, ethanol, acetonitrile and ethyl acetate) were involved to Evaluate antimicrobial of 13 different plants including different plant parts against 12 fungal species. The results showed promising antifungal activity of the ethanolic extract of *C. verum* and *S. aromaticum* that exhibited the strongest effect against *P. marneffie*, *C. albicans*, and *A. niger*. Two active compounds were purified using silica gel column chromatography followed by thin layer chromatography (TLC), and the characterization of active compounds was carried out by using IR, NMR and Mass spectroscopic analysis followed by determination of the minimum inhibitory concentration (MIC). First compound (A) belongs to ethanolic extract of *C. verum* was identified as (raffinose), The second compound (B) belong to ethanolic extract of *S. aromaticum* was identified as (oleanolic acid). Additionally, cytotoxicity of both purified compounds was carried out, where compound (B) showed high inhibitory activity against all tested carcinoma cells, while compound (A) did not produce any antitumor activity. Also, an antioxidant test was done for both compounds and the results showed that both compounds (A and B) have antioxidant activity. In conclusion, compound (B) had antifungal, antitumor and antioxidant activity while compound (A) had antifungal and antioxidant activity but, it had not antitumor activity against all tested carcinoma cells.

***Keywords*:** Antimicrobial activity; Plant extracts; Raffinose; Oleanolic acid; Antioxidant; Antitumor.

1. Introduction

For many years, natural medicine has provided health support to many people around the world. In some countries, few people whose percentage does not exceed 20 % can obtain medicines and health supplies, so many people resort to use natural products because of their beneficial effect and the absence of side effects for them in addition to their low price compared with chemical medicines [1].

Fungal diseases have caused serious losses to crop yield and quality. The annual economic losses caused by phytopathogenic fungi are estimated to exceed $200 billion, which is significantly higher than the losses caused by any other microbial groups [2].

Also, pathogenic fungi have a very harmful effect on human health, and infection with these fungi is more dangerous for humans who have immunodeficiency, cancer and AIDS. [3]

In the late nineteenth century, the use of many herbs and natural compounds as

antifungal was documented [4], For example, garlic, lemon, basil, datura and alfalfa have been used in the treatment of many fungal diseases because they are safe for humans and the environment and because they are easily obtained and used in medicine and to enhance flavor of foods in addition to its low price value [5].

The current study aims to estimate the antifungal activity of thirteen plants collected from the Egyptian market against twelve types of pathogenic fungi using different polar solvents, as well as purification and characterization of the most active compounds using IR, NMR and GC Mass instill the hope of applying these extracts as a natural source for application Therapeutic instead of chemotherapy, which has undesirable side effects.

2. MATERIALS AND METHODS

2.1. Fungal strains

Fungal strain were tested (this stains were obtained from The Regional Center for Mycology and Biotechnology, Al Azhar University) (Table 1)

Table 1: fungal strains.

No.	Test microorganisms (Fungi)	RCMB Number
1	*Candida albicans*	RCMB (005003) [1]
2	*Candida tropicalis*	RCMB (005 004"1")
3	*Candida lipolytica*	RCMB (003005)
4	*Cryptococcus neoformans*	RCMB (0049001)
5	*Aspergillus fumigatus*	RCMB (002008)
6	*Aspergillus flavus*	RCMB (002002)
7	*Aspergillus niger*	RCMB (002005)
8	*Penicillium aurantiogriseum*	IMI 89372
9	*Penicillium marneffie*	RCMB (001019 "1")
10	*Penicillium expansum*	RCMB (001001"1")
11	*Penicillium italicum*	RCMB (001018 "1")
12	*Syncephalastrum racemosum*	RCMB (016001"1")

2.2. Plants used for bioassays

Plants were obtained from Agriculture Research Center in Giza and subjected to the antifungal activity test. (Table 2)

Table 2: Plants and their parts involved in the antifungal assay

No.	Scientific plant name	Common name	part
1	*Allium Sativum*	Garlic	Cloves
2	*Aloe vera*	Cactus	Leaves
3	*Cinnamomum verum*	Cinnamon	Bark
4	*Coriandrum sativum*	Coriander	Seeds
5	*Eichhornia crassipes*	Water hyacinth	Leaves
6	*Hibiscus sabdariffa*	Roselle	Flowers
7	*Lawsonia inermis*	Henna	Leaves
8	*Nigella sativa*	Black seed	Seeds
9	*Piper nigrum*	Black pepper	Seeds
10	*Punica granatum*	Pomegranate	Pell
11	*Punica granatum*	Pomegranate	Seeds
12	*Syzygium aromaticum*	Clove	Flowers
13	*Zingiber officinale*	Ginger	Rhizome

2.3. Extraction of antifungal agents

Plant parts were collected and left to dry out at 20 °C for 7 days away from sunlight and then grinded to powder form, 100 gm of Plant powder was macerated in 300 ml of different solvents individually (water, methanol, ethanol, acetonitrile and ethyl acetate) for 48 h, after 48 h extracts were filtered by using (Whatman filter paper No.1), the previous repeated three times to obtain a clear crude extract. Filtrate was concentrated through rotary evaporator at 40 °C and crude extract was achieved [6].

2.4. Assay of antifungal Activity

The antimicrobial potentialities were determined by agar diffusion method as the diameter of the inhibition zones; 6mm diameter wells cut by cork borer in malt extract agar sterile plates (15cm), which had seeded with fungal test the holes were filled by 50 µl with each extracts, while control hole was filled with solvent used in the extraction. Plates were left in a cooled incubator at 4 °C for two h and then incubated at 28 °C for fungi and at 37 °C for yeast.

Zones of inhibition were measured after 24 h for the yeasts and 48 h for filamentous fungi [7].

2.5. Purification of the active antimicrobial compound

2.5.1. Column chromatography

A glass column (50 cm× 1.5 cm) was prepared by packing silica gel (40 g, 100–200 mesh), which was previously activated for 30 min at 105°C. The column was equilibrated with a series of solvent solutions with n-hexane, ethyl acetate, methanol and ethanol subsequently.

The most Tow active crude extracts of *C. verum* and *S. aromaticum* were loaded separately on the top of the column, then the following solvent systems were made for each crude extract:

System no.1: n-hexane (100 % v) followed by System no.2:n-hexane - ethyl acetate (3:1 v/v), System no.3: n-hexane - ethyl acetate (2:1 v/v) System no.4: n-hexane - ethyl acetate (1:1 v/v), System no.5: ethyl acetate (100 % v), System no.6: ethyl acetate - methanol (3:1 v/v), System no.7: ethyl acetate - methanol (2:1 v/v), System no.8: ethyl acetate - methanol (1:1 v/v), System no.9: ethyl acetate -methanol (1:2 v/v), System no.10: ethyl acetate - methanol (1:3 v/v), System no.11: methanol (100 % v) and System no.12: ethanol (100 % v). and 75 fractions from crude extract of *C .verum*, 80 fractions from crude extract of *S. aromaticum* were collected (5 ml of each fraction) [8].

2.5.2. Thin layer chromatography (TLC)

Fractions of *C. verum* and *S. aromaticum* were spotted on silica gel plate (60 SF 254e. Merch20x20) using a clean capillary tube individually.

Different types of mobile phases were investigated under the technique of try and error concept (T/E). Since the ethanol as an extracting solvent gives a polar nature of the crude extract so, multiple running systems related to polar systems were selected such as butanol – acetic acid – water 3:1:1 (BAW), as well as those related to slightly non polar ones such as chloroform – ethanol 9:1 (CE) and chloroform – acetone - butanol 85:15:20 (CAB).

Development chamber containing developing solvent (BAW) was prepared, then a TLC plate was placed inside the closed chamber. When the solvent phase reached the top, the plate was air dried and visualization process was accomplished by both naked eye and long UV (365nm). (By the same way), both of CE and CAB solvent system were settled in development chamber individually [9].

2.6. Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antifungal or antibacterial that inhibits the growth of the microorganism. The active fraction was screened to determine its lowest effective concentration against three fungal species (*A. niger*, *C. albicans*, *P. marneffie*). Double folded dilutions (1000 – 3.9 ug/ml) were examined by agar diffusion method [10].

2.7. Identification of the most effective antifungal compounds

The Infra-Red test of the fractions was estimated using BRUKER, Vector 22, at the National Research Center in Giza.

The active fractions of interest were subjected to the proton (^{1}H) NMR, ^{13}C analysis and were recorded in Deuterated dimethyl sulfoxide (DMSO-d6) and chloroform (CDCL3) on NMR spectrometer (Bruker Avance DRX 500, Germany). The mass spectrum of the active fractions was analyzed using the direct inlet unit in the Shimadzu QP-5050 GC-MS at the Regional Center for Mycology and Biotechnology, Al- Azhar University to ensure the purity of the active compound and give the mass fragmentation of its constituents.

2. 8. Evaluation of cytotoxic effect of certain chemical compounds

In vitro determination of antitumor activities of fennel ethanolic extract against 3 different cell lines; the breast tumor cell line (MCF-7), lung cancer cell line (A549), and hepatocellular carcinoma cell line (HEPG-2) in corresponding to the normal cell line (WI-38) to give a satisfactory impression of cytotoxicity the fennel ethanolic crude extract. MTT protocol was applied to evaluate cytotoxicity as follow; inoculation of 1 x 105 cells/ml (100 µl/well) for each well in the 96 well tissue culture plate and incubated at 37 ºC for 24 h, washing twice, two-fold dilutions of the tested sample were made in maintenance medium (RPMI) medium with 2% serum, added to the wells in corresponding to the negative control, incubation at 37 ºC and examined. MTT solution was prepared (5 mg/ml in PBS) (BIO BASIC CANADA INC), 20 µl of MTT solution were added, shaking at 150 rpm for 5 min, incubate (37 ºC, 5% CO2) for 1-5 h, the media was discarded, re-suspend formazan in 200 µl DMSO, shaking at 150 rpm for 5 min and read optical density at 560 nm [11].

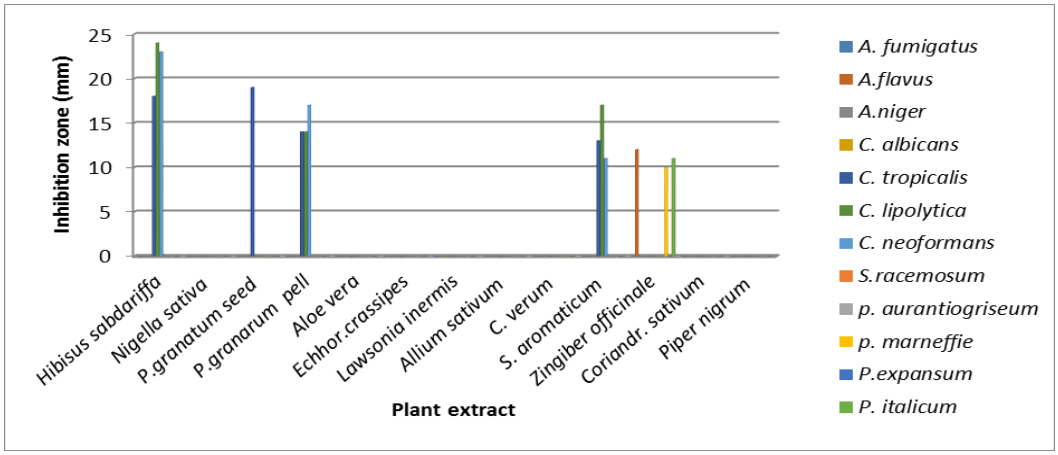
2.9. Anti oxidant Assay

Freshly prepared (0.004% w/v) methanol solution of DPPH was prepared and stored at 10°C in the dark. A 3 ml of freshly prepared DPPH solution was added to 40 ul of seed extract with well shaking, absorbance was recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The reduction in absorbance at 515 nm was recorded at 1 and 16min in corresponding to the control and ascorbic acid as a reference standard. All the determinations were performed in three replicates [11].

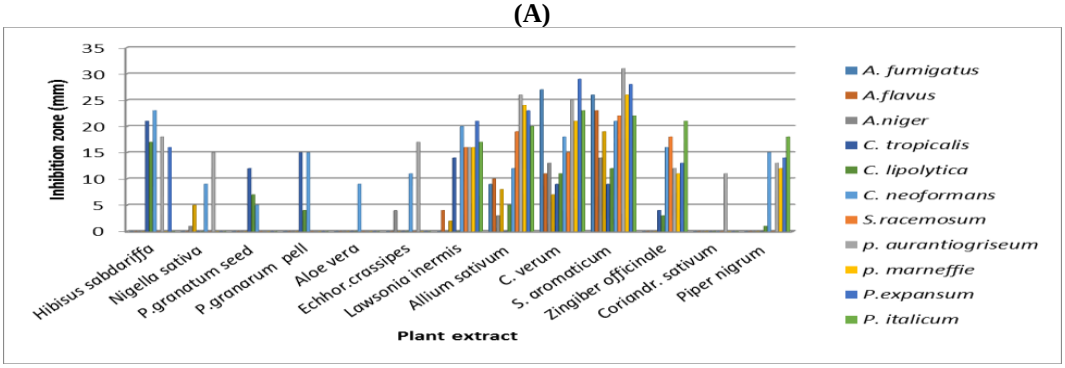
3. RESULTS AND DISCUSSION

3.1. Antifungal activity of plant extract

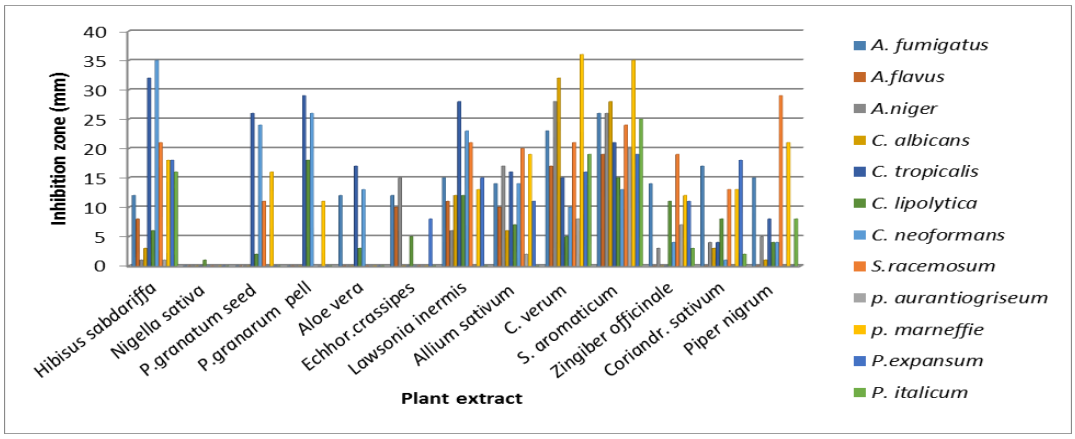
Five extracting solvents (water, methanol, ethanol, acetonitrile and ethyl acetate) were used to extract antimicrobial compounds of 13 different plants against 12 fungal species, and the results were shown in figure (1).



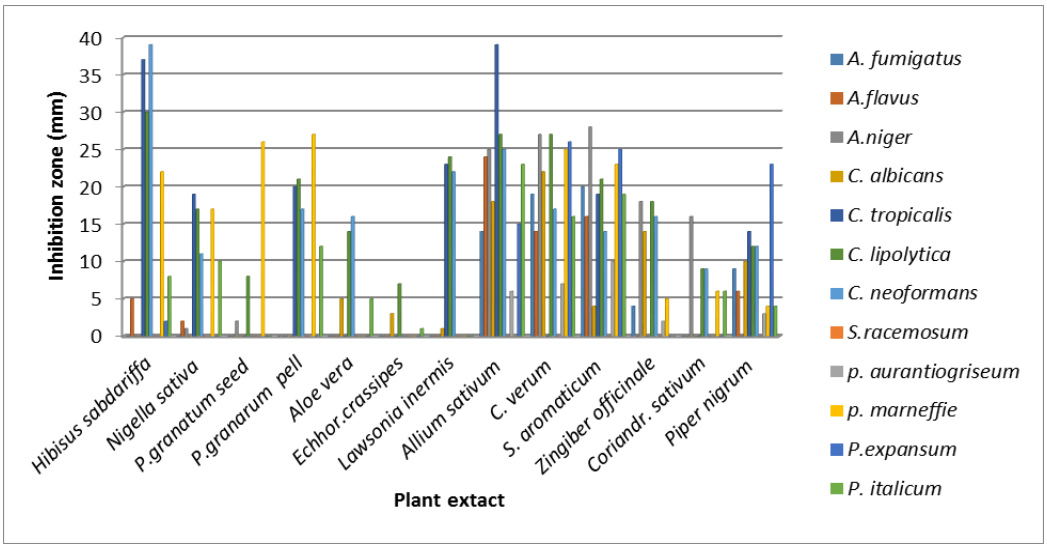
(A)



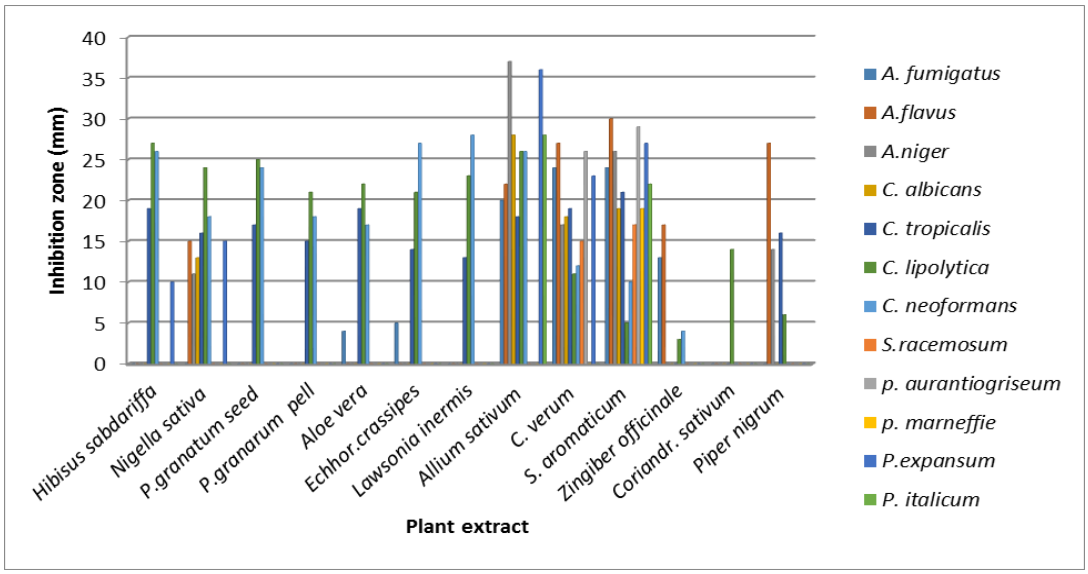
(B)



(C)



(D)



(E)

Fig. (1). Inhibitory effect of different extracts against certain fungal species. A: Aqueous extract, B: Methanolic extract, C: Ethanolic extract, D: Acetonitrile extract, E: Ethyl acetate extract

Figure (1) revealed that the strongest extract in terms of effectiveness against most of the fungi tested was the ethanolic extract of both of *C. verum* and *S. aromaticum.*

Antifungal activity of *C. verum*

The ethanolic extract of *C .verum* was produced inhibition zone with diameter equal 32 mm in case of *C. albicans* and 28 mm in case of *A. niger*, the minimum inhibitory effect of ethanolic extract of *C. verum* Bark against the growth of *C. lipolytica* and showed inhibition zone with diameter equal 5mm.

Antifungal activity of *S. aromaticum*

The ethanolic extract of *S. aromaticum* Flowers was produced inhibition zone with diameter equal 35 mm on *P. marneffie* followed by and *C. albicans* that showed inhibition zone of fungal growth with diameter equal 28 mm.

In addition, the growth of *A. niger* and *A. fumigatus* was inhibited when inoculated in contact with this ethanolic extract and produced inhibition zone with diameter equal 26 mm, while in case of *P. italicum* diameter of inhibition zone was 25mm. Other fungal test organisms showed mixed results by ethanolic extract of *S. aromaticum* Flowers under the experimental conditions.

3.2. Separation and purification of the most active antimicrobial compound

The ethanolic extract of *C.* verum and *S. aromaticum* were subjected to column chromatography with silica gel. Among the 75 fractions collected from *C. verum* and 80 from *S. aromaticum*, the Fraction number (10) from ethanolic extract of *C. verum* was found to be the most active fraction that inhibited the growth of tested microorganism's strains (Figure 2).

While, fraction number (13) from ethanolic extract of *S. aromaticum* was found to be the most active fraction that inhibited the growth of tested strains. (Figure 3). Both of fraction no. (10) and (13) showed a single band on the TLC plate This indicates that each of them contains only single compound.

Fig. (2). Effect of fraction number (10) on growth of *A. niger*, *C. albicans* and *P. marneffie.*

Fig. (3). Effect of fraction number (13) on growth of *A. niger*, *C. albicans* and *P. marneffie.*

3.3. Describe of the pure compounds by IR, NMR, mass spectroscopies and ^{13}C for fraction number (10) (compound A)

IR Spectroscopy

IR spectroscopy is clear and indicates the appearance of hydroxyl group (OH) by band at 3402cm-1and also the presence of alkane (–CH) stretching band or (CH2) at 2952 cm-1 This is an indicative for a compound with an aliphatic nature with a hydroxyl group (alcoholic hydroxyl groups).

Nuclear Magnetic Resonance (NMR) spectroscopy

To confirm the identity of compound (A), the ^{1}H NMR was recorded in dimethyl sulfoxide (DMSO-d6). ^{1}H NMR spectrum (Figure 7) displayed many multiple signalsin the region of oxygenated protons between δ3.03 and 5.15 ppm indicating mostly sugar moiety. Also, the downfield shifted signals at δ6.57, 6.19 and 5.29 ppm are indicative for the anomeric protons. The three downfield shifted signals are indicative for a three sugar units connected through glycosidic linkages and hence indicating the presence of trisaccharide.

According to the ^{13}C NMR spectrum (Figure 8), compound (A) exhibited eighteen carbon signals; all of them are of sp3hybridization. The three down field shifted carbons δ92.7, 97.4 and 102.5 ppm are representative for the three anomeric carbons. The remaining fifteen carbons have chemical shifts between δ61.8 and 82.4 ppm indicating all of them

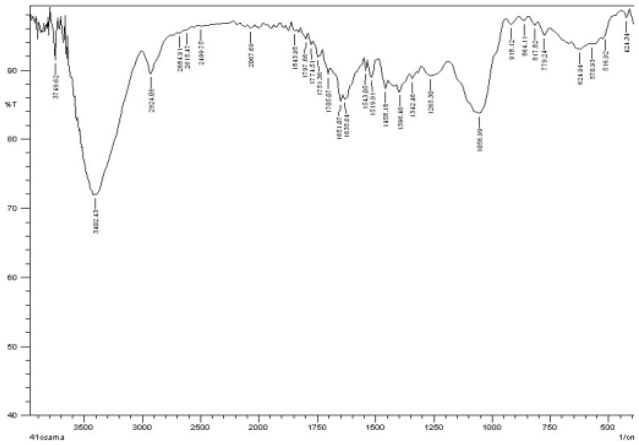


Fig. (4). IR spectrum of compound (A)

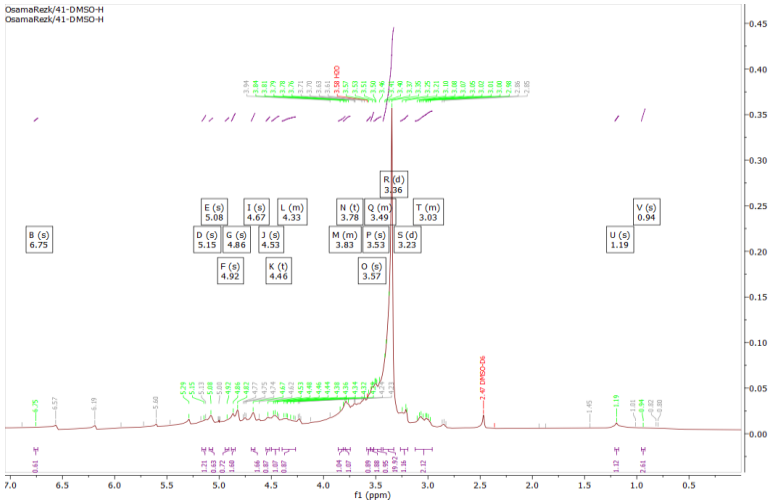


Fig. (5). ^{1}H NMR (500 MHz, CDCl3) spectrum of compound (A).

are oxygenated carbons. These information are a rather confirmation of a sugar moiety consisting of a three units.

Mass Spectroscopy

Compound (A) showed molecular ion peak at m/z504. The molecular weight of (A) was then established as 504 Dalton and the corresponding molecular formula as C18H32O16, bearing three double bond equivalents (DBE).

Based on the above revealed chromatographic and spectroscopic data, compound A was identified as: (3R,4S,5S,6R)-2-(((2S,3S,4S,5R)-3,4-dihydroxy-2,5bis(hydroxymethyl)tetrahydrofuran-2-yl)oxy)-6((((3R,4S,5R,6R)-3,4,5 trihydroxy-6(hydroxymethyl)tetrahydro-

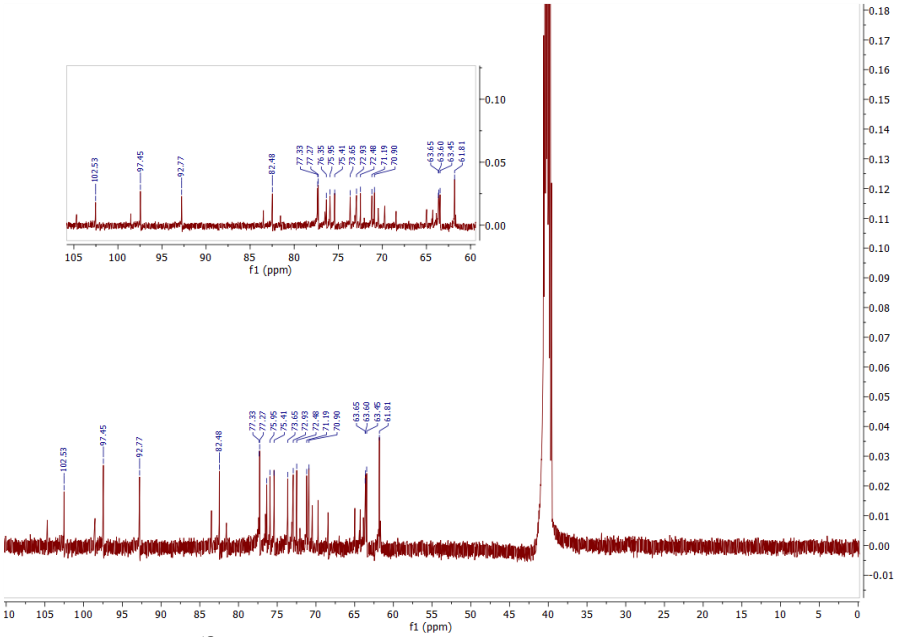


Fig. (6). ^{13}C NMR (125 MHz, CDCl3) spectrum of compound (A).

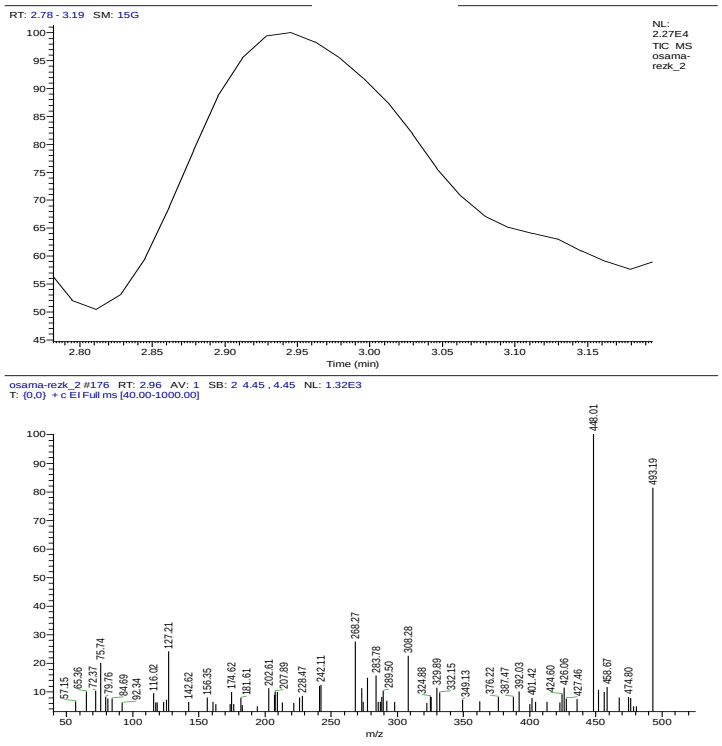


Fig. (7). Mass spectroscopic analysis of compound (A).

2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-3,4,5-triol which has the trivial name, raffinose .

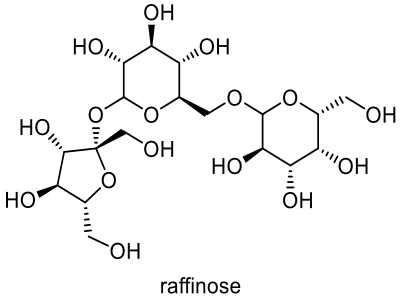


Fig. (8). Raffinose (Compound A).

3.4. Description of the pure compounds by IR,NMR, mass spectroscopies and ^{13}C for fraction number (13) (compound B)

IR Spectroscopy: IR spectroscopy is clear and indicates the appearance of hydroxyl group (OH) by band at 3418 cm-1and also the appearance of carbonyl group (C=O) at 1696cm-1mostly of a carboxylic acid group. The presence of olefinic bond can be detected by stretches at 3039 cm-1While band at 2952 cm-1is indicative for the presence of alkane (–CH) stretching band or (CH2).

Nuclear Magnetic Resonance (NMR)Spectroscopy

The ^{1}H NMR spectrum for compound (B) was recorded twice in two different deuterated solvents.

In dimethyl sulfoxide (DMSO-d6)

^{1}H NMR spectrum (Figure 10) displayed one highly downfield shifted broad singlet signal at δ12.02 mostly for a carboxylic group. Another triplet signal was displayed at δ5.16 which can be assigned to olefinic proton while the doublet signal at δ4.29is corresponding to a hydroxyl group. Another two signals appeared as doublets of doublet were resonating at δ3.00and δ2.75 ppm assigned to an oxy-methine and another de-shielded sp3methine. Multiple signals were observed between δ0.86-1.94 integrated for 44 proton which can be classified as follow: seven singlet methyls, ten sp3 methylene and three sp3methines. This is matching with the proposed molecular formula.

In chloroform (CDCL3):

The ^{1}H NMR spectrum (Figure 11) of compound (B) in chloroform displayed a similar pattern to that of DMSO-d6 except for the disappearance of the two signals corresponding to the acidic protons. It is noteworthy to refer herein that it is mandatory for acidic protons to be resolved in each measurement. So, there are no contradictions between the two measurements as the remaining protons of the compound are well resolved in both of them. This is a further confirmation of proposed structure.

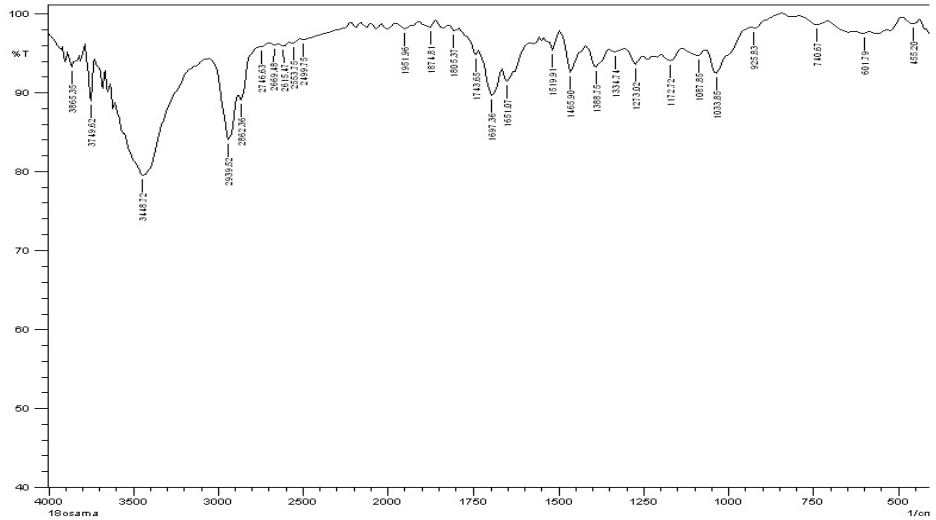


Fig. (9). IR spectrum of compound (B)

According to the ^{13}C NMR spectrum (Figure 12), compound B exhibited thirteen carbon signals, among them three sp2 and twenty sevensp3carbon atoms. The threesp2 carbon atoms are including one carboxylic carbon at δ179.2 ppm and the remaining two are constituting an olefinic bond (122.7 and 143.6 ppm). The twenty sevensp3carbon atoms are classified as follow: one oxy-methine at δ79.1 ppm, ten methylene groups, three methines and seven methyl groups.

Mass Spectroscopy

Ccompound B showed a molecular ion peak at m/z 456 followed by expulsion of (OH) group so that a fragment ion peak was available at m/z439 and water molecule was expelled so that fragment ion peak was available at m/z439.The molecular weight of (B) was determined as 456 Dalton and the corresponding molecular formula as C30H48O3, which bears seven double bond equivalents (DBE).

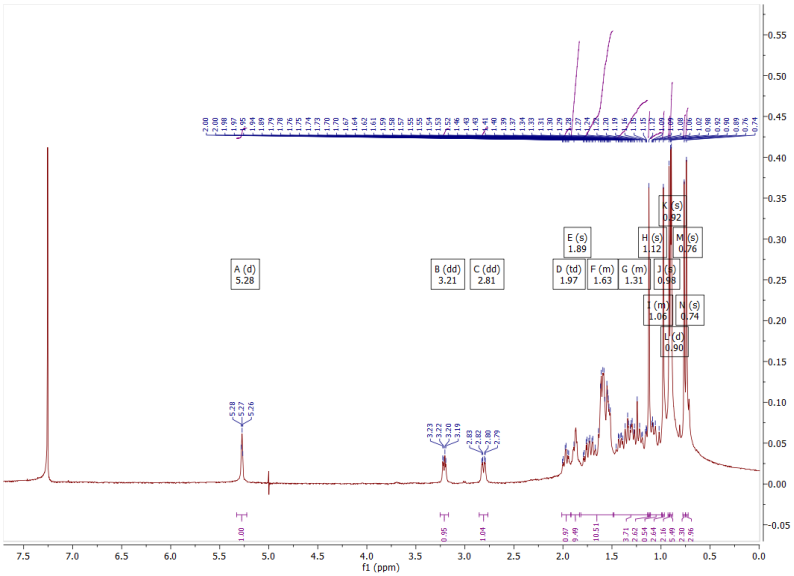


Fig. (10). ^{1}H NMR (300 MHz, DMSO-d6) spectrum of compound (B).

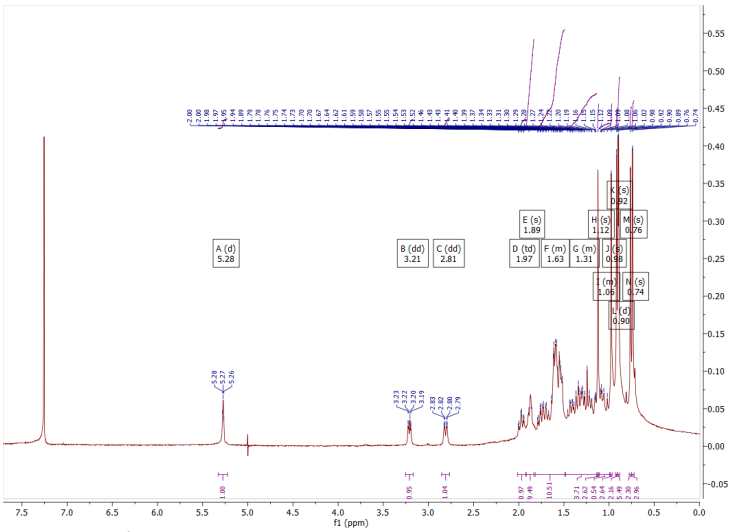


Fig. (11). ^{1}H NMR (500 MHz, CDCl3) spectrum of compound (B).

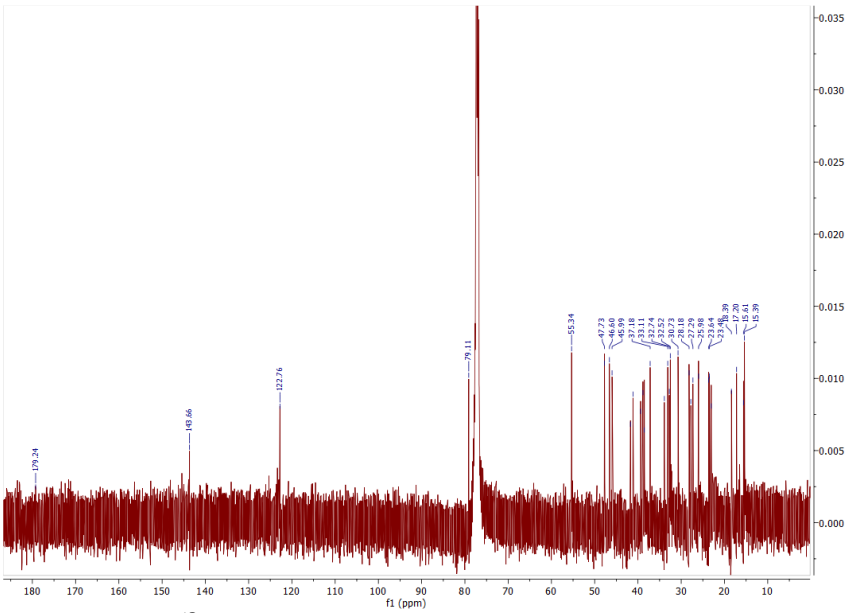


Fig. (12). ^{13}C NMR (125 MHz, CDCl3) spectrum of compound (B).

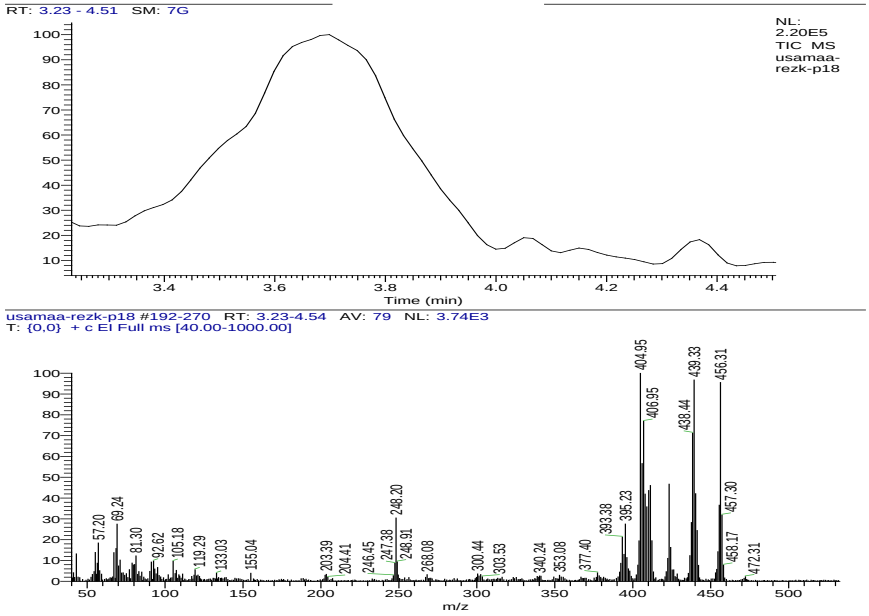


Fig. (13). Mass spectroscopic analysis of compound (B).

Based on the characterizations by IR, NMR, mass spectroscopies and ^{13}C as described above, we suggest that this compound to be (4aS,6aS,6bR,8aR,10S,12aR,12bR,14bS)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylic acid which has the trivial name, oleanolic acid**.**

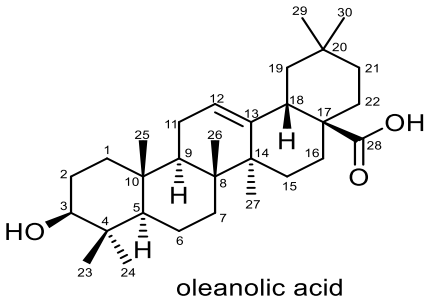


Fig.(14). Oleanolic acid/ Compound B).

3.5. Determination of MIC for the most active antimicrobial compound:

Ccompound (A) was tested against three fungal strains (*C. albicans*, *A. niger* and *P. marneffie*). the MIC was 12.5 µg/ml in case of *C. albicans* While, the MIC was 25 µg/ml in case of; *A. niger* and *P. marneffie*.

Furthermore, the MIC of compound (B) was 12.5 µg/ml for *Candida albicans* and 50 µg/ml for *A. niger*, while 25 µg/ml was the MIC compound (B) in case of *P. marneffie*.

Table (3): Determination of the MIC of the most active antimicrobial compounds against certain fungi

Active compounds	Test microorganisms		
	C. albicans	*A. niger*	*P. marneffie*
	Concentration of active compound (µg/ml)		
A	12.5	25	25
B	12.5	50	25

Table (4): showed the IC_{50} for both compound A and B against the carcinoma cell lines.

Carcinoma cell lines	Compound A IC_{50} (µg/ml)[1]	Compound B IC_{50} (µg/ml)[2]
WI-38 cells	>500	78.1 ± 5.6 *
A-549 cells	>500	27.2 ± 1.6*
MCF-7 cells	>500	53.3 ± 3.4*
HepG-2 cells	>500	19.7 ±1.2*

3.7.Antioxidant activity by Compound A and B

An antioxidant test was done for both compound (A) and (B) and the results showed the following : both compound (A) and (B) reveal superior DPPH scavenging activities with IC_{50} of 15.1 ± 0.86 and 25.9 ± 1.32 µg/ml, respectively (Figure 15)

Finally in this research many important results were concluded; the strongest extract in terms of effectiveness against most of the fungi tested was the ethanolic extracts specially the ethanolic extract of *C. verum* and *S.aromaticum*, The most sensitive fungal strain to this ethanolic extract were *A. niger* ,*C. albicans*, and *P. marneffie*. This conclusion was in agreement with (**Ayyasamy et al., 2012)** [12] who reported that better inhibitory potential was obtained using ethanolic extract of *p. florida*.

The results of the present study indicate that ethanolic extracts of *C. verum* and *S. aromaticum* exhibited the highest antifungal activity against tested fungi compared to other solvents (water, methanol, acetonitrile and ethyl acetate). This is not compatible with (**Mohamed & El-Hadidy., 2008)** [13] who reported that compounds extracted from crude extract of *comos caudatud* with solvents with intermediate polarity ethyl acetate had the highest inhibition.

In our study, the ethanolic extract of *C. verum* Bark showed high antifungal activity against all test fungal species, it inhibit growth of *P. marneffie* showing 36 mm diameter of inhibition zone followed by 32 mm diameter of inhibition zone in case of *A. fumigates* and *C. albicans*. (The sentence has been removed after this paragraph)

This result tends to agree with the investigation studied by (**Al-Hakami et al.,**

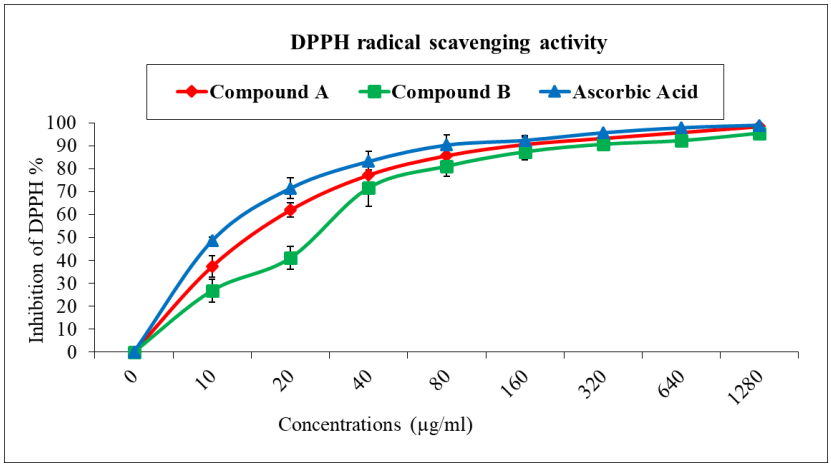


Fig. (15). DPPH radical scavenging of Compound A, Compound B and Ascorbic acid.

2016) [14] who isolated wide spectrum antifungal agents from *C. verum* Bark inhibited the growth *C. albicans, P. marneffie* and *A. fumigates*.

In this study, The ethanolic extract of *S. aromaticum* flowers produced inhibition zone with diameter 35 mm on *P. marneffie* followed by *C. albicans* (that showed) inhibition zone of fungal growth with diameter equal 28 mm.

In addition, the growth of *A. niger* and *A. fumigatus* was inhibited when inoculated in contact with the ethanolic extract of *S. aromaticum* flowers and produced inhibition zone with diameter equal 26 mm, while in case of *P. italicum* diameter of inhibition zone was 25mm.

Other fungal test organisms showed mixed results by ethanolic extract of *S. aromaticum* Flowers under the experimental conditions. This result was in the same line with those reported by (**Taguchi et al., 2005)** [15], assessed anti *C. albicans* activity of *S. aromaticum*. They found that oral intake of clove oil *(S. aromaticum*) may suppress the overgrowth of *C. albicans* in the alimentary tract including oral cavity.

In the present study, two active compounds were obtained from the *C. verum* and *S. aromaticum* extracts and were characterized using IR, NMR, and Mass spectrometric analysis. The first compound (A) isolated from *C. verum* Bark was identified as (raffinose) with high antimicrobial activity against tested microorganisms. The MIC of compound (A) was 12.5 µg/ml in case of *C. albicans* and 25 µg/ml in case of *A. niger* and *P. marneffie*. This result tends to agree with the investigation studied by (**Kim et al., 2016)** [16]. Who suggest that raffinose effectively, It inhibits grows of gram-positive, gram-negative bacteria and some fungi such as *P. aurantiogriseum.* But this is not compatible with (**Chirife et al., 1983)** [17] Who have demonstrated throgd their experiments that raffinose does not have any antimicrobial properties.

In the present study, the second compound (B) isolated from *S. aromaticum* flowers was identified as (oleanolic acid) with MIC equal 12.5 µg/ml in case of *C. albicans* and 50 µg/ml in case of *A. niger* while 25 µg/ml was the MIC of compound (B) in case of *P. marneffie*. This compound (B) has free hydroxyl group and three free, these free hydroxyl group may have high antimicrobial activity against tested fungal species.

This result was in line with those reported by (**Aslam et al., 2009)** [18] who isolated an antimicrobial compound from the roots of chickpea, Cicer spp. and demonstrated that the free hydroxyl groups in the isolated antimicrobial agent were responsible for the activity against both bacteria and fungi.

In this study compound (B) (oleanolic acid) showed high inhibitory activity against Breast,Lung and Hepatocellular carcinoma cells ,but no effect by compound (A) against A-549 cells, MCF-7 cells and HepG-2 cells.

These result was agree with those of (**Wang et al., 2013)** [19] who demonstrated that oleanolic acid inhibit the growth of transplanted tumour in mice and proliferation of liver hepatocellular cells (HepG2).

Furthermore, (**Amara et al., 2016)** [20] recorded that, oleanolic acid had anti tumar activity against breast cancer.

In addition, (**Zhao et al., 2015)** [21] found that oleanolic acid inhibit the multiplication of lung cancer cells after treatment with sixty g/mL of oleanolic acid for eight h.

In the present investigation An antioxidant test was done for both compound (A) and (B) and the results showed the following: both compound (A) and (B) reveal superior DPPH scavenging activities with IC50 of 15.1 ± 0.86 and 25.9 ± 1.32 µg/ml, respectively.

This result was in line with those reported by (**Gao et al., 2009)** [22] who revealed that oleanolic acid from

Ligustrum lucidum was shown to decrease the malonaldehyde (MDA) level and increase superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) activities in alloxan induced-diabetic rats.

CONCLUSION

In the current study, we performed several phytochemical investigations of some plants including different plant parts. This study demonstrated that ethanolic extracts of *C. verum* and *S. aromaticum* possessed antimicrobial and antitumor activities thus, ethanolic extracts of *C. verum* and *S. aromaticum* can be used as a potential source of antimicrobial and antitumor compounds. However, more investigations are needed to exploit the therapeutic use of these extracts.

Acknowledgements

Many thanks to Dr. Sayed Abdelkariem, Researcher at the Regional Center for Mycology and Biotechnology, Al Azhar University, for his help, support and continuous encouragement, as well as all the members of The Regional Center for Mycology and Biotechnology Al-Azhar University.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Osman MY, Taie HA, Helmy WA, Hassan Amer H. Screening for antioxidant, antifungal, and antitumor activities of aqueous extracts of chamomile (Matricaria chamomilla). Egyptian Pharmaceutical J. 2016; 15(2): 55-61.

[2] Horbach R, Navarro-Quesada AR, Knogge W, Deising HB. When and how to kill a plant cell: infection strategies of plant pathogenic fungi. Plant Physiol J. 2011; 168(1): 51-62.

[3] Nii-Trebi NI. Emerging and neglected infectious diseases: insights, advances, and challenges. BioMed Intern Res. 2017; 13(1): 1-15.

[4] Saadabi AM. Antifungal activity of some Saudi plants used in traditional medicine. Asian Plant Sci J. 2006; 5(5): 907-909.

[5] Bokhari FM. Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia .Mycopath J. 2009; 7(1): 51-57.

[6] Andleeb S, Alsalme A, Al-Zaqri N, Warad I, Alkahtani J, Bukhari SM. In-vitro antibacterial and antifungal properties of the organic solvent extract of Argemone mexicana L. King Saud University Science J. 2020; 32(3): 2053-2058.

[7] Hou L, Shi Y, Zhai P, Le G. Antibacterial activity and in vitro anti-tumor activity of the extract of the larvae of the housefly (Musca domestica). Ethnopharmacol J. 2007; 111(2): 227-231.

[8] Zhao X, Li RJ, Chuang ZH, Zhang J, Cheng-hua HE, Zheng Y, Wen-da W, Zhang H. Separation and purification of deoxynivalenol (DON) mycotoxin from wheat culture using a simple two-step silica gel column chromatography. Integrative Agriculture J. 2016; 15(3): 694-701.

[9] Soliman W, Ibrahim M, El Baz H. In vitro Evaluation of Syzygium aromaticum L. Ethanol Extract as Bio control Agent against Postharvest Tomato and Potato Diseases. Egypt Bot J. 2019; 59(1): 81-94.

[10] Suleiman WB. In vitro estimation of superfluid critical extracts of some plants for their antimicrobial potential, phytochemistry, and GC–MS analyses. Ann Clin Microbiol and Antimicrob J. 2020; 19(1): 1-2.

[11] Suleiman WB, Helal EE. Chemical constituents and potential pleiotropic activities of Foeniculum vulgare (fennel) ethanolic extract; in vitro approach. Egypt Chem J. 2022; 65(7): 5-6.

[12] Menaga D, Mahalingam PU, Rajakumar S, Ayyasamy PM. Evaluation of phytochemical characteristics and antimicrobial activity of Pleurotus Florida mushroom. Asian pharmaceutical and clinical Research J. 2012; 5(4): 102-106.

[13] Mohamed NH, EL-Hadidy AM. Studies of biologically active constituents of Verbascum eremobium Murb. and its inducing resistance against some diseases of cucumber. Egypt phytopathol J. 2008; 36(1): 133-150.

[14] Al-Hakami EE, Al-Kassari OA, Al-Helali MF. Antimicrobial activity of Cinnamon barks (Aqueous and Ethanolic Extracts) and some antifungals against Candida albicans Isolated from Oral Candidiasis in Leukemia patients. PSM Microbiol J. 2016; 1(1): 18-25

[15] Taguchi Y. Ishibashi H, Takizawa T, Inoue S, Yamaguchi H, Abe S. Protection of oral or intestinal candidiasis in mice by oral or

intragastric administration of herbal food, clove (Syzygium aromaticum). Nippon Ishinkin Gakkai Zasshi J. 2005; 46(1): 27-33.

[16] Kim HS, Cha E, KimY, Jeon YH, Olson BH, Byun Y, Park HD. Raffinose, a plant galactoside, inhibits Pseudomonas aeruginosa biofilm formation via binding to LecA and decreasing cellular cyclic diguanylate levels. Sci Rep J. 2016; 6(1): 18-53.

[17] Chirife J, Herszage L, Joseph A, Kohn ES. In vito study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. Antimicrob Agents Chemother J. 1983; 23(5): 766-773.

[18] Aslam N, Stevenson PC, Kokubun T, Hallm DR. Antibacterial and antifungal activity of cicerfuran and related 2-arylbenzenofurans and stilbenes. Microbiol Res. 2009; 164(2): 191-195.

[19] Wang X, Bai H, Zhang X, Liu J, Cao P, Liao N, Zhang W, Wang Z, Hai C. Inhibitory effect of oleanolic acid on hepatocellular carcinoma via ERK–p53-mediated cell cycle arrest and mitochondrial-dependent apoptosis. Carcinogenesis J. 2013; 34(6): 1323-1330.

[20] Amara S, Zheng M, Tiriveedhi V. Oleanolic Acid Inhibits High Salt-Induced Exaggeration of Warburg-like Metabolism in breast cancer cells. Cell Biochem Biophys J. 2016; 74(3): 427- 434.

[21] Zhao X, Liu M, Li D. Oleanolic acid suppresses the proliferation of lung carcinoma cells by miR-122/Cyclin G1/MEF2D axis. Mol Cell Biochem J. 2015; 400(1): 1-7.

[22] Gao D, Li Q, Li Y, Liu Z, Fan Y, Liu Z, Zhao H, Li J, Han Z. Antidiabetic and antioxidant effects of oleanolic acid from Ligustrum lucidum Ait in alloxan-induced diabetic rats. Phytother Res. 2009; 23(9): 1257-1262.

تنقيب وتوصيف مضادات الفطريات المستخرجة من بعض النباتات (معملياً)

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الملخص

تم استخدام خمسة مذيبات استخلاص (ماء ، ميثانول ، إيثانول ، أسيتونيتريل ، أسيتات إيثيل) لاستخلاص مضادات الميكروبات من 13 نباتًا مختلفًا بما في ذلك أجزاء نباتية مختلفة مقابل 12 نوعًا من الفطريات. أظهرت النتائج أن أقوى مستخلص من حيث الفاعلية ضد معظم الفطريات المختبرة هو المستخلص الإيثانولي من لحاء القرفة وزهور القرنفل حيث أظهرا أقوى تأثيرلهما ضد تلك الفطريات (كانيدا ألبيكانز، أسبيرجيلس نايجر ، بنسيليوم مارنيفيى) . تم تنقية هذين المركبين باستخدام هلام السيليكا(داخل عمود الفصل) وتنقيتهما بواسطة كروماتوجرافيا الطبقة الرقيقة وتم توصيف تلك المركبات النقية باستخدام تحليل الأشعة تحت الحمراء والرنين المغناطيسي النووي والتحليل الطيفي الشامل متبوعًا بتحديد الحد الأدنى للتركيز المثبط ، تم التعرف على المركب الأول (أ) من المستخلص الإيثانولي للقرفة وتم تعريفه بأنه (الرافينوز) وكذلك تم التعرف على المركب الثانى من المستخلص الإيثانولى للقرنفل وتم تعريفه على أنه (حمض الأولينوليك) ، بالإضافة إلى ذلك ، تم إجراء السمية الخلوية لكلا المركبين النقيين ، حيث أظهر المركب (ب) نشاطًا مثبطًا عاليًا ضد جميع الخلايا السرطانية المختبرة ، لكن المركب (أ) لم يكن له أي نشاط مضاد للأورام. كما تم إجراء اختبار مضاد للأكسدة لكل من المركب (أ) و (ب) وأظهرت النتائج أن كلا المركبين (أ) و (ب) لهما نشاط مضاد للأكسدة. في الختام ، تم استنتاج أن للمركب (ب) نشاط مضاد للفطريات ومضاد للأورام ومضاد للأكسدة بينما كان للمركب (أ) نشاط مضاد للفطريات ومضاد للأكسدة ولكن لم يكن له أى فاعلية مضادة للأورام ضد جميع الخلايا السرطانية المختبرة