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Antifungal susceptibility testing of *Malva parviflora* yield extracts, against *C. albicans* and *A. fumigatus*

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ABSTRACT

The increasing on the incidence of drug-resistant pathogens has drawn the attention of researchers towards screening the antimicrobial action of plant-derived compound. This study aimed to determine the antifungal activity of *Malva parviflora* extracts, which is widespread and used by the general population in Libya. Extraction was carried out using a cold maceration method by several different polarity solvents (petroleum ether, ethyl acetate, methanol and distilled water). Their total-phenolic and flavonoid content were assessed. The content of total phenolic and flavonoids were found to be the highest in petroleum ether fraction extract (119.558mg GAEg-1) and (227.029mg RU g-1) respectively. The antioxidant activity was estimated using DPPH as free radical scavenging and sugar reducing power. The methanol fraction extract displayed the highest IC50 (50 mg/ml). The results indicate that the increase in the percentage of DPPH free radical inhibition is directly proportional with the increase in the extract concentration. Reducing power increased with the increase on extracts concentration. *M. parviflora* leave extracts were tested against fungal species Aspergillus. *fumigatus* and *Candida. albicans* using antifungal susceptibility assay of yeast and filamentous fungi. Extracts had strong antifungal activity against *C. albicans* and *A. fumigates*. Petroleum ether fraction extract has the strongest antifungal activity against *A. fumigatus* with 94.96% percentage of inhibition. The percentage of inhibition is 96.48% was shown against *C. albicans* for aqueous extract.

KEYWORDS: *Malva parviflora* extracts, *C. albicans, A. fumigatus,* Antifungal

INTRODUCTION

The search for effective, safe and reliable sources of medication has been an ever driving force to develop new forms of pharmaceuticals to treat different diseases. Since the mid Fifties of the 20th century, the phytochemicals produced from plants have been used to develop antimicrobial drugs that are widely used after being strictly tested in humans. Clinical microbiologists are very much interested in phytochemicals of plant origin to develop new drugs (1**).** *Candida* infections of the mouth, throat, and esophagus which is caused by the *yeast Candida*, which is the most common types of fungi that cause serious life threatening fungal infection. *Aspergillosis* is a disease caused by *Aspergillus spp*. It most often affects people with lung disease or a weakened immune system. *Candidiasis* is a disease caused by Candida spp when candida enters the blood system, it is called *invasive candidiasis*. Plants have been used as medicines for more than 5000 years, as a source of antibiotics, cardio protective, analgesics, antineoplastic, among others (2). Medicinal plants are used for antibacterial, antifungal, and antiviral activities in many parts of the world. Major pharmaceutical companies look for new therapeutic agents from alternative sources like medicinal plants.

Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, various studies have expected to explain the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials (3). Libya has a strategic location along the southern coast of the Mediterranean and extends Deep South into the Sahara Desert. The total area of around 1,759,540 km2, lies between latitudes 19o and 34oN, and longitudes 9o and 26oE. The climate is typically Mediterranean. The well-known and the largest plant area in Libya is Jabal Al Akhdar located in the north eastern part of the country, Jabal Nafusa, Jabal Al Owainat which are located in the North West. Libya is rich with medicinal plants that are used in traditional medicine. The Libyan flora contains 2118 plant species that belong to 864 genera and 161 families. According to literature, there are 450

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medicinal plants growing in Libya and 208 are recognized and extensively used by people (1).

M. parviflora has been used by people from ages in different ways and for many purposes. Mallow leaves are edible raw in salads and they impart a slightly mucilaginous texture. The leaves are commonly cooked and eaten like spinach; they can also be added to soup and it has been used as coloring agent. The whole plant was used as emollient and pectoral and the seeds as demulcent (2). The widespread of medicinal plants in Libya could provide the basis for an alternative source to control and treat infectious diseases and reduce the use of conventional drugs, minimizing their undesirable side effects. To reach that goal solid and detailed research of such plants is

mandatory in order to understand the composition of their constituents and their detailed effectiveness along that line. *M. parviflora* is widely used traditionally for different purposes and the detailed study of such plant is the driving force in this study. This study will focus on of the chemical composition; antioxidant and antimicrobial activity of *M. parviflora* leaves *yeast* and *filamentous* fungi.

MATERIAL AND METHODS:

COLLECTION OF *M. PARVIFLORA* LEAVES FROM PLANT:

The fresh plant of M. parviflora was collected from the city of Sabha, south of Libya at the end of December 2018. Leaves were separated from their stem, washed with water and then shade dried at room temperature for two weeks. After that, they were hand crushed to get a homogeneous fine powder and then kept in airtight container in a dry place at room temperature until used to prepare the different extractions. The reason for choosing the south of Libya as a collection location of the raw material is because of very dry weather in the area which helped drying in a relatively very short time with no spoilage of the material.

CHEMICALS, SOLVENTS AND REAGENTS USED IN THIS STUDY:

The following chemicals have been used at a certain stage of the current study. They include: Petroleum ether (40-60 0C), ethyl acetate HPLC grade, methanol (99%) HPLC grade, distilled water, lead acetate, potassium dichromate, chloroform, ammonia, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, Mayer's reagent, folin ciocalteus reagent, sulfuric acid, ferric chloride, potassium ferric cyanide, diphenyl-1-picrylhydrazyl, hydrochloric acid, sterile 0.9% normal saline No.0.5 McFarland standard, Phosphate-buffered saline and 20% (v/v) Tween 80. The role of each of these chemicals will be discussed later.

PREPARATION OF EXTRACTS:

In order to investigate the bioactive compounds that are responsible for the anti-oxidant and the anti-microbial activities of the plant powder, five extracts were prepared.

PETROLEUM ETHER FRACTION EXTRACT:

In order to separate the non-polar compounds that are present in the sample, petroleum ether was used as a solvent, where 400g of powder was extracted by cold maceration method with ratio (10g powder: 100ml solvent). The powder was extracted twice using petroleum ether with boiling points ranging between (40-60oC) for four days. The extract was filtered and the filtrate was concentrated under reduced pressure by rotatory evaporator at 40ºC. The extract was weighted and stored in a refrigerator at 4ºC. In order to prepare another extract, the residual powder was oven dried for 1hour. The oven-dried powder in the above step was extracted for the second time using ethyl acetate to isolate the semi-polar components. First, 3liters of the solvent ethyl acetate were added to the powder and thoroughly mixed for two days and then the fourth liter was added. At the end of the fourth day, the mixture was filtered, dried, and the extract was weighed and stored in a refrigerator at 4oC. After that, the powder was dried again by an oven for 2 hours for the preparation of next extract.

METHANOL FRACTION EXTRACT:

The dried powder was macerated by methanol to isolate the polar compounds that are retained in the powder. Three liters of methanol were added to the powder in a flask, mixed well for two days and after that one liter of methanol was added. The extract was filtered and the filtrate was dried, weighed and stored in a refrigerator in the same temperature as above.

AQUEOUS EXTRACT:

To simulate the use of the plant by an ordinary person, an aqueous extract was prepared. A 300g sample of the powdered leaves was placed in a flask with 3liters of distilled water and left for 2days. The mixture was then filtered, frozen and lyophilized by using freeze drying.

Methanol Crude Extract:

The leaves powder (200g) was extracted by cold maceration method with ratio 10g powder: 100ml solvent. The powder was extracted twice using 99% methanol for four days and then it was filtered. The filtrate was concentrated by vacuum evaporator. The weight of the dry extract was calculated, and the extract was stored in refrigerator at 4ºC. The methanol solvent was used to separate the non-polar, semi-polar and polar components from the leaves powder. In each case the extract yield was determined.

DETECTION OF FLAVONOID:

In order to investigate the presence of flavonoid, lead acetate test was used. here The alcoholic solution of extract was treated with 10% lead acetate solution, the formation of bulky white or yellow color precipitate was taken as a positive test for flavonoid (4).

DETECTION OF ALKALOIDS:

Mayer's reagent test was used, where an alcoholic solution of the extract was treated with 5ml of diluted HCl 0.2N, and then filtered. The filtrate was tested with Mayer's reagent. A white or creamy precipitate indicates the test as positive (5).

DETECTION OF TANNINS:

In order to detect the tannins in the extract, Potassium dichromate test was used, where a few drops of 10% K2Cr2O7 were added to an alcoholic solution of the extract and observed for yellowish brown precipitate, which is evinced the presence of tannins (6).

DETECTION OF SAPONINS:

The Forth test was carried out to detect the presence of saponins. The crude extract was treated with 20ml of distilled water and shacked in graduated cylinder for 15minutes, 1cm layer of foam indicates the presence of saponins (7).

DETECTION OF QUINONS:

To detect the presence of quinons the Sulphuric acid test was used. Here the alcoholic extract was treated with concentrated H2SO4, a pink color considered as a positive result (8).

DETECTION OF TERPENOIDS:

The extract placed in a test tube was treated with chloroform, then concentrated H2SO4 was added. A violet color implied the presence of terpenoids such experiment is known as Salkowski's test (8).

DETECTION OF COUMARINE:

In order to detect coumarine Ammonia solution test was used. The alcoholic solution of the extract was treated with drops of ammonia solution. The formation of greenish blue fluorescence indicates the presence of coumarins (9).

DETECTION OF ANTHRAQUINONE:

Borntrager's Test is usually used to verify the presence of anthraquinone. Here about five mg of the extract was boiled with 10% HCl for few minutes in a water bath. The mixture was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates the presence anthroquinones (8).

DETERMINATION TOTAL PHENOLIC CONTENT:

Total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method (10). Finally, the absorbance of the extract solution was measured at a wavelength of 765nm using UV-VIS spectrophotometer. The total phenolic content extracts were expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE /g).

DETERMINATION OF FLAVONOID CONTENT:

Total flavonoid content was estimated according to (11). The absorbance was measured at 510nm using UV-VIS spectrophotometer. Total flavonoid content of the extracts was expressed as milligrams of rutin equivalent per gram of extract (mg RE/g).

IN VITRO ANTIOXIDANT EVALUATION:

Plants contain a wide variety of free radicals scavenging molecules include phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity which possess the ability to scavenge the free radicals in human body. Free radical scavenging activity evaluations were carried out by the following methods.

DETERMINATION OF ANTIFUNGAL ACTIVITY:

In order to determine the antifungal activity of extracts, *A. fumigatus* ATCC 26933 obtained from American Type Culture Collection, Maryland, USA and Candida albicans MEN were used, these types were chosen because Candida albicans is commonly used as model organisms for fungal pathogens which is ranked as one of the most common groups of organisms that cause hospital-acquired infections, while Aspergillus fumigatus is one of the most common Aspergillus species to cause disease in individuals with an immunodeficiency.

HARVESTING *A. FUMIGATUS* CONIDIA:

Conidia of A. fumigatus were harvested in a safety cabinet Class II. 10ml Phosphate-buffered saline PBS-T [0.1% (v/v) Tween80] was used to wash the plates. Conidia were harvested by centrifugation at (2,056xg) for 5minutes at room temperature on a Beckmann GS-6 bench centrifuge. The supernatant was removed and the conidial pellet was washed twice in sterile PBS to remove excess Tween80 and re-suspended in sterile PBS. The concentration of conidia in the suspension was determined by counting with a haemocytometer.

C. ALBICANS STRAIN GROWTH AND HARVEST:

YEPD agar was prepared by dissolving Glucose (2% w/v), Yeast extract (1% w/v), and bacteriological peptone (2% w/v), in deionized water and autoclaving at 105 oC for 30 minutes.

For agar plates 2% (w/v) agar was added and autoclaved as described above. In some cases, erythromycin was added to the hand warm agar prior to pouring to control bacterial contamination. This was prepared by dissolving 5ml of a 5 mg/ml solution of erythromycin in DMSO when the agar solution was hand warm. Once in the agar solution, the plates were spread as per normal and stored at 4oC. All erythromycin supplemented plates were used within 3 days.

Susceptibility of *C. albicans* and *A. fumigatus* to Extracts:

C. albicans cells were grown to stationary phase in YEPD. Cells were counted by using Haemocytometer, and diluted to a density of 5 x 106 cells/ml. Cell suspensions of 100µl were added to each well of a 96 well plate row two was used control media. IC50 was determined as the lowest concentration of extract required to reduce growth by 50% relative to the growth of the control.

All experimental work for susceptibility testing of A. fumigatus to caspofungin was conducted according to the reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard with some modification is detailed in (5). To improve the reading all Aspergillus plates were shaken before reading the result. The optical density was recorded at 540 nm using a microplate reader (Synergy HT, Bio-Tek). The results will be expressed as percentage reduction in fungi viability compared to controls and concentration that gave the 50% inhibition (IC50) will be calculated by Probit analysis (12). The mean value will be calculated from three separate experiments.

RESULTS:

EXTRACTION YIELDS:

The extraction yield is a measure of the efficiency of solvent to extract the bioactive compounds from the original material. Extracts of *M. parviflora* were dried by rotatory evaporator and vacuum drier to allow maximum removal of solvents that was used in extraction methods. The solvents used in the present study were selected based on different polarity ranges. In term of chemistry, polar components would dissolve in polar solvents and non-polar substance will dissolve in non-polar solvents. Petroleum ether, ethyl acetate and methanol (in order of increasing polarity, respectively) were selected to enable the extraction and separation of a wide range of components that are

present in the M.parviflora powder. Application of different extraction solvents used in this study was an attempt to produce best separation of compounds in each extract. This was achieved by gradual isolation of compound groups following different solvent particles. At first, *M. parviflora* was macerated by methanol as preliminary step to isolate all compounds that are present in the powder (non-polar, semi-polar and polar) which is considered as a crude extract, then fractionation of compounds was done as follows: Powdered sample was first soaked in petroleum ether in order to extract out the non-polar compounds. The percentage of P.E. extract yield was based on the weight of dried and ground plant material. After that, the powder was soaked in ethyl acetate solvent to isolate the semi-polar compounds and then extracted by methanol, which is classified as a high polarity solvent. After employing a cold maceration method, the yields of *M. parviflora* leaves in different extractions are shown in Table (1).

Table 1: Extraction yield of *M.parviflora* extracts

Extract	Yield of Extraction % w/w
Methanol crude extract	9.75%
Petroleum ether fraction extract	1.54%
Ethyl acetate fraction extract	1.60%
Methanol fraction extract	8.03%

PHYTOCHEMICALS INVESTIGATION:

The medicinal properties of plants are due to the presence of different bioactive components. Ultimately, the goal in surveying plants for biologically active or medicinally useful compounds should be to isolate the one or more constituents responsible for a particular activity.

The different extracts of *M. parviflora* have been subjected to several qualitative tests for identifying the bioactive compounds.

The results revealed the presence of various phytoconstituents, which could be further involved in drug discovery and development. All extracts displayed the presence of the same phytochemicals such as flavonoid, alkaloid, terpenoid, quinone and tannins as shown in Table (2). The quantitative Analysis of Phytochemicals: Total phenolic were determined with respect to standard curve of gallic acid (r2= 0.9988). The content is found to be the highest in petroleum ether fraction extract (119.558mg GAEg-1) while ethyl acetate fraction extract showed lower concentration (87.555mg GAEg-1). The total phenolic contents of different extracts are presented in Figure (4). Antioxidant Assay by DPPH is used to measure the scavenging activity of different extracts, Figure (1) shows that a methanol fraction extract possesses the highest radical scavenging activity.

The efficiency of the sample in scavenging the DPPH radicals is expressed by IC50 value, which is defined as the inhibitory concentration at which 50% of free radicals are scavenged. The values of IC50 were calculated from the regression equations of linear graph. A small IC50 value implies a high antioxidant activity. The IC50 values of different extracts of *M. parviflora* and the L-ascorbic acid are shown in Table (3). The methanol fraction extract displayed the highest IC50 (50 mg/ml) in comparison to the petroleum ether extract IC50 (138.5 mg/ml). For DPPH method validity, the assay has been applied on the L-ascorbic acid as a positive control. The resulted IC50 was found to be 0.78mg/ml, which is the lowest value among all extracts. The results indicate that the increase in the percentage of DPPH free radical inhibition is directly proportional with the increase in the extract concentration.

.Table (2): Qualitative phytochemical evaluation of M.parviflora extracts

Plytochemical	Test	Methanol crude extract	Petroleum ether fraction extract	Ethyl acetate fraction extract	Methanol fraction extract	Aqueous extract
Flavonoid	Lead acetate	+ve	+ve	+ve	+ve	+ve
Alkaloid	Mayer's reagent	+ve	+ve	+ve	+ve	+ve
Saponin	Forth	-ve	-ve	-ve	-ve	-ve
Terpenoid	Salkowskis	+ve	+ve	+ve	+ve	+ve
Anthraquinone	Borntragers	-ve	-ve	-ve	-ve	-ve
Quinon	Sulphuric acid	++ve	++ve	++ve	++ve	++ve
Tannin	Potassium dichromate	++ve	++ve	++ve	++ve	++ve
Coumarin	Fluorescence	-ve	-ve	-ve	-ve	-ve

*+ve= low concentration., *++ve= moderate concentration,*-ve= absence

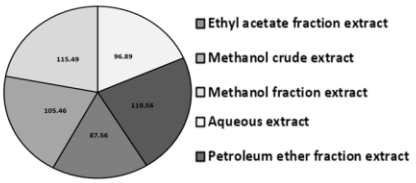
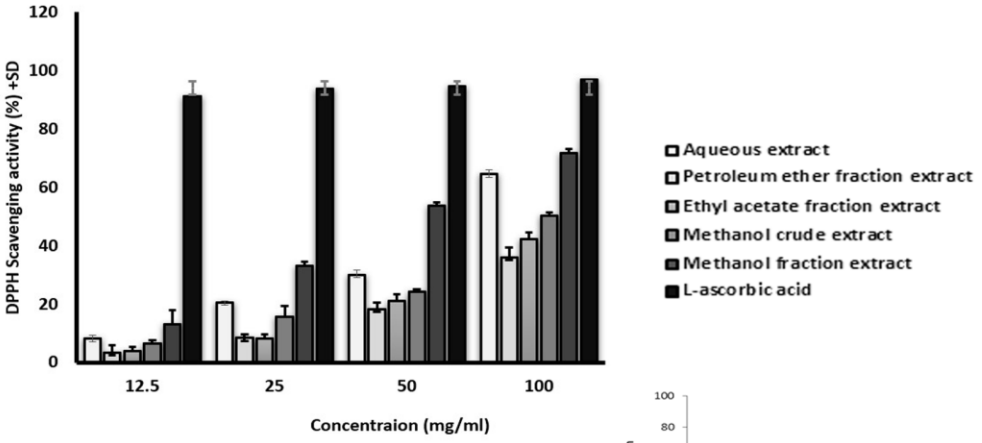


Figure 1: Total phenolic contents (mg/g) of different extracts



.Figure (2): Antioxidant activity of different M.parviflora extracts

Table 3. IC50 values for each extract of M. parviflora

Extract	IC_{50}(mg/ml)± SD
Methanol crude extract	100±0.57
Petroleum ether fraction extract	138.5±0.28
Ethyl acetate fraction extract	120±0.56
Methanol fraction extract	50±0.81
Aqueous extract	75±0.54
L-ascorbic acid	0.78±0.01

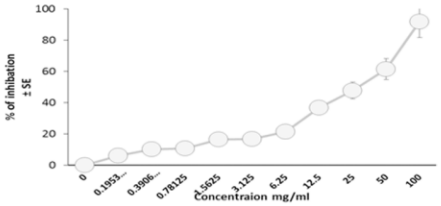


Figure 3: percentage of inhibition of methanol crude extract against A.fumigatus.

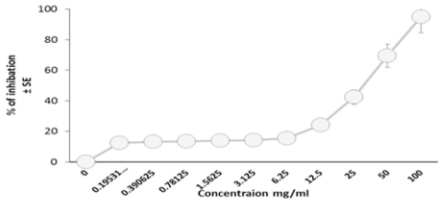


Figure 4: percentage of inhibition of petroleum ether fraction extract against A.fumigatus.

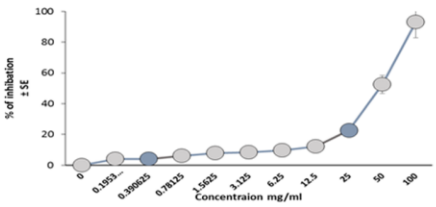


Figure 5: percentage of inhibition of ethyl acetate fraction extract against A.fumigatus.

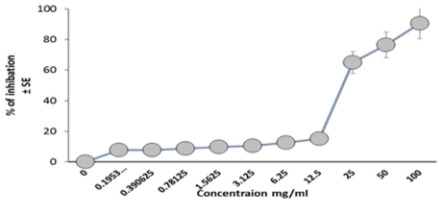


Figure 6: percentage of inhibition of methanol fraction .extract against A.fumigatus

ANTIFUNGAL ACTIVITY STUDY:

A. FUMIGATUS:

Figures (3, 4, 5, and 6) show the antifungal activity of all extracts of *M.parviflora* against *A.fumigatus* by determine the % of inhibition, using amphotericin B as positive control. The IC_{50} is measured for each extract in table (4).

The efficiency of the sample in scavenging the DPPH radicals is expressed by IC50 value, which is defined as the inhibitory concentration at which 50% of free radicals are scavenged. The values of IC50 were calculated from the regression equations of linear graph. A small IC50 value implies a high antioxidant activity. The IC50 values of different extracts of M. parviflora and the L-ascorbic acid are shown in Table (3). The methanol fraction extract displayed the highest IC50 (50 mg/ml) in comparison to the petroleum ether. MIC50 were calculated for each extract, the result show that all extract have an anti-fungal activity agains the strain used of *A. fumigatus*.

C. ALBICANS:

Figures (7, 6, 5, and 10) show the antifungal activity of all extracts of *M.parviflora* against *C.albicans* by determine the % of inhibition, using amphotericin B as positive control. The IC_{50} is measured for each extract in table (5).

Result shown that extracts of this plant have strong antifungal activity against *C. albicans* strain which used in this study.

Table 4. IC50 of extract against A. fumigatus

Extract	IC50 mg/ml±SD
Methanol crude extract	27.8766±1.23
Petroleum ether fraction extract	30.3579±2.12
Ethyl acetate fraction extract	47.2022±1.20
Methanol fraction extract	20.3228±1.23
Aqueous extract	28.0375±2.11
Amphotericin B	6.5297±2.10

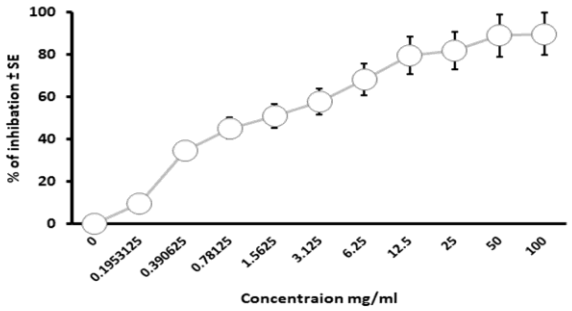


Figure 7: percentage of inhibition of methanol crude extract against C.albicans.

Table 5. IC50 extracts against C. albicans

Extract	IC50 mg/ml±SD
Methanol crude extract	1.3939±0.65
Petroleum ether fraction extract	1.4607±1.84
Ethyl acetate fraction extract	12.5682±2.33
Methanol fraction extract	0.9568±1.10
Aqueous extract	1.6669±1.89
Amphotericin B	0.1966±2.05

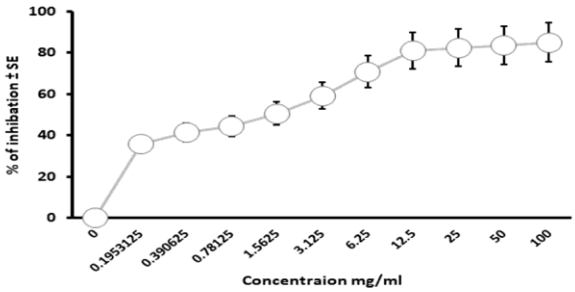


Figure 8: percentage of inhibition of petroleum ether fraction extract against C.albicans

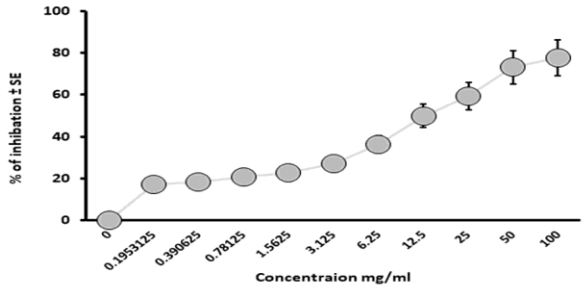


Figure 9,against C.albicans: percentage of inhibition of ethyl acetate fraction extract against C.albicans

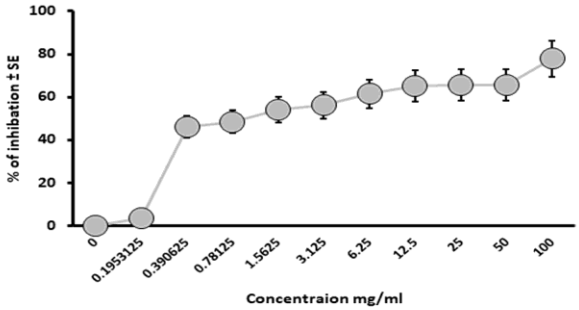


Figure 10: percentage of inhibition of methanol fraction extract ,against C.albicana.

DISCUSSION

The World Health Organization (WHO) estimates that about 80% of populations in developing countries use extensively traditional medicine, mainly of plant origin for health care. Although higher plants play a major role in drug manufacturing by providing principle active ingredients, medicinal plants still have to contribute significantly for that purpose (13). Since they are rich with secondary bioactive compounds that possess antimicrobial activity, as an alternative source for safe, cheap and effective treatment of infectious diseases. Medicinal plants are rich in a wide variety of secondary metabolites that possess antimicrobial properties and may serve as an alternative, effective, cheap and safe antimicrobial for treatment of microbial infection.

The medicinal value of the plant is due to the presence of various bioactive chemical constituents such as alkaloids, tannins, flavanoids and phenolic compounds. In present study, phytochemical screening of the *M. parviflora* showed the presence of most important bioactive compounds such as flavonoid, alkaloid and tannin. Recent reports showed that plant products including polyphenolic compounds and various plant extracts exhibit antioxidant and antimicrobial activities. In this study, the total phenolic contents were determined in all extracts of *M.parviflora*, The highest phenol content is found in petroleum ether fraction extract (119.558mg GAEg-1) compared with other extracts. Remarkable phenol content is found in methanol fraction extract (115.493 mg GAEg-1), followed by methanol crude extract (105.456 mg GAEg-1), (96.893 mg GAEg-1) is found in aqueous extract and the minimum amount is found in ethyl acetate fraction extract (87.555 mg GAEg-1). All extracts contain high amounts of total phenolic content.

The flavonoid synthesized by plants as a response to microbial infection, the findings of the present study explored the presence of broad range of flavonoids in different leave extracts. Petroleum ether fraction extract shows remarkably superior flavonoid content (227.029mg RU g-1). The antioxidant activity of the plant could be related to hydroxyl group due to their polar nature.

Aspergillus is the second most common pathogenic fungus after Candida. However, *A. fumigatus* and other species have emerged as important causes of invasive fungal infection in immunocompromised individuals and represents a major cause of morbidity and mortality in these individuals, reports showed that medicinal plants had antifungal activity due to presence of different secondary metabolites, researches they are looking for new antifungal agents to treat the fungal infections (14). Antifungal activity of the tested extracts of *M. parviflora* expressed broad-spectrum antifungal activity against the tested fungal pathogens. The percentage of inhibition and IC_{50} is measured for different extracts and compared with amphotericin that is used as positive control.

Petroleum ether fraction extract has the strong antifungal activity against *A. fumigatus* with 94.96% percentage of inhibition, and the rest of the extracts have similar results and the least effective is methanol fraction extract with 90.50% percentage of inhibition. IC_{50} is 20.32mg/ml for methanol fraction extract and it is the closet to amphotericin then the methanol crude extract, aqueous extract, petroleum ether extract and the least extract is ethyl acetate extract. These differences are could due to the difference in composition and percentage of bioactive compounds in each extract but in general the results are close and the differences are slight and all extract have strong antifungal activity which is in agreement with literature. The percentage of inhibition is 96.48% against *C. albicans* for aqueous extract and the least effective extract is ethyl acetate fraction extract with 77.81% percentage of inhibition. The methanol fraction extract has the lowest IC_{50} 0.95mg/ml which deal with the results of *A. fumigatus*.

All extracts of *M. parviflora* have strong antifungal activity, there are many factors affect the potency of each extract as antifungal agent. Overall activity of extracts can result from a mixtures of compounds with synergistic, additive, or antagonistic activity, research methodology (15), inoculum size of tested fungi, the type of growth medium, the incubation time and temperature (4). According to the literature review, it is apparent that it is difficult to compare the current results with the previous once due to the difference in the fungal strains, extraction method and the types of solvents being used, and the plant part.

CONCLUSION

As a conclusion of the current study it is clear that medicinal property of *M. parviflora* extracts results from the presence of secondary metabolites. The leave extracts proved to have bioactivity action against yeast and filamentous fungi. Our results of this study show a strong antifungal activity against *Candida albicans* and *Aspergillus fumigates*. Phytochemical and quantitative analyses of flavonoid, polyphenols and the antioxidant activity prove such effect of all leave extracts.

FURTHER WORK:

For any future work, it is recommended that a detailed study regarding identifying the bioactive compounds by using GC-MS and LC-MS techniques should be carried out in order to be able to understand the mechanisms of action behind the response of different strains of yeast and mold to the constituents in the plant. Furthermore, such detailed study should lead to the possibility of manufacturing more drugs related to treat different types of opportunistic fungal infection to help immunocompromized individual.

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