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Antifungal Activity Effect of Methanolic Extracts of Myrrha and Samwah Medicenal Plants

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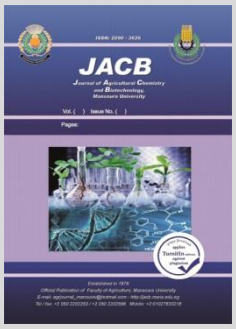

ABSTRACT

A lot of several plants have therapeutic potentials. Some of these plants myrrha (*Commiphora myrrha*) belongs to Burseraceae family and samwah (*Cleome droserifolia*) belongs Cleomaceae family. These two plants were extracted with methanol. Methanolic extracts of these two plants were prepared. The extracts of myrrha and samwah plants were tested at different concentrations to know its ability to inhibit the growth of different tested fungi in two ways based on the estimation of mycelia dry weight in liquid media and the measurement of the diameter of the inhibition zone diameter of growth in solid media. Four microoraganisms were examined in this investigation. These fungal strains were**:** *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus and Fusarium moniliforme*. Results indicated that terpenes, tannins, flavonoids, saponins, resins, alkaloids, resins, carbohyrates and phenolicglycosides were found in myrrha methanolic extract.However samwah contained the same components as myrrha except resins. Furthermore, various minerals were found in the extracts of the studied plants, including Ca, Mg, Fe, K, Zn, Mn, Cu, Sr, Ni, B ,V, Ag ,Li and Co .

Keywords: Myrrha,samwah, *flavus, parasiticus, ochraceus.*

INTRODUCTION

Medicinal plants have antifungal, antimicrobial, antibiotic, antiviral, anti-inflammatory, antiarthritic, antirheumatic, and antihemorrhoidal properties.Aflatoxin B1 synthesis was suppressed by plant phenolic substances such as syringaldehyde, sinapic acid, and acetosyringone. However, vanillyl acetone, cinnamic acid, salicylic acid, thymol, and vanillin were phenolic compounds that ceased *A. flavus* growth by targeting oxidative mitochondrial stress as defense system. Medicinal plants have been used from centuries ago, for the treatment of various diseases. There are about 53,000 medicinal plants around the world. 70 % of synthetic medicines are currently produced from plants (Saleem *et al*., 2017).

Myrrha plant (*Commiphora myrrha*) is a small tree or a large shrub, grows in small sandy and rocky regions of Somalia, Sudan,Ethiopia, Kenya and Saudi Arabia. Myrrh gum-resin is a reddish brown found in the stem of various Commiphora species. *Commiphora myrrha* has been used to treat a variety of diseases such as obesity and lipid disorders. In addition, it has anti-hyperglycemic, antioxidant, hepatoprotective, analgesic, anti-inflammatory, hypolipidemic, cholesterol lowering activities through inhibiting LDL oxidation.The myrrh's Gum-resin-volatile oil are the major useful contents, where it contains (30–60%) gum including acidic polysaccharides, resin (25–40%) and volatile oil (3–8%), eugenol (Alqahtani *et al*. 2019) .

The secand plant under investigation named Samwa (*Cleome droserifolia*) it belongs to the family Cleomaceae it is a fragrant flowering shrub of the genus Cleome with about 200 species. Cleome species flourish in similar environments with varying soil conditions. Samwa is found in North Africa and the subcontinent of India. Because of its historical use in traditional medicine, Samwa is becoming increasingly endangered. Cleome plants relieve stomach problems and heal a variety of diseases such as rheumatic fever and scabies.They provide instant relief from stomach and reduce inflammation, rheumatic pain, aid wound healing, bites of scorpions and snakes.Their rubefacient, antibacterial, analgesic, antipyretic, antioxidant, and anti-inflammatory properties are responsible for these effects.Because the essential oils of Cleome droserifolia are high in sulphur and nitrogen-containing chemicals, they have been demonstrated to have powerful antibacterial capabilities. Phytochemicals abound in Cleome droserifolia, and various bioactive compounds have been identified (Panicker *et al*. 2020) .

The present research is deals also with some fungal species that produce aflatoxin.The most frequent fungus species capable of producing mycotoxins in food and feedstuffs is *Aspergillus* sp. *.Aspergillus flavus* can befound in soil and other substrates . Fungal secondary metabolites(mycotoxins) that have been linked to animal and human health risks. Many human diseases have been linked to Aspergillus flavus, the most serious of which being invasive aspergillosis.It can also spread disease to insects and crops such as rice, maize, and peanuts. Cereals such as maize, wheat, and sorghum are examples of agricultural products.Major ingredients of poultry feed include by-products and a variety of oil seeds.Animals and humans can be harmed by agricultural commodities contaminated with toxigenic fungi like *A. flavus* that produce mycotoxin.Because mycotoxins are produced by distinct species, thorough identification and characterization of fungus is critical for developing any preventative approach.As a contamination in chicken feed, many mycotoxins have been reported, aflatoxin B1, B2, G1, G2, and Ochratoxin A are the most common aflatoxins (OTA). These aflatoxins are the major four effected components whileaflatoxin B1 is the most studied aflatoxin

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since it is the most poisonous and potentially carcinogenic (Fakruddin *et al*. 2015) .

The present work aimed to study the antifungal activity effect of myrrha and samwah methanolic extracts on harmful fungi which produced aflatoxin and mycotoxins in food and feedstuffs.Also phytochemicals screening were evaluated.

Terpenes, tannins, flavonoids, saponins, phenolicglycosides, carbohydrates, resins, and alkaloids were identified for the two plant methanolic extracts.

MATERIALS AND METHODS

Experimental medicinal plants:

Medicinal plants used in this study were Myrrha (*Commiphora myrrha*), family Burseraceae and Samwah (*Cleome droserifolia*), family Cleomaceae.These plants were bought in a local markets in Mansoura, Egypt's Dakahlia governorate.

Phytochemical screening :

Preliminary phytochemical screening tests were performed on the two plant methanolic extracts. Terpenes, tannins, flavonoids, saponins, phenolic glycosides, carbohydrates, resins, and alkaloids in each sample (Harborne,1988).

Extraction with methanol :

The Lis-Balchin *et al*. (1998) approach was used as follows: Each powdered plant sample was extracted by soaking for 48 hours in methanol at a ratio of 1:1 (w/v) 100g/100ml.Under high hand pressure, the extracts were filtered through cheese cloth, and the solvent was removed under vacuum at 60-65˚C to give a crude methanolic extract using a rotary evaporator.The crude extract was stored in the refrigerator until it was needed, and serial concentrations were prepared.

Minerals content :

Minerals constituent as : Ca, Mg, Fe, K ,Zn, Mn, Cu, Sr, Ni, B ,V, Ag ,Li and Co were determined using Inductivity Coupled Plasma (iCAP™ 7000 Plus Series ICP-OES, Thermo Scientific™) after acid digestion using HNO3 (70%) and H2O2 (30%) in a microwave digestion apparatus (model Milestone MLS 1200 Mega)(Bettinelli *et al*., 2000).

Antimicrobial activites :

In this study the antifungal activities of the tested extracts were evaluated against four mycotoxigenic fungi. The changes in mycelial dry weights were used to evaluate these activities after 14 days incubation period at 28ºC,after incubation at 28ºC/7 days, the presence or absence of inhibition zones, as well as the acquired diameter in which no growth was detected, were measured.

The tested fungi.

In this study four strains of aflatoxin producing fungi named: *A.flavus, A. parasiticus, A.ochraceus* and *F.moniliforum*, were obtained from the mycotoxins-Lab., National Research Centre, Egypt.

Studying the influence of the tested extracts on Mycelium growth.

Mycelium growth and sporulation of the tested fungi was evaluated on yeast extract sucrose broth media (YES). The 100 ml of culture media were prepared in 250 ml conical flasks. To each flask, the tested extracts were incorporated into the broth media with 0.5% DMSO as an emulsifying agent to final concentrations of extract: 500; 1000; 1500, 2000 and 2500ppm, respectively.Later, a 10 mm actively growing culture disc of each investigated fungus cultured on potato dextrose agar (PDA) plates was injected aseptically into flasks. The flasks were incubated at 28±2ºC in the incubator. After 14 days, the mycelium dry weight and sporulation were measured. The experiment was carried out in triplicates.Mycelial mat was collected after the incubation days and filtered through preweighed Whatman no. 1 filter paper individually to assess fungus growth. It was dried in an incubator at 50±2ºC until it reached a consistent weight.The formula was then used to determine the actual weight of dried fungal mycelium. (Arey, 2010).

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper).

The degree of sporulation of fungi was determined using standard methods as recommended by Wilson and Knight, 1952 and Tuite, 1969.

Determination of Minimum Inhibitory Concentration (MIC):

To determine the MIC values of the tested extracts against the all tested fungi, the technique of disc diffusion was used according to the recommendations described in the M44-A method (NCCLS, 2004) with minor modification. In brief, potato dextrose agar (Difco, USA) medium was employed, which was formulated following instructions from the manufacturers and divided (150 mL) into sealed flasks, heated and placed in Petri plates at the time of use. Petri dishes were used to disperse the cultures (150 x 6 mm) and inoculaned, in the surface. After complete absorption of the inocula,disks with 10 different myrrha concentrations levels (50 up to 500 ppm) were placed in equil distant points. Disks with the samwah methanolic extracts were assessed using the same methods. Plates were incubated upside down at 28 ± 2ºC. The diameter (mm) of the inhibition zone produced around the disc after 96 hours of inoculation was used to determine each fungus sample's level of sensitivity to the tested antifungal drugs.We use tetracycline(20ppm) as reference drug.

RESULTS AND DISCUSSION

I. Phytochemical screening of different plant methanolic extracts:

The results preliminary phytochemical study of myrrha and samwah methanolic extract are shown in Table (1).These results revealed the presence of terpenes, tannins, flavonoids, saponins, resins, alkaloids, carbohyrates and phenolic glycosides in myrrha plant extract.Samwah, on the other hand, has the same components as myrrha, with the exception of resins.

Table 1. Preliminary phytochemical screening of crude methanolic extracts .

Examined components	Methanolic extracts of different plant samples	
	Myrrha	Samwah
Terpenes	+	+
Tannins	+	+ +
Flavonoids	+	+ +
Saponins	+ +	+
Resins	+	-
Alkaloids	+	+
Carbohyrates	+	+
Phenolic glycosides	+ +	+

From Table (1) it is noted also that myrrha plant extract is rich in saponins and phenolic glycosides. While samwah plant extract is rich in tannins and flavonoids.

More than 8000 phenolic compounds as naturally occurring substances from plants. It is very interesting to note that half of these phenolic compounds are flavonoids presenting as aglycone, glycosides and methylated derivatives .Many other phenolic components, including flavonoids, have been studied

for their effectiveness as antibacteria, anticancer, antioxidants, cardioprotective agents, immune system boosting, anti-inflammation, skin protection from UV radiation are just a few of the terms that come to mind.It can be interested that pharmaceutical and medical application candidate.These phytochemical compounds can be found in foods and herbal remedies (Tungmunnithum *et. al.*,2018).

II. Elements content:

Data in Table (2) showed that znic in both sample had the highest content . Average values of 83.94 and 81.44 ppm in myrrha and samwah samples, respectively . In myrrha sample Mn, Cu and Sr are found in moderat amount of 57.72, 38.93, and 38.09 respectively. Results for element content inform that Sr ion had the highest concentration in samwah plant extract followed by Zn , B and Mn .They recorded average values of 228.27,81.44, 75.06 and 76.25 ppm respectively .

Table 2. Elements content (ppm) of investigated medicinal plant samples.

element	Investigated plants	
	Myrrha	Samwah
Ca	17.28	33.66
Mg	3.38	6.59
Fe	3.14	4.70
K	1.11	11.20
Zn	83.94	81.44
Mn	57.72	76.25
Cu	38.93	24.48
Sr	38.09	228.27
Ni	29.44	7.04
B	24.37	75.06
V	18.62	16.21
Ag	12.13	9.96
Li	6.95	6.59
Co	2.12	3.27

III- Effect of Myrrha and Samwah extracts on mycelia dry weight of some fungal

Data represented in Table 3 the growth of the organisms tested was significantly slowed by myrrha and samwah. When methanolic extracts were added and cultivated on yeast extract broth medium, they suppressed mycelial weight (mg) of the various fungal strains tested, and suppression increased as concentration was raised (Table 3). Myrrha methanolic extract was found to be the highest antifungal activity when compared to that of methanolic extract of samwah. The minimum value percentage reduction in mycelial dry weights when 500 ppm methanol extract was added, the results were obtained to the culture of *A.ochracues* with 163.5 mg/50ml mycelial dry weight (Table 3). Meanwhile, the highest effect in growth inhibition was found for *F.moniliforum* and *A. ochraceous* with 2000ppm of myrraha extract. However, 2500ppm of the same extract completely inhibited the mycelial growth of *A. parasiticus.*

The Commiphora myrrha plant's oleo-gum resins were shown to have broad spectrum action against all of the fungus in both of the extracts tested. The terpenoids determined in phytochemical screening are mostly responsible for this effect (Mothana *et al*., 2009).

The present study identifies myrrha and samwah as a potential biological antifungal, due to its great activity against a broad range of fungi, allowing only human pathogenic fungus to be killed, and this selectivity appears to be an extra point in natural antibiotics research.

In regard to the obtained data in Table 4, the tested methanolic extracts showed antifungal activity against all tested fungal strains, with variable degrees of inhibition. The most pronounced activity with inhibition zones greater than 10 mm was found with myrrha methanolic extracts of all tested organisms were*: A.parasiticus* (13 mm), *A.flavus* (11.7mm), *A.ochraceus* (18.3 mm) and *F.moniliforum* (19.6 mm). However, the inhibition zone of samwah methanolic extract greater than 10 mm were found against *A. ochraceous* (21.3mm), *A.parasiticus* (18.3 mm), *A.flavus* (13.3 mm). However for *F. moniliforum* it was 9.7mm, which means a weak antifungal activity. Myrrh oleo-gum resins were found to be highly suppressive against all four fungus groups examined (Table 4).

Table 3. Mean values of Mycelia dry weight (MDW) (mg/50ml yeast extract broth medium).

* Tested organism	Concentration In ppm	Mean values of Mycelia dry weight, in mg and % inhibition				
		Control	Myrrha		Samwah	
		MDW	MDW	%, Inhibition	MDW	%, Inhibition
A.flavus	500	235.7	143.7	36.3	193.3	14.3
	1000	224	123.3	45	144.3	35.6
	1500	236	54	77.12	120.3	49
	2000	211.3	27.3	87.1	123	41.8
	2500	177.7	24	86.5	76	57.2
A. parasiticus	500	284.3	135.3	45.5	189.3	23.8
	1000	257	131.3	48.9	175.3	31.8
	1500	233.7	18.3	92.17	109	53.4
	2000	235	3.7	98.4	80	66
	2500	222	0.0	100	59.6	73.1
A. ochracauesand	500	167	118.7	27.5	163.5	2.1
	1000	172.3	37	78.5	149	13.5
	1500	172.3	3.0	98.3	97.3	43.5
	2000	159.7	0.0	100	74.3	53.5
	2500	133	0.0	100	46.7	64.9
Fusarium moniliforum	500	143	66	53.8	105	26.6
	1000	137.7	22	84	61	55.7
	1500	117	26	77.8	28.3	75.8
	2000	112	0.0	100	22.7	79.7
	2500	99	0.0	100	13	86.7

The myrrha methanolic extract at concentration of 500 ppm were effective against *A.parasiticus* (13 mm), compared to the same extract for samwah (18.3 mm). The least MIC values were found for *Fusarium moniliforum* with the least IZDG (19.6 and 27 mm; for myrrah and samwah, respectively). When compared to the samwah methanolic extract, the plant's myrrha oleo-gum resins Commiphora myrrha was effective against all of the fungal species.

In comparison to antibiotics findings; the obtained results could be concluded that methanolic extracts of Myrrha and Samwah was less effective than the standard antibiotics. Dolara *et al*., (2000) and Adam and Selim (2013) mentioned that in vitro investigation of two sesquiterpenes produced from myrrh (furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one) found antibacterial efficacy against *Pseudomonas aeruginosa* (minimum inhibitory concentration (1.4 mg mL^{-1}), *Staphylococcus aureus* (MIC 0.18 mg mL^{-1}), and *Escherichia coli* (MIC 2.8 mg mL^{-1}). Furthermore, these sesquiterpenes were found to have antifungal properties against *Candida albicans* (MIC 1.4 mg mL^{-1}). Mammalian nerve cells showed local anaesthetic action as well.Some investigations have revealed that myrrha contains essential oil (myrrhol), resin (myrrhin), gum, and bitter principles. The antimicrobial effects in myrrha is due a mixture of furanosesquiterpenoids mainly furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one (El-Ashry *et al*., 2003).

When the inhibitory zone is larger than 6 mm or the growth inhibition is moderate or higher, plant extracts are considered active against bacteria and fungus (Groove and Randall, 1955 and Saadabi *et al*., 2006).

Although there are many studies that evaluate the antifungal activity of myrrha extracts against *A.flavus, A.niger and Penicillium citrinum* (Batool Z. Ali, 2007), there are no studies that evaluated the antifungal activity of myrrh methanol extract against the tested fungi used in this study.

Table 4. Minimum inhibitory concentration values of the tested mycotoxigenic fungi as affected by the tested methanolic extracts of Myrrha and Samwah plants.

Tested organism	MIC values in ppm with IZDG (mm) of the tested extracts against the tested mycotoxigenic fungi					
	Myrrha		Samwah		Tetracycline	
	MIC	IZDG	MIC	IZD	MIC	IZDG
A.flavus	350	11.7	400	13.3	20	4.7
A. parasiticus	500	13	550	18.3	20	6
A. ochracauesand	650	18.3	600	21.3	20	6.3
Fusarium moniliforum	800	19.6	950	27	20	7

MIC: minimum Inhibitory concentration (ppm).
IZDG: inhibition zone diameter of growth, mm.

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التأثير المضاد للفطريات للمستخلصات الميثانولية لنباتات المره و السموة الطبية

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هناك أعداد كبيرة من النباتات الطبية لها تأثير مضاد للكائنات الحية الدقيقة. من هذه النباتات المره و السموه التي تم دراستها في هذا البحث من خلال المستخلص الميثانولي لهذه النباتات . و قد تم عمل تركيزات مختلفة من هذه المستخلصات لكل نبات لدراسة تأثيرها علي الكائنات الحية الدقيقة . و قد تم في هذا البحث عمل اختبارات قياسية لاربعة(4) فطريات و هي علي التوالي : *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus and Fusarium moniliforme* . و قد اظهرت النتائج ان المستخلص الكحولي لكل من نباتي المره و السموه يحتوي علي تربينات, تانينات, فلافونيدات , سابونينات ,قلويدات, كربوهيدرات, جليكوسيدات . علاوة علي ذلك فان مستخلص المرة يحتوي علي الراتنجات ,كما تحتوي هذه النباتات علي بعض المعادن الهامة مثل الكالسيوم ,الماغنيسيوم , الحديد ,البوتاسيوم ,الزنك ,المنجنيز النحاس , ألاسترانشيوم , النيكل , البورون , الفناديوم , الفضة , الليثيوم و الكوبلت. تم أختبار المستخلص الميثانولي لنباتي المرة و السموه بتركيزات مختلفة لمعرفة قدرتها علي تثبيط نمو الفطريات بطريقتين : الاولي علي أساس تقدير الوزن الجاف لنمو الفطريات في بيئة سائلة MDW (mg/50ml) و الثانية علي اساس قياس قطر هالة التثبيط الخاصة بنمو الفطر في بيئة صلبة IZDG (mm).