In Vitro Screening for Antimicrobial Activity of Some Medicinal Plant Seed Extracts

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Abstract: Phytochemical screening (saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides) of four medicinal plant seeds (*Jatropha curcas, Simmondsia chinensis* (Jojoba), *Moringa oleifera* and *Datura metel*) extracted by aqueous, ethanol and Folch solvents, were examined for their antimicrobial activity against three types of plant pathogenic fungi namely; *Botrytis cinerea, Fusarium oxysporum* and *Rhizoctonia solani,* in addition to four types of bacteria, namely; *Bacillus cereus, Staphylococcus aureus, Ralstonia solanacearum* and *Pesudomonas aeruginosa* using disc diffusion paper. Results revealed that different concentrations of aqueous extracts were more effective against bacterial activity compared to fungal activity, except for *D. metel* aqueous extract which showed no antifungal effect and very weak effect on only two of the tested bacteria. *B. cereus* was more sensitive to *J. curcas* aqueous extract, while *P. aeruginosa* was more sensitive to *S. chinensis* and *M. oleifera* aqueous extracts. On the other hand, results showed that *J. curcas* and *M. oleifera* ethanol extracts were more effective on *Staph. aureus* growth, while *S. chinensis* and *D. metel* did not have any effect on any of the fungi or bacteria under study. The evaluation of the antifungal and antibacterial effect did not confirm the broad spectrum of *S. chinensis* Folch extract, while *M. oleifera and D. metel* were more effective on reducing *R. solani* growth. Also *F. oxysporum* was affected by *J. curcas* Folch extract only at high concentrations. These findings support that the traditional use of the plant extracts in the treatment of different infections caused by pathogenic microbes is valuable and should be taken in consideration.

Keywords: Phytochemical screening, antimicrobial activities, *Jatropha curcas, Simmondsia chinensis* (Jojoba), *Moringa oleifera, Datura metel.*

INTRODUCTION

Under field conditions, pesticides have minor effect and are considered as a source of chemical pollution and a toxin of human diets [1]. Using these chemical pesticides for a long time, developed pathogens resistance towards pesticides and they became useless [2-4]. Plants and microbial tissues have defensive antibiotics and natural compounds against these pathogens [5-7]. Recently, many researchers have focused on the investigation of plant extracts and their uses as antimicrobial agent by nature. The fact that using plant extracts as traditional medicine continues to provide health converges for over 88% of the world's population, especially in the developing world [8]. The actions of these plants on microorganisms have been found to be due to the presence of phytochemical substance such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, flavonoids and a host of other chemical compounds referred to as secondary metabolites that are present in them [9-11]. According to further studies, phytochemical compounds in medicinal plants, which have antibacterial effect, provide information on nature extracts as inhibitory factors (microbiocidal or microbiostatic), and thier efficiency depends on the extracts solvent, concentration and cell damage inflicted to the tested microorganisms [8]. There are over thousands of varieties and species of medicinal plants used globally as antimicrobial agents and for curing different infections [12]. These plants include *Jatropha curcas,* which is believed to be a native of South America and Africa but later introduced to other continents of the world [13]. Many methods were used for *J. curcas* extraction from stem bark and roots to be used as an antimicrobial agent [14]. The ethanolic and aqueous extracts of *J. curcas* plant were analyzed phytochemically and screened against different microorganisms responsible for various human infections [15].

Simmondsia chinensis Schneider, commonly known as Jojoba, is a semi-arid evergreen shrub [16]. It grows in the desert of south-western United States and north-western Mexico. However, this plant is cultivated in

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ISSN: 1927-3037/16

Australia, Brazil, Argentina and Middle East countries [17-18]. Jojoba is unique among plants in the fact that 50% of its seeds weight consists of oil. This oil contains quantities of sterols, stanols and different tocco phenols [19-20]. The ethanolic and aqueous extracts of *S. chinensis* root contained some phytochemical compounds like tannins, phenols and flavonoids, but void of alkaloids, glycosides and saponins [21]. Also, different parts of *S. chinensis* namely; testa, seeds, male and female leaves were extracted by soxhlet apparatus using ethanol and methanol. Results showed no activity against *Staph. aureus, Bacillus subtilis, E. coli, Klebsiella pneumoniae* and *Candida albicans* [22].

Moringa oleifera is a member of the family *Moringaceae*, native to Africa, South Asia, America, Himalayan region, India, Pakistan, and the pacific and Caribbean Islands [23]. Moringa plants provide a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemical compounds [24]. It was also studied for its antibiotic effect [25] and antimicrobial properties [26]. The phytochemical screening of *M. oleifera* leaf extracted by water and ethanol indicated the presence of flavonoids, steroids, alkaloids and saponins. Also, antifungal activity of ethanolic and aqueous extracts of *M. oleifera* leaf was highly active against some fungal strains [27]. *M. oleifera* seeds are rich in oil ß-carotene, plant sterols and lecithin, and also its oil contains unusual kinds of fatty acids [28]. In Sudan, powdered seeds have been used in water purification [29]: this powder also works as a natural coagulant, which clarifies very turbid water [30]. Reports have been elucidated on the findings of the antibiotic principle of *M. oleifera* seeds through their purification, and antimicrobial properties [31]. *M. oleifera* seeds extracted by aqueous, petroleum ether and methanol at different concentrations, inhibited growth of two species of fungi and four types of bacteria to varying degrees, but the aqueous extract was strong and superior on antibacterial activity especially Gram positive as compared to methanol or petroleum ether, while no activity was observed against some fungal strains [29]. The ethanolic extract of *M. oleifera* seeds was inhibitory to both *Shigella flexneria* and *E. coli*, while *Salmonella typhi* was not affected. Also, various concentration of the aqueous extract was inactive against the tested organisms [32].

Datura plant belongs to the family *Solanaceae* which is distributed worldwide and includes 85 genera and about 2800 species. There are approximately 25 different species of *Datura* throughout the world usually called as Jimson weed [33]. *Datura* leaves and seeds are widely used in herbal medicines as anesthetic, antispasmodic, antitussive, bronchiectasis and hallucinogenic [34]. Aqueous extracts of *D. mete;* roots, stems, leaves and seeds, were used as antibacterial agent against five human pathogens, each of the root and stem aq. extract was less effective on tested bacteria compared with leaf and seed extracts [35]. Leaf, stem and roots of *D. metel* were extracted by ethanol and water: aqueous extract contained all the phytochemical compounds, while tannins and steroids were absent in the ethanolic extract. *P. aeruginosa* and *S. typhi* were most inhibited by leaf and stem aqueous extract of *D. metel*, while leaf ethanolic extract demonstrated an inhibitory potency against *P. aeruginosa, K. pneumoniae* and *B. cereus.* Also, the root aqueous extract of the plant showed no antibacterial activity on all tested bacteria [36]. Some new antibacterial agent was isolated from *D. metel* leaves by ethanol using soxhlet apparatus at different concentrations, which successfully inhibited *P. aeruginosa, B. subtilis, S. typhi, K. peneumonia, Staph. aureus* and *Proteus mirabl,* but could not inhibit *E. coli* [37]. Leaves aqueous extract of *D. metel* contained all phytochemical compounds, except alkaloids, glycosides and phytosterols. This extract has an inhibitory activity against *Flavobacterium psychrophilium,* cold water bacteria, which causes some diseases at high concentration, while leaves chloroform extract has more inhibitory effect on these bacteria, but hexane extract showed less effect on bacterial growth at higher concentration [38]. This study aims to investigate the phytochemical content and evaluate the antibacterial and antifungal activity of *J. curcas, S. chinensis, M. oleifera* and *D. metel* using aqueous, ethanol and Folch seed extracts.

MATERIALS AND METHODS

Source of Samples

Four medicinal plant seeds were used in this study namely; *Jatropha curcas, Simmondsia chinensis* (Jojoba), *Moringa oleifera* and *Datura metel,* which were supplied by Orman herbarium garden, Agricultural Research Center (ARC), Cairo, Egypt, during year 2015. Seeds were grounded to pass through a 100 mesh sieve and stored in sealed glass bottles under dry and dark conditions at room temperature for latter extraction.

Preparation of Extracts

Aqueous extraction was made with some modifications [39]: 150 ml of double distilled water was

added to 25g of the grounded seed samples in a flask, stirred, then flasks were sealed with aluminum foil and allowed to stand for 72 hrs at room temperature. Ethanol extraction was done by mixing 25g of grounded seeds with 150ml ethanol (95%) for 7 days under shaking. Also, Folch extraction was done by extracting 25 g of grounded seeds with 150 ml of Folch solvent (Chloroform : Methanol 2:1 v/v) for 24 hours at room temperature [40]. Extracted contents were then filtered with whatman No. 1 filter paper, then filtrates were concentrated using rotary evaporator (Yamato Scientific Ltd., RE 504, Japan; using pump, JEIO TECH, VE-11, Korea) at 40ºC until dryness. Extracts were dissolved in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, Co. 3050 spruce street, St. Louis, MO 63103, USA) to make serial dilutions of extracts (250, 500, 1000, 2000 and 4000ppm) [41]. Concentrated extracts were stored at 4 ºC in sealed glass bottles prior to use.

Phytochemical Screening

Simple qualitative phytochemical screening of the major groups of saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides were tested according to standard methods [42-44].

Collection, Maintenance and Enumeration of Organisms

Three cultural strains of fungi and four cultural strains of bacteria used in this study, were kindly provided by Plant Pathogen Institute (PPI), Agriculture research center (ARC), Cairo, Egypt. Fungal strains namely; **Botrytis** *cinerea* (Gray rot), Fusarium oxysporum *(Fusarium white wilt) and Rhizoctonia solani* (Root rot), were grown on slant potato dextrose agar (PDA) at 24° C for 12 days until complete sporulation [45]. The slants were maintained under sterilized paraffin oil as stock culture. The spore suspension was obtained from slant agar with 0.1% peptone water. The number of spores was determined by indirect technique for cell count [46], one touch from pure colony was taken and added to 10ml peptone water, then dilutions were made (suspension solution), after that, 1 ml from each dilution was added in petri dish and media was poured on the suspension. The number of spores from suspension solution was 4 x 10^6 spores/ml.

Bacterial strains used in present study included: *Bacillus cereus, Staphylococcus aureus, Ralstonia solanacearum* (Potato brown rot) and *Pesudomonas aeruginosa,* which were grown on slant nutrient agar (NA) (Oxoid, 40, England) [45]. The same indirect technique was used for enumeration of colonies forming unit (CFU/ml) by making many dilutions where each one ml of suspension contains 10^5 to 10^6 CFU. The antibacterial potential of seed extracts was evaluated by disc paper diffusion [47-48].

Antifungal and Antibacterial Tests

1 ml of each fungal strain suspension was added to Petri dishes containing liquefied PDA (45-50 ^oC). Also, 1ml of every bacterial strain was incubated in NA, after being solidified in Petri dishes (2 hrs). Filter paper discs (5 mm) whatman No. 3 containing 20 µl from each concentration of seed extract were put on the media's surface. Plates containing PDA and NA were then incubated at 28 ^oC for 48 hr and at 37 ^oC for 24 hrs respectively, and the diameter of the inhibition zone (DIZ) was measured in mm and recorded. The inhibition zones obtained were compared with the positive control (25 µl of tetracycline 10µg/disc, cipla ltd, Mumbai, India) and the negative control disks were saturated with 20 µl of 10% DMSO solution [41]. Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (250, 500, 1000, 2000 and 4000 µg/ml). Inhibition of the growth was indicated by a clear solution or a definite decrease in color reaction.

RESULTS AND DISCUSSION

Four medicinal plant seeds, extracted by three extraction methods, were used in this study to test their antimicrobial effect on 3 pathogenic fungi and four strains of bacteria.

Phytochemical Screening of Extracts

Table **1** showed simple qualitative phytochemical analysis of seed extracts for *Jatropha curcas, Simmondsia chinensis* (Jojoba), *Moringa oleifera* and *Datura metel,* were performed by three extraction methods. *J. curcas* aqueous extract had the highest amount of all phytochemical compounds; it was rich in glycosides content followed by flavonoids and phenols with no alkaloids. Ethanol and Folch extracts had a reduced amount of components than aqueous extract. Tannins and flavonoids were absent in ethanol extract and glycosides was absent in Folch extract. It was found that *J. curcas* ethanol stem extract contained saponins, tannins, cordic glycosides and flavonoids [14].

On the other hand, each of *S. chinensis* and *M. oleifera* aqueous extracts had more phenols than other

Table 1: Simple Qualitative Phytochemical Analysis of Extracts from Seeds of *Jatropha curcas, Simmondsia chinensis, Moringa oleifera* and *Datura metel*

J. curcas	**Saponins**	**Tannins**	**Steroids**	**Alkaloids**	**Flavonoids**	**Phenols**	**Glycosides**
Aqueous extract	++	++	++	-	++	++	++
Ethanol extract	+	-	+	+	-	+	+
Folch extract	+	+	+	+	+	+	-
S. chinensis	**Saponins**	**Tannins**	**Steroids**	**Alkaloids**	**Flavonoids**	**Phenols**	**Glycosides**
Aqueous extract	-	++	-	-	++	++	-
Ethanol extract	-	-	-	-	+	-	-
Folch extract	-	-	+	-	+	-	-
M. oleifera	**Saponins**	**Tannins**	**Steroids**	**Alkaloids**	**Flavonoids**	**Phenols**	**Glycosides**
Aqueous extract	+	-	+	+	++	+++	-
Ethanol extract	++	++	++	++	++	++	-
Folch extract	+	-	+	+	+	+	-
D. metel	**Saponins**	**Tannins**	**Steroids**	**Alkaloids**	**Flavonoids**	**Phenols**	**Glycosides**
Aqueous extract	+	+	+	+	+	+	+
Ethanol extract	+	-	-	+	+	+	+
Folch extract	+	-	+	+	+	-	-

+ = slightly present, ++ = moderately present, +++ = highly present, - = absent.

phytochemical compounds, while glycosides was not detected in the three extracts of both plants. Also, most components were absent in ethanol and Floch extracts of *S. chinensis* seeds. Results in the same Table illustrated that only *D. metel* aqueous extract had all phytochemical compounds in fewer amounts than other aqueous extracts. Tannins were not detected in ethanol and Folch extracts, while phenols and glycosides were absent from Folch extract only. Such preliminary phytochemical screening is helpful in predicting drugs nature and useful for detecting different constituents in different polarity solvents as mentioned [21].

Jatropha curcas Seed Extracts

The diameter of inhibition zone (DIZ) in millimeters (mm) at different concentrations of *J. curcas* seed extracts compared with tetracycline at 10µl as positive control, were demonstrated in Table **2**. Results showed that, the inhibition of bacterial growth by aqueous extract was more than fungal growth inhibition at different concentrations. *B. cereus* was the most sensitive bacteria and was affected by this extract, followed by *Staph. aureus* at all concentrations, while *R. solanacearum* was less sensitive. *B. cereus* and *Staph. aureus* recorded DIZ 25.33 and 24.89mm at the highest concentration, respectively, while *R. solanacearum* recorded 7.67 mm at the same concentration of *J. curcas* aq. extract. Also, fungal growth of *F. oxysporum* was affected (21.0 mm) followed by *R. solani* (13.67 mm) at the highest concentrations (4000ppm).

It worth mentioning that, *J. curcas* aqueous extract at 4000 ppm had more effect on *B. cereus, Staph. aureus* and *F. oxysporum* growth than tetracycline (positive control), which recorded DIZ 20, 21, and 17mm, respectively. These results explain the effect of high amounts of phytochemical compounds in aqueous extract of *J. curcas* especially glycosides. *J. curcas* water and ethanol extraction from root was done [15, 49]. Results was that each of the alkaloids and tannins were present in all root extracts and the plants contained active compounds against microorganisms and inhibited the growth of all bacteria tested, except *P. aeruginosa*. Results in Tables **1** and **2** came in agreement with previous reports [15]. Also, it was observed that aqueous extract of *J. curcas* root was effective against *Staph. aureus* with 17mm at 1.0 ml [15, 50]. *J. curcas* ethanol extract was found to be inactive against all the tested microorganisms used in this study except *Staph. aureus,* which recorded DIZ 10.67, 10.00, 7.67 and 6.33mm at 4000, 2000, 1000 and 500ppm, respectively. Previous researches used the ethanol stem extract of *J. curcas* against *Staph. aureus* [14], they noted that this extract showed no

significant difference in the zone of inhibition against *Staph. aureus* compared with the positive control ciprofloxacin. These results were revealed in the absence of tannins and flavonoides in ethanol extract beside the presence of little amount of phytochemical compounds (Tables **1** and **2**). Also, tetracycline recorded 21 mm as a positive control with *Staph. aureus.*

The effect of aqueous and ethanol extracts from *J. curcas* roots on some bacteria and fungi were studied [15]. It was found that ethanol extracts were rich in tannins, alkaloids, steroids and saponins, whereas aqueous extract contained the same phytochemical compounds but a lower level, therefore the ethanol extract has effect on *Staph. aureus* and *P. aeruginosa* growth at high concentrations, while aqueous extracts

Table 2: Antifungal and Antibacterial Activity of *Jatropha curcas* Seed Extracts

Strains		Aqueous extract (ppm)					Tetracycline**	DMSO
		4000	2000	1000	500	250		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	21.00±1.41	17.67±0.91	14.00±0.97	10.67±0.97	5.67±0.84	17	-
	Rhizoctonia solani	13.67±0.69	10.33±1.33	7.67±0.91	4.67±0.77	-	14	-
Bacteria	*Bacillus cereus*	25.33±0.59	21.33±0.91	15.00±0.77	12.67±1.28	8.33±1.08	20	-
	Staphylococcus aureus	24.89±1.37	22.00±1.41	14.35±0.97	10.67±0.91	8.00±0.97	21	-
	Ralstonia solanacearum	7.67±0.84	2.67±0.77	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-
Strains		**Ethanol extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	10.67±0.91	10.00±0.69	7.67±0.49	6.33±0.77	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-
Strains		**Folch extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	13.33±0.69	10.67±0.91	8.33±0.97	-	-	17	-
	Rhizoctonia solani	7.67±0.77	3.67±0.59	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-

*: Diameter of inhibition zone (DIZ mm) including disc diameter of 4 mm.
**: Diameter of inhibition zone (mm) observed by tetracycline as a positive control (10µg/disc).
-. Not active.
DMSO: Dimethyl sulfoxide [(CH3)2SO] as a negative control.
Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

have no effect on only *Staph. aureus* at various concentrations.

J. curcas Folch extract affects the growth of two fungi only, *F. oxysporum* and *R. solani* up to 1000 and 2000 ppm, respectively, while it did not show any antibacterial effect. These results may be due to the low level of phytochemical compounds and the lack of others like glycosides.

Simmondsia chinensis (Jojoba) Seed Extracts

Results in Table **3** revealed that *S. chinensis* aqueous extract had an effect against only one fungus

Table 3: Antifungal and Antibacterial Activity of *Simmondsia chinensis* Seed Extracts

Strains		Aqueous extract (ppm)					Tetracycline**	DMSO
		4000	2000	1000	500	250		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	17.00±1.08	12.33±1.33	8.67±1.08	4.00±1.14	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	15.00±0.69	12.33±0.97	8.00±0.77	5.67±0.84	-	20	-
	Staphylococcus aureus	19.67±0.97	15.39±1.24	10.48±1.15	4.94±0.24	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	32.33±0.59	25.33±1.03	19.87±1.57	11.40±1.09	5.32±0.77	26	-
Strains		**Ethanol extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-
Strains		**Folch extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-

*: Diameter of inhibition zone (DIZ mm) including disc diameter of 4 mm.
**: Diameter of inhibition zone (mm) observed by tetracycline as a positive control (10µg/disc).
-. Not active.
DMSO: Dimethyl sulfoxide [(CH3)2SO] as a negative control.
Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

and three bacterial strains. At the highest concentration of aqueous extract, *F. oxysporum* recorded DIZ 17.00 mm, while *B. cereus, Staph. aureus* and *P. aeruginosa* recorded DIZ 15.00, 19.67 and 32.33 mm, respectively. Results illustrated that the increased concentration of *S. chinensis* aqueous extract decreased the growth of microorganisms tested. On the other hand the phytochemical compounds in *S. chinensis* aqueous extract were mostly absent except tannins, flavonoids and phenols which were present in high levels (Table **1**). Therefore, this extract has less effect on microbial growth compared with *J. curcas* aqueous extract (Tables **2** and **3**).

Results in other researches [21] came in agreement with the results obtained in Table **1**, where it was found that water extract of *S. chinensis* root contains tannins, phenols and flavonoids but is void of alkaloids, steroids and saponins, while ethanol root extract contained the same phytochemical compounds of ethanol seed extract for tannins and phenols only.

Also, Table **3** showed no effect of both *S. chinensis* ethanol and Folch extracts on all tested microorganisms due to the absence of the major phytochemical compounds in these extracts especially tannins and phenols.

The *S. chinensis* ethanol extract had no activity against many kinds of microorganisms including *Staph. aureus* at different concentrations (Al. Oizwini *et al.,* 2014). These results came in agreement with results obtained in Table **3**.

Moringa oleifera Seed Extracts

Concerning *M. oleifera* aqueous extract, results in Table **4** showed that, the DIZ of *B. cereus* were 18.23, 15.98, 12.20 and 8.07 mm. Similarly *R. solani* had DIZ of 15.33, 14.39, 11.54 and 7.4 mm for fungal strains at concentrations of 4000, 2000, 1000 and 500 ppm, respectively. Meanwhile *F. oxysporum* was not affected by 1000 ppm concentration. *Staph. aureus* and *R. solanacearum* were unaffected by all the concentrations of aqueous extract with no zones of inhibition compared with positive control antibiotic, but *P. aeruginosa* was more sensitive to aqueous extract at all concentrations. It recorded DIZ 29.67, 19.67, 11.33, 9.00 and 6.33 mm, while *B. cereus* was the least microbe tested affected by this extract. The presence of high levels of flavonoids and phenols in *M. oleifera* aqueous extract was more effective on growth of all fungi, in addition to *P. aeruginosa* (Tables **1** and **4**). It was previously mentioned that, the flavonoids were known to be biologically active against liver toxins, viruses and other microbes [51]. Also, others found that, *M. oleifera* aqueous seed extract was strong and superior to antibacterial activity against *Staph. aureus* and *P. aeruginosa* [29], these results came in agreement with the results obtained in this study except for *Staph. aureus.*

On the other side, results of *M. oleifera* ethanol extracted had no effect on *B. cereus* and *R. solani* growth within all concentrations. Also, it had no effect on *F. oxysporum* along 500 ppm. As for *Staph. aureus,* it was more sensitive to ethanol extract at all concentration levels. The DIZ recorded 20.33, 18.33, 15.00, 10.33 and 8.00 mm followed by *B. cereus* (14.67, 13.33, 9.67 and 8.33 at 4000 to 500 ppm). *P. aeruginosa* was the least bacterium affected by ethanol extract. With reference to results in Table **1**, ethanol extract contained the highest amounts of all phytochemical compounds except glycosides which were absent, that may explain the effectiveness of ethanol extract on bacteria rather than fungi. Earlier, it was found that *M. oleifera* aqueous extract had no effect on the tested organisms at various concentrations but there was appreciable antimicrobial activity demonstrated by the ethanol extract with *E. coli* and *Shigella flexneri* being susceptible, while *S. typhi* showed no susceptibility to both extracts[32]. Others used methanolic and aqueous extracts of *M. oleifera* seeds and found considerable effect on bacteria isolated from wound infections including *E. coli*.. Therefore, they reported that the extracts had broad spectrum of activity [52]. Also, researchers reported that *M. oleifera* ethanol extract have high antibacterial activity against *S. typhi* while the aqueous extract had low activity against the same organism [53]. Nevertheless results in this study illustrated that *M. oleifera* aqueous extract had effect on all tested fungi till 2000 ppm and an even stronger effect on bacteria *B. cereus* and *P. aeruginosa* until 1000 and 250 ppm, respectively. In addition, *M. oleifera* ethanol extract inhibited the growth on only one fungi and three bacteria namely; *B. cereus, Staph. aureus* and *P. aeruginosa.*

Results in Table **4** showed that all fungi and bacteria tested were not affected by *M. oleifera* Folch extract except for *R. solani* and *P. aeruginosa*. *R. solani* with DIZ recorded 24.33 and 19.21mm at 4000 and 2000 ppm, respectively compared with the positive control (tetracycline) which was 14 mm.

Table 4: Antifungal and Antibacterial Activity of *Moringa oleifera* Seed Extracts

Strains		Aqueous extract (ppm)					Tetracycline**	DMSO
		4000	2000	1000	500	250		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	18.23±0.73	15.98±1.08	12.20±0.73	8.07±1.21	3.72±0.82	15	-
	Fusarium oxysporum	17.00±0.59	8.22±0.88	-	-	-	17	-
	Rhizoctonia solani	15.33±1.14	14.39±1.04	11.54±1.54	7.40±0.61	-	14	-
Bacteria	*Bacillus cereus*	9.00±0.49	6.32±1.08	2.76±0.65	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	29.67±0.49	19.67±0.49	11.33±1.14	9.00±0.77	6.33±0.49	26	-
Strains		**Ethanol extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	14.33±0.91	11.33±0.84	7.33±0.91	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	14.67±1.14	13.33±1.19	9.67±0.91	8.33±1.08	-	20	-
	Staphylococcus aureus	20.33±0.49	18.33±1.53	15.00±0.77	10.33±0.77	8.00±0.69	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	10.33±1.28	8.00±0.69	-	-	-	26	-
Strains		**Folch extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	24.33±1.85	19.21±0.81	13.54±0.98	9.02±0.34	2.00±0.84	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	18.33±0.69	15.00±0.69	10.67±0.84	7.67±0.49	-	26	-

*: Diameter of inhibition zone (DIZ mm) including disc diameter of 4 mm.
**: Diameter of inhibition zone (mm) observed by tetracycline as a positive control (10µg/disc).
-. Not active.
DMSO: Dimethyl sulfoxide [(CH3)2SO] as a negative control.
Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

Datura metel Seed Extracts

Extracts of *Datura spp.* were known for their phytochemical compounds which affect antibacterial activity beside their use against diseases caused by some pathogenic bacteria [35-39]. *D. metel* aqueous extract contained all the phytochemical compounds but in decreased levels compared with other extracts under

Table 5: Antifungal and Antibacterial Activity of *Datura metel* Seed Extracts

Strains		Aqueous extract (ppm)					Tetracycline**	DMSO
		4000	2000	1000	500	250		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	7.00±1.19	3.75±0.73	0.80±0.55	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	8.52±0.40	6.22±0.88	-	-	-	26	-
Strains		**Ethanol extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	-	-	-	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-
Strains		**Folch extract (ppm)**					**Tetracycline****	**DMSO**
		4000	**2000**	**1000**	**500**	**250**		
		Diameter of inhibition zone (DIZ mm)*						
Fungi	*Botrytis cinerea*	-	-	-	-	-	15	-
	Fusarium oxysporum	-	-	-	-	-	17	-
	Rhizoctonia solani	12.00±0.77	9.00±0.59	4.84±0.62	-	-	14	-
Bacteria	*Bacillus cereus*	-	-	-	-	-	20	-
	Staphylococcus aureus	-	-	-	-	-	21	-
	Ralstonia solanacearum	-	-	-	-	-	9	-
	Pseudomonas aeruginosa	-	-	-	-	-	26	-

*: Diameter of inhibition zone (DIZ mm) including disc diameter of 4 mm.
**: Diameter of inhibition zone (mm) observed by tetracycline as a positive control (10µg/disc).
-. Not active.
DMSO: Dimethyl sulfoxide [(CH3)2SO] as a negative control.
Inhibition > 15 mm (strong inhibition), 15 – 10 mm (moderate), and <10 mm (weak).

study (Table **1**). Therefore, this extract had no effect on fungi growth, but it affected bacterial strains in weak inhibitory zones. Table **5** illustrated that the only two bacterial strains inhibited were *Staph. aureus* which recorded DIZ 7.00, 3.75 and 0.80mm at 4000, 2000 and 1000 ppm, respectively, and *P. aeruginosa* which recorded DIZ of 8.52 and 6.22 mm at 4000 and 2000 ppm respectively, while DIZ was 26 mm as displayed by tetracycline (positive control antibiotic). It was mentioned that, *D. metel* aqueous seed extract was effective against *Staph. aureus,* it showed inhibition of 14 and 17mm at the concentrations of 50 µl and 100 µl, respectively [35]. It was clear from Table **5** that *D. metel* ethanol extract had no effect on all tested microorganisms. This might be due to the absence of tannins and steroids. The remnant phytochemical components were found also in low levels (Table **1**). It was found that, *Staph. aureus* was one of the weakest bacteria affected by *D. metel* ethanol leaf extract [37]. These results agreed with ethanol seed extract used in this study.

Folch seed extract had effect only on one fungal strain, *R. solani,* at concentrations 4000, 2000 and 1000 ppm, with DIZ 12.00, 9.00 and 4.84mm, respectively, compared with positive control which was 14mm at 10µl of tetracycline (Table **5**).

CONCLUSION

Results in this study revealed finally that aqueous seed extracts of *Jatropha curcas* and *Simmondsia chinensis* exhibited high degree of antibacterial and antifungal activities compared with ethanolic and Folch extracts, while aqueous and ethanol seed extracts of *Moringa oleifera* had more activities against bacterial and fungal growth. Seed extract of *Datura metel* showed no effect on all tested microorganisms. These results can be attributed to the presence of some phytochemical compounds in these extracts. Results in this work confirmed greatly that seeds of *J. curacas, S. chinensis* and *M. oleifera* can be used as an antibacterial and antifungal agent against the tested micro-organisms.

ACKNOWLEDGMENTS

The authors would like to thank the collaboration between the Faculty of Agriculture, Alexandria University, Egypt and the Horticulture Research Institute, Egypt.

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Received on 24-08-2016 Accepted on 01-11-2016 Published on 05-12-2016

DOI: http://dx.doi.org/10.6000/1927-3037.2016.05.04.4