World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:8, No:5, 2014

Antimicrobial Activity and Phytochemicals Screening of Jojoba (*Simmondsia chinensis*) Root Extracts and Latex

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Abstract**—**Plants are rich sources of bioactive compounds. In this study the photochemical screening of hexane, ethanolic and aqueous extracts of roots and latex of jojoba (*Simmondsia chinensis*) plant revealed the presence of saponins, tannins, alkaloids, steroids and glycosides. Ethanolic extract was found to be richer in these metabolites than hexane, aqueous extracts and latex. The extracts and latex displayed effective antimicrobial activity against *Salmonella typhimurium, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus flavus*. The increase in volume of the extracts and latex caused more activity, as shown by zones of inhibition. *Candida albicans* growth was inhibited only by hexane extract. Jojoba latex was not effective against *Candida albicans* at 0.1 and 0.5 ml extracts concentration but showed 5mm zone of inhibition at (1.0 ml). Lower volume (0.1ml) of latex encouraged *Aspergillus flavus* growth, while at (1.00 ml) reduced its mycelial growth. Thus, jojoba root extracts and latex can be of potential natural antimicrobial agents.

Keywords**—**Antimicrobial activity, Jojoba (*Simmondsia chinensis*), latex, photochemical, root Extracts.

I. Introduction

CONCERN over pathogenic and spoilage microorganisms in foods are increasing due to the increase in outbreaks of food borne disease. Currently there is a growing interest in using natural antibacterial compounds, like essential oils and extracts of various species of edible and medicinal plants, herbs, and spices which have long been used as natural agents for food preservation due to the presence of antimicrobial compounds [1]

Anti-microbial agents are substances that kill microorganisms or inhibit their growth and they are widely employed to cure bacterial diseases [2]. Plants are rich sources of bioactive compounds. Many of these naturally compounds were of antimicrobial functions and serve as a source of antimicrobial agents against food-borne pathogens [3].

There are thousands of species of medicinal plants used globally for the cure of different infections [4]. These plants are used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body.

Plants have the capacity to produce a large number of organic chemical called as phytochemicals [5]. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins, essential oils and other aromatic compounds. In addition, many biological activities and antibacterial effects have been reported for plant tannins and flavonoids. Some of these phytochemicals can significantly reduce the risk of cancer due to poly-phenol antioxidant and anti-inflammatory effects as reported by [6].

According to the current trend of giving value to natural and renewable resources, the use of natural antimicrobial compounds particularly in food and biomedical applications, becomes very common [7]. Numerous efforts were conducted to find natural alternatives to prevent bacterial and fungal growth in foods. It was reported that plant extracts and phytochemicals with known antimicrobial properties can be of great effects towards therapeutic treatments [8]. Extracts of various plant parts such as leaf, stem, root, fruit and seeds were found to be effective against seed-borne pathogenic fungi [9].

It is known, that latex has been used as a term for the fluid substance in plants [10]. It is usually secreted after tissue injury. In most plants, latex is white, but some have yellow, orange, or scarlet. It was reported that latex as found in nature is a milky fluid found in 10% of all flowering plants (angiosperms) [11]. Latex is a complex emulsion consisting of proteins, alkaloids, starches, sugars, oils, tannins, resins, and gums that coagulates on exposure to air. The study of [5] cleared that the leaf and latex extracts of *Calotropis gigantea* was effectively inhibited the growth of some organisms. Also, the latex of *Jatropha curcas* showed antibacterial activity against *Staphylococcus aureus* [12].

With the intent of exploring bioactive compounds from plant origin, jojoba (*Simmondsia chinensis*) plant has been selected in the present work. *Simmondsia chinensis* (Link) C. Schneider commonly is known as jojoba and belongs to family *Simmondsiaceae*. It is mostly woody, evergreen, perennial shrubs that produce small seeds containing waxy liquid. The plant is native to Southern Arizona, Sonora and Baja California. Now, it is cultivated in different parts of the world including Egypt due to its high economic value [13]. The phytochemicals investigation of different jojoba extracts and the wax obtained from its seeds led to identification and characterization of different components belonging to

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different classes of secondary metabolites [14]. The flavonoid profile of jojoba plant fruits may place this family among other families which are rich in flavones methyl ethers and flavonoid content which make the pericarp a valuable source for antioxidant and hepato-protective compounds [15]. This plant extract has been reported to be useful as a dietary supplement for use in a weight control regiment in humans, a component of functional food, a food additive, a medical food, or as a therapeutic agent [16]. Different extracts from jojoba plant are widely used in many folk medicinal uses. Most of the previous work was directed only on the seeds, fruits and leaves of this medicinally important plant [13]. Also, jojoba liquid wax generally used in folk remedies however, there are no reports on the roots of jojoba (*Simmondsia chinensis*) [17]. The same authors also reported that a lot of works as suggested by literature survey had been carried out on seeds and seed oil but, there are no reports on roots. Worthy to note, other investigators [18] reported through their studies that the roots of other jojoba kinds e.g. *Zizyphus oxyphylla* Edgew *(Rhamnaceae*) was used to cure different ailments [19]. Moreover, a cold suspension of dried roots powder of Ampelozyziphus *amazonicus* was used to prevent malaria [20].

There is no doubt that, microbial growth is a major concern because some micro-organisms can potentially cause food-borne illness. Due to the great consumer awareness and special concern regarding synthetic chemical additives, foods preserved with natural additives have become very popular. Therefore, the aim of the present study was to screen the most important phytochemicals and antimicrobial activity of various extracts (hexane, ethanol and aqueous) of jojoba (*Simmondsia chinensis*) root and latex against some food-borne pathogenic microorganisms

II. Materials and Methods

A. Plant Material

Roots of jojoba (*Simmondsia chinensis*) plant and latex were obtained from the farms of the Egyptian Natural Oil Company, Ismailia Desert Road, Egypt. The roots were washed under running tap water, air dried and then grounded to fine powder and stored in airtight bottles. The latex was collected and kept into sampling bottles and stored in the refrigerator at 4ºC.

B. Extracts Preparation

Hexane, ethanolic and aqueous extracts of jojoba roots were prepared as follows: Air-dried powder of jojoba roots (10 g), 50 ml of hexane was added and placed into an air tight cork bottle then kept on a rotary shaker at 190-220 rpm for 24 hrs. The extract was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was sterilized by using membrane filtration unit. The obtained filtrate was stored in sterile bottles and kept in the refrigerator at 4ºC until use. Similarly, ethanolic and aqueous extracts were prepared using ethanol (95%) and sterile distilled water respectively. Solvents (analytical grade) for extraction were obtained from Sigma–Aldrich, St. Louis, MO, and (USA).

Latex from the plant was collected into sterile sampling bottles and centrifuged at 5000 rpm for 15 min. then sterilized using the membrane filtration unit. The sterile filtrate was stored in sterile bottles and kept in the refrigerator at 4ºC until use.

C. Phytochemicals Screening

Tests for the presence of saponins, tannins and alkaloids were carried out according to [21]. Lieberman Burchad reaction was used to test steroids as described by [22] while the Salkowski test was used for the presence of glycosides.

D. Microorganisms

The microorganisms used in this study: *Salmonella* typhimurium, *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus flavus* were obtained from Naval Medical Research Unit 3 (NAMRU 3) Cairo, Egypt. The bacteria and *Candida albicans* were maintained on nutrient agar slant and Sabouraud's Dextrose Agar (SDA) respectively then stored at 4ºC. Both bacteria and *Candida albicans* were sub-cultured onto fresh media at regular intervals while, *Aspergillus flavus* was cultured on Potato Dextrose Agar (PDA) and sub-cultured at regular intervals until used for the antimicrobial tests.

E. Antibacterial Activity

The antibacterial activity of the root extracts and latex of jojoba were determined using the agar diffusion method of [23]. Fresh broth culture of test organisms (standardized inocula) was swabbed onto sterile Mueller Hinton Agar in Petri dishes. A sterile stainless steel cork borers (12 mm) was used to make the wells on the plates. The holes were filled with the root extracts and the latex in different concentration (0.1 ml, 0.5 ml and 1.0 ml for each). For control experiments the holes were filled with sterile distilled water. The inoculated Petri dishes were left for an hour at room temperature for the extracts to diffuse before the growth of organisms commenced, then incubated at 37ºC for 24 h. The microbial growth was determined by measuring the diameter of zone of inhibition (mm).The experiment was done three times and the mean values are presented.

F. Antifungal Activity

The pour plate method was used for the assay of the effect of the root extracts and the latex of jojoba against *Aspergillus flavus*. Sterile root extracts were introduced into test tubes containing sterile Potato Dextrose Agar. For the latex 0.1 ml, 0.5 ml, and 1.0 ml were incorporated into 19.9 ml, 19.5 ml and 19 ml of the medium respectively. These were dispensed of Petri dishes and allowed to set. Each plate was bored with sterile cork borer of 12 mm in diameter. The plates were inoculated by using a sterile cork borer 12 mm to cut the advancing edges of young cultures of *Aspergillus flavus* which were used to replace the ones cut on the plate using sterile forceps. Control experiments were also setup performed

without jojoba root extracts and latex on 20 ml medium. Plates were incubated in upright position at 30ºC for 72 h.

G. Determination of Minimum Inhibition Concentration (MIC)

After determining the inhibition, the MIC of the test samples at the concentration of 0.1, 0.5 and 1.0 ml were measured against the test organisms.

Agar diffusion method described for antibacterial test was also used in determining antifungal action of the jojoba root extract and latex against *Candida albicans*. Control experiments were also carried out where the holes on the agar plates were filled with sterile distilled water. Plates were left at room temperature for one hour before incubation at 30ºC for 48h. MIC determination of *Aspergillus flavus* followed antifungal test. Plates were incubated in upright position at 30ºC for 72 h. Diameter of zone of inhibition due to the activity of the extracts and latex was measured (mm) after the period of incubation.

III. Results and Discussion

A. Phytochemicals Screening

Three extracts (hexane, ethanol and aqueous) of jojoba (*Simmondsia chinensis*) roots and latex were subjected to qualitative phytochemicals screening in the present study. The results revealed the presence of saponins, tannins, alkaloids, steroids and glycosides as shown in (Table I).

TABLE I
PHYTOCHEMICALS SCREENING OF JOJOBA ROOT EXTRACTS AND LATEX

Phytochemicals	Extracts			
	Hexane Extract	Ethanol extract	Aqueous extract	Latex
Saponins	+	++	+	+
Tannins	+	++	+	++
Alkaloids	+	++	++	+
Steroids	-	+	+	+
Glycosides	+	++	+	-

(++)= positive; (+) = Slightly Positive; and (−) = Negative

Saponins, tannins and alkaloids were present in all the root extracts which is in agreement with a previous report by [24] who found that plants contained components which were active against microorganisms. The ethanol extract was richer in these metabolites than both hexane and aqueous extracts. This may be due to the ability of ethanol to extract more components. However, all the extracts were slightly rich (slightly positive +) in steroids except in the case of hexane extract which showed no steroids. On the other hand, latex was of no glycosides but was found of slight saponins, alkaloids, steroids and has rich tannin contents. Such preliminary phytochemicals screening may be useful for detection of different constituents in the used polar and non-polar solvents. It was reported by [25], [26] that some herbal preparations were found to contain phytochemicals such as saponins, tannins, alkaloids, anthraquinone and cardiac glycosides, these phytochemicals are known to have antimicrobial effects. Most antibacterial substances have the following modes of action: inhibition of cell wall synthesis, disruption of cell membrane function, inhibition of protein synthesis, inhibition of nucleic acid synthesis and also acting as anti-metabolites [27]-[29]. Also, Igbinosa et al. [30] reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention. Moreover, the inhibitory effect of saponins on inflamed cells was revealed. Furthermore, alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications. In addition, steroidal extracts from some medicinal plants exhibited antibacterial activities on some bacterial isolates.

B .Antimicrobial Activity

Antimicrobial activity of various extracts (hexane, ethanol and aqueous) of jojoba (*Simmondsia chinensis*) roots and latex against the employed microorganisms was qualitatively assessed by the presence or absence of inhibition zones in this study. The obtained results cleared that the three extracts of jojoba roots and latex showed differences in antimicrobial activities against the tested microbial species. Data in (Table II) shows the antimicrobial activity of hexane extracts of jojoba root on different tested organisms. The data cleared that control samples were not sensitive towards all the used microbial species. *Escherichia coli* were not sensitive with no zone of inhibition at all hexane concentrations. *Salmonella typhimurium* was the lowest sensitive bacterium with 13 and 3 mm zones of inhibition at concentration of 1.0 and 0.10 ml respectively. *Bacillus cereus* was the most sensitive with 6 mm zone of inhibition at low concentration (0.10 ml) extract and with 18 mm at 1.0ml. *Staphylococcus aureus* was sensitive at 0.10ml with 5 mm zone of inhibition and reached to 17mm at 1.0ml concentration. *Candida albicans* acquired sensitivity to hexane extract with 20 mm zone of inhibition at 1.0 ml, but with 4 mm zone of inhibition at 0.10 ml extract concentrations. The sensitivity of *Staphylococcus aureus* for hexane extract was found (14 mm) of inhibition at concentrations at 0.50 ml followed by *Clostridium perfringens* (13mm) then *Bacillus cereus* (11.5mm).

TABLE II
ANTIMICROBIAL ACTIVITY OF THE HEXANE EXTRACT OF JOJOBA ROOT ON DIFFERENT TESTED MICROORGANISMS

Organisms	Volume of Hexane Extract (mL)			
	Control	0.10	0.50	1.0
Salmonella typhimurium	-	3*	10*	13*
Bacillus cereus	-	6	11.5	18
Clostridium perfringens	-	4	13	16
Staphylococcus aureus	-	5	14	17
Escherichia coli	-	-	-	-
Candida albicans	-	4	14	20

* = Zone of inhibition in (mm). − = No zone of inhibition.

Regarding the ethanolic extract of jojoba root it was found to show different range of activity against all the tested microbial species except *Candida albicans* which showed no activity (Table III). Meanwhile, *Escherichia coli* was the most

sensitive to ethanolic root extract at the high concentration of (1.0 ml) with 17 mm inhibition zone followed by *Clostridium perfringens & Salmonella typhimurium* with 16 mm and *Bacillus cereus* with 15 mm then *Staphylococcus aureus* came in the last order of activity with 11mm.

TABLE III
ANTIMICROBIAL ACTIVITY OF THE ETHANOLIC EXTRACT OF JOJOBA ROOT ON DIFFERENT TESTED MICROORGANISMS

Organisms	Volume of Ethanolic Extract (mL)			
	Control	0.10	0.50	1.0
Salmonella typhimurium	-	5*	11*	16*
Bacillus cereus	-	6	11	15
Clostridium perfringens	-	5	10	16
Staphylococcus aureus	-	3	6	11
Eschrerichia coli	-	4	12	17
Candida albicans	-	-	-	-

* = Zone of inhibition (mm). − = No zone of inhibition

Aqueous extracts of jojoba roots was effective against *Staphylococcus aureus* with 16 mm at concentration of 1.0 ml and 6 mm zone of inhibition at 0.1ml (Table IV), this result was in accordance with the experiment carried out by [31]. Aqueous extract inhibited the growth of all the other tested bacteria with different variations except *Candida albicans* which showed no inhibition.

TABLE IV
ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF JOJOBA ROOT ON DIFFERENT TESTED MICROORGANISMS

Organisms	Volume of Aqueous Extract (mL)			
	Control	0.10	0.50	1.0
Salmonella typhimurium	-	4*	10*	15*
Bacillus cereus	-	5	10	14
Clostridium perfringens	-	4	7	12
Staphylococcus aureus	-	6	12	16
Escherichia coli	-	4	10	12
Candida albicans	-	-	-	-

*= Zone of inhibition (mm). − = No zone of inhibition

The latex of jojoba was not effective against *Candida albicans* at 0.1and 0.5 ml concentration of extracts but showed 5mm inhibition zone (Table V) at higher concentration (1.0 ml). *Salmonella typhimurium* appeared to be of the lowest zone of inhibition at 1.0 and 0.5 ml, with no inhibition zone at 0.1ml. *Staphylococcus aureus*, *Clostridium perfringens, Bacillus cereus* and *Escherichia coli* had successive activities at 1.0 ml; the inhibition at 1.0 ml was higher than at 0.5 ml concentrations. *Staphylococcus aureus* was of the highest zone of inhibition (12mm and 9 mm) at 1.0 ml and 0.5 ml concentrations respectively, whereas its growth showed to be the only bacteria with 3 mm zone of inhibition at the lower concentration (0.1 ml).

TABLE V
ANTIMICROBIAL ACTIVITY OF THE JOJOBA LATEX ON DIFFERENT TESTED MICROORGANISMS

Organisms	Volume of Latex Extract (mL)			
	Control	0.10	0.50	1.0
Salmonella typhimurium	-	-	3*	6*
Bacillus cereus	-	-	5	12
Clostridium perfringens	-	-	8	16
Staphylococcus aureus	-	3*	9	12
Escherichia coli	-	-	6	11
Candida albicans	-	-	-	5

*= Zone of inhibition (mm). − = No zone of inhibition

Worthy to note that Candida *albicans* was not sensitive to ethanolic and aqueous extract but its growth was inhibited by hexane extract. On the other hand, the hexane extract of the jojoba root is the most potent against *Candida albicans*, *Bacillus cereus* and *Staphylococcus aureus.* It was observed that an increase in the volume of the extracts and latex caused more activity, as shown by the diameters of inhibition zones. The noticed differences in the antimicrobial effects of the investigated samples may be due to the phytochemical properties and differences among the samples. It was reported by [32] that it is quite possible that some of the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in solvents.

The antifungal activity of the root extracts and latex of jojoba against *Aspergillus flavus* was shown in (Table VI). The results showed that lower volume (0.1ml) of latex encouraged the growth of *Aspergillus flavus*, while higher volume (1.0 ml) reduced its mycelial growth. Thus latex from Jojoba (*Simmondsia chinensis*) possessed antifungal activity, since the growth of the test fungus was inhibited and this agreed with [33].

TABLE VI
ANTIFUNGAL ACTIVITY OF THE ROOT EXTRACTS AND LATEX OF JOJOBA AGAINST ASPERGILLUMS *FLAVUS*

Extracts	Volume of Extracts (mL)			
	Control	0.10	0.50	1.0
Hexane	18*	18*	16*	12*
Ethanolic	19	22	18	16
Aqueous	16	14	15	14
Latex	19	23	21	17

*=Zone of inhibition (nm)

C. Minimum Inhibitory Concentration (MIC)

As shown in (Table VII) and (Fig. 1) the minimum inhibitory concentration (MIC) indicated that *Salmonella typhimurium*, *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* and *Candida albicans* were sensitive (+) at concentration 0.1ml, 0.5 ml and 1.0 ml of hexane extract of jojoba root (1.5 - 9.5 mm). Meanwhile, *Escherichia coli* at these concentrations were not sensitive.

On the other hand, it was cleared that both ethanol and aqueous extracts at all their concentrations (0.1 ml, 0.5 ml and 1.0 ml) gave the same pattern whereas *Salmonella typhimurium*, *Bacillus cereus, Clostridium perfringens,*

Staphylococcus aureus and *Escherichia coli* were able to inhibit the growth (1.4 -7.8 mm), on the contrary *Candida albicans* was not able to inhibit the growth.

Concerning jojoba latex, it was noticed that *Salmonella typhimurium*, *Bacillus cereus, Clostridium perfringen, Escherichia coli* and *Candida albicans* were not sensitive at low concentration (0.1 ml), while *Staphylococcus aureus* was sensitive (2.5-5.5 mm) at 0.5 ml and 1.0 ml concentrate respectively. In the meantime, the tested bacteria showed inhibition minimum inhibitory concentrations (MIC) of the zones (1.5 - 4.2 mm) at 0.5 ml concentrate except *Candida albicans*. At the higher concentration (1.0 ml) of all the tested microorganisms revealed growth (3.0 -10.0).

TABLE VII
MINIMUM INHIBITORY CONCENTRATION (MIC) OF JOJOBA ROOT EXTRACTS AND LATEX

Organisms	Hexane Extract			Ethanol Extract			Aqueous Extract			Latex			Control
	Extracts in (mL)												
	0.10	0.50	1.0	0.10	0.50	1.0	0.10	0.50	1.0	0.10	0..50	1.0	1.0
Salmonella typhimurium	+	+	+	+	+	+	+	+	+	−	+	+	−
Bacillus cereus	+	+	+	+	+	+	+	+	+	−	+	+	−
Clostridium perfringens	+	+	+	+	+	+	+	+	+	−	+	+	−
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	−
Escherichia coli	−	−	−	+	+	+	+	+	+	−	+	+	−
Candida albicans	+	+	+	−	−	−	−	−	−	−	−	+	−

(+) = Growth indicated inhibition. (-) = No growth indicated no inhibition

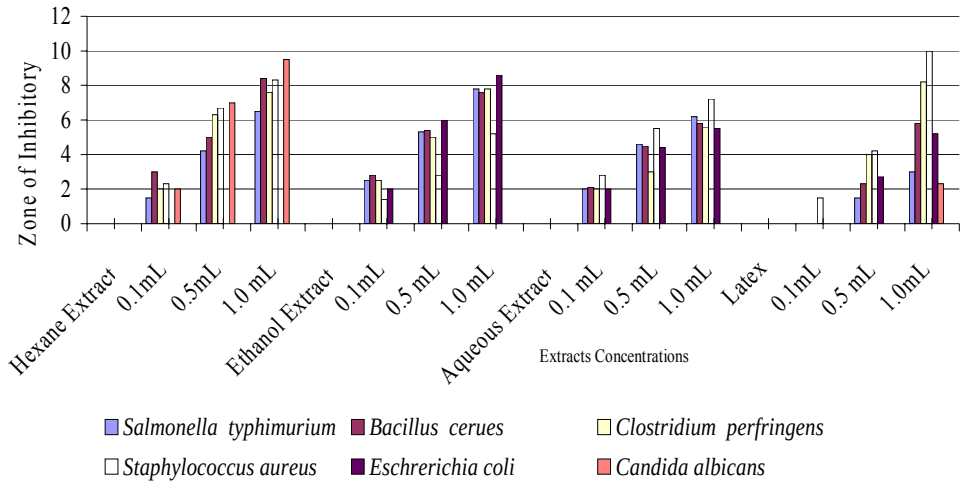

Fig. 1 Minimum inhibitory concentration (MIC) of jojoba root extracts and latex

The latex of jojoba was observed to be more effective at 1.0 ml concentration than each of the extracts due to its ability to inhibit all the tested organisms. The observation based on the minimum inhibitory concentration (MIC) of the extracts (Table VII) was correlated with the reports which found that bacteria varied widely in the degree of their susceptibility [34], [35], and [29].

TABLE VIII
MINIMUM INHIBITORY CONCENTRATION OF JOJOBA ROOT EXTRACT AND LATEX AGAINST *ASPERGILLUS FLAVUS*

Extracts	Volume of Extracts (ml)			
	Control	0.10	0.50	1.0
Hexane	+	+	+	+
Ethanol	+	+	+	RMG
Aqueous	+	+	+	+
Latex	+	+	+	RMG

(+) = Growth indicated no inhibition
RMG = Reduction of mycelia growth

The screening of the jojoba root extracts and latex showed activities against *Aspergillus flavus.* The latex and extracts of jojoba root were effective due to their ability to inhibit the entire tested organism. Both ethanol extract and latex reduced the mycelia growth o*f Aspergillus flavus* (Table VIII).

IV. CONCLUSION

The findings of the present study revealed that the root extracts and latex of jojoba contain potent antimicrobial property. Their antimicrobial tests showed that they exhibited broad spectrum of activities by inhibiting the growth of four investigated bacteria and two fungi. The phytochemicals screening indicated that the tested jojoba root extracts (hexane, ethanol and aqueous) and latex contained saponins, tannins, alkaloids, steroids and glycosides. This can suggest that these extracts and latex have potential use as natural preservatives in food against the well-known causal agents of food-borne diseases and food spoilage.

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