EVALUATION OF THREE ESSENTIAL OILS ACTIVITIES AS ANTIMICROBIAL AND INSECTICIDAL AGENTS

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ABSTRACT: The present work aimed to study the physicochemical properties, chemical composition antimicrobial and insecticied of three essential oils, namely cedar wood (Guniperus virginiana) belonging to famly (Pinaceae), rose wood (Aniba rosaeaodora) belonging to family (Rosaceae) and sandal wood (Santalum album) belonging to family (Santalaceae). These plants are considered to be medicinal and aromatic plants which are well grown in the Egyptian newly reclaimed lands. Evaluation of the oils activity as antimicrobial was done against Grampositive and Gram-negative bacteria, fungi, yeast and larval fourth cotton leaf worm (Spodoptera littoralis). Our results showed that Cedar wood oil was distinguished by highest dextro rotation mean (+43°) while the specific gravity of sandal wood oil was higher than cedar wood oil and rose wood oil. The highest acid value recorded was cedar wood essential oil (2.45). The identified components in cedar wood essential oil were 20. The major compound was Thugopsene (27.5%), also the identified components in rose wood essential oil were 14. The major component was (Linalol 68.174%). As for the identified components in sandal wood essential oil, they were 13. The major component was α-Santalol (50.3%).

Increasing the concentration of essential oils to 100% at least doubled the inhibition zone for most studied bacteria, except Escherichia coli. Also increasing the concentration of essential oils from (10:50%) increases the mortality of larvae, the death of the virgin and the proportion of altezr.

Key words: Medicinal, aromatic, essential oil, antimicrobial, insecticidal,

INTRODUCTION

Medicinal and aromatic plants have always important components for human activities, i.e. spices, medicinal drugs, antioxidants for keeping quality of oils and fats. antimicrobial to some harmful microorganisms (bacteria, fungi and yeast) as well as being used for food preservation. Cedar wood (Guniperus virginiana) is belonging to famly (Pinaceae), rose wood (Aniba rosaeaodora) belonging to family (Rosaceae) and sandal wood (Santalum album) belonging to family (Santalaceae). These plants are considered to be medicinal and aromatic plants which are well grown in the Egyptian newly reclaimed lands. physico-chemical Undoubtedly, the properties of the essential oils are often used to verify whether an oil was really natural, reconstituted or even compounded. The physico-chemical properties of cedar wood essential oil are: specific gravity at 27°C (0.898 to 0.927), optical rotation (-14° to +54°), refractive index at 27°C (1.4752 to 1.4902), acid value (6) and ester value (13.5 to 34). (Lawrence 1985). meanwhile sandal wood essential oil was: specific gravity at 27°C (0.992 to 1.034), optical rotation (-50° to -130°), refractive index at 27°C (1.5132 to 1.5242), acid value (40), ester value (105 to 160), and solubility in 80% (by volume) ethyl alcohol (one to two volumes). (Bruno et al. 2006). Previous research, sandal wood oil was carried out for 38 h using pre immersed whole sandal wood chips in hot water at 95°C for 24 h . The yield of the oil obtained was I.56 wt% and the colour of the oil was pale yellow with pleasant odour with asantalol 56.73%, β -santalol 27.10%, α santalene 0.30% and β-santalene 0.91%. Structures of major constituents responsible

for woody odour and medicinal values are presented (Tiwari, 1989). Also, cedar wood oil was carried out, and major components were: α - cedrene (27.2), β -cedrene (7.7), thujopsene (27.6), cuparene(6.3), cedrol (15.8), and widdrol (1). (Adams 1987). While Linalool was the main component in the essential oil of A. rosaeodora (Adam et al 1998). Sandal wood oil is used in Indian traditional medicine system Ayirveda as antiseptic, antipyretic, antiscabietic, diuretic, expectorant, simpulant and for the treatment of bronchitis, dysuria for the treatment of dysuria, urinary bronchitis, infection, gonorrhoeal recovery owing to antibacterial and antifungal properties (Takaishi et al. 2005). Also Sandal wood oil constituents and their synthetic analogues are known to be strongly antimicrobial and antibacterial (Rodriguez Vaguero et al. 2009). Beside that, rose wood oil showed significant antimicrobial activity (Reynolds and Martindale 1996). Plant essential oils have been described as being of value as insecticides, and clearly the activities of some of the essential oils appear to be the result of effects on the insect nervous system, either by inhibition acetylcholinesterase or by antagonism of the octopamine receptors. The rapid action against some pests is indicative of a neurotoxic mode of action. In this regard they may be acting in ways similar to that of conventional synthetic insecticides. Several reports indicate that essential oils and monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme (ACHE) activity. For example, suggested that the toxic effect may be attributed to competitive reversible inhibition of acetylcholinesterase by occupation hydrophobic site of the enzyme's active centre. Also toxicity of constituents of essential oil is related to the octopaminergic nervous system of insects. Enan (2001). Insecticidal properties for cedar wood shavings from Juniperus virginiana arrested the life cycle at the 1st instar stage of the Peanut Trash Bug (Elasmolomus sordidus).

It also caused the death of colonies of Indian Moths (Plodia interpunctella) and Forage Mites (Tyrophagus putrescentiae). Virginia cedarwood oil (3%), cedrene (2%), and cedrol (2%) were all highly toxic to Peanut Trash Bug colonies. Cedar wood oil and cedrene also affected the reproductive behavior of adults or hatchability of eggs. Colonies of German cockroaches (Blatella germanica) were not affected by cedar wood from Juniperus virginiana. Sabine (1975). Pesticides based on plant essential oils could be used in a variety of ways to control a large number of pests. Moreover, sublethal modification of insect behaviors. including mating, host-finding, feeding and oviposition, by selected blends of essential oils may enhance their overall efficacy. Akhtar et al. (2010). Sandal wood was screened for their acaricidal and oviposition deterrent activities against two-spotted spider mite (TSSM), Tetranychus ur-ticae Koch (Acari: Tetranychidae), in laboratory using a leaf-dip bioassay. Sandal wood oil was observed to be the most effective against TSSM adult females (87.2 ± 2.9% mortality) . Sandal wood oil also demonstrated oviposition deterring effects based on a 89.3% reduction of the total number of eggs on leaf disks treated with the oil. Badawy et al. (2010). Also sandal wood oil demonstrated the highest acaricidal activity against T. urticae adults. Kumral et al. (2010). The present work aimed to study the physicochemical properties, chemical composition antimicrobial and insecticied of three essential oils, namely cedar wood (Guniperus virginiana), rose wood (Aniba rosaeaodora) and sandal wood (Santalum album).

MATERIALS AND METHODS MATERIALS:

The essential oils of cedar wood (*Juniperus* <u>virginiana</u>), rose wood (*Aniba rosaeaodora*), and sandal wood (*Santalum album*) plants were obtained from the local markets.some information about the plants are shown in Table (1).

Plant	Latin names	Family name	
Cedar wood	Juniperus virginiana	Pinaceae	
Rose wood	Aniba rosaeaodora	Rosaceae	
Sandal wood	Santalum album	Santalaceae	

Table (1): listed the latin and family names of the medicinal and aromatic plants under study:

METHODS

Physical and Chemical Properties of Essential Oils Physical Properties: Solubility in 80% ethanol

The solubility in 80% ethanol was determined by titrating a known volume (1ml) of essential oil with ethanol 80% to the point of homogeneity and is calculated as volume / volume.

Specific gravity

The specific gravity of the oils were determined using a pycnometer (1 ml-capacity) as described by Guenther (1960).

Refractive index

The refractive index was determined using Abbe refractometer, Model 60 according to the procedure described in A.O.A.C. (1975).

Optical rotation

The optical rotation of the oils was determined by a polarimeter at room temperature using sodium lamp. The specific optical rotation of the oils were calculated according to the following equation as stated by Guenther (1960):

 $^{t}d = /dI$

Where t = observed optical rotation

d= specific gravity of the oil measured at 20°C

L = length of polarimeter tube in decimeters,

Chemical characteristics: Acid value

The acid value was determined according to the method described in A.O.A.C. (1975). A known weight of the oil (1g) was dissolved in a neutral ethanol (10 ml) and directly titrated by ethanolic potassium hydroxide (0.1N) using phenolphthalein as an indicator.

Acid value =
$$\frac{V \times N \times 56.1}{W}$$

Where

V = volume in millileters of KOH solution.

N = normality of KOH solution.

W= weight of the oil in grams.

Saponification number:

The saponification number of the various substances encountered in the present investigation was determined according to the following procedure which was essentially similar to the standard procedure previously reported by Gunther (1961).

Gunther used phenolphthalein as an indicator which proved to be successful with the end point determination .

About 1.5 gram of the oil was accurately weighed in a 150 ml flask The oil was treated with a known volume (10 ml or 20 ml) of ethanolic potassium hydroxide (about 0.5 N). The mixture was heated on a waterbath at 100°C for one hour, using an air cooled condenser. At the end of this period, the excess potassium hydroxide was back titrated with hydrochloric acid (0.15 N) using the indicator. A blank determination was carried out using the same quantity of ethanolic potassium hydroxide.

The saponification number was calculated from the following equation:

saponification number =

56.1(Blank titr.-titr.ofsample)xN of HCl weight of the sample(oil) in grams

Ester value:

The saponification number represents the ester value of essential oil when the acid value of the sample is small. However, in the case of the sample with high acid value, the ester value represents the difference between the saponification value and the acid value.

Ester value = Saponification number - Acid value

Chemical composition:

In order to study the chemical composition of cedar wood, rose wood and sandal wood essential oils, gas chromatography / mass spectrometry technique was used.

Antimicrobial activity of cedar wood, rose wood and sandal wood essential oils:

The microorganisms Citrobacter sp., klebsiella sp., sallmonilla sp., Protus sp., Lacto bacillus. Escharichia coli, Pythium sp., Rhizopius sp., Beauveria bassiana, Saccharomyces cervisiae and rhodotorula sp. were obtaind from microbiology department, faculty of agriculture, Zagazig university.

Disc-diffusion method:

The disc-diffusion method was used for detection of the antimicrobial activity of essential oils as described by Conner and Beuchat (1984).

The essential oils of cedar wood, rose wood and sandal wood were diluted in tween 80% to give solutions with different concentrations 40%, 70%, and 100% (V/V). The appropriate medium was poured into sterile plates (100 mm diameter), left to solidify at room temperature, the organisms

were inoculated on the surface of the previous media. Sterile 6 mm diameter of whatman (No.1) filter paper were dipped in the appropriate oil solution, blotted and then placed on the surfaces of inoculated plates. The inhibitory effect of the tween 80 was also tested by placing disc saturated with tween 80 on each inoculated plate. The plates were incubated at 37°C (bacteria), 28°C(fungi), 30°C (yeast), for 48-72 hours. Finally the inhibition zones around the disc was measured (mm). All testes were conducted in triplicates.

Antifungal activity of essential oils:

The pathogenic Fungi to several crops: (Rhizopius (*Pythium* sp.), sp.) (Beauveria bassiana) were obtained from department microbiolay. faculty agriculture, zgazeg university. Determination of the inhibitory effect of essential oils. The filter paper disk diffusion plate method of Loo et al. (1945), was employed. Essential oils of cedar wood, rose wood and sandal wood were prepared and potato dextrose agar medium (P.D.A) in petri-dishes was used throughout this study. Discs (5mm diam) were taken from the margin of each fungal colony into P.D.A plates grown for six days at 28°C. The disc was placed into the center of each plate under a septic condition. On the other hand, filter paper discs (whatman No 1mm. diam) was impregnated with each essential oil in different concentrations then the discs were placed. 2cm for from the fungal disc. the inoculated PDA plates were Then inverted and incubated at 28°C for 48-72 until surface of the control ones (plates containing filter paper discs without the essential oils). This test was performed three successive times for each sample.

Insecticidal activity of essential oils

This experiment was conducted to identify some alternative natural materials such as essential oils, against larval fourth

to cotton leaf worm (Spodoptera littoralis), (Zaki and Abd EL-Raheem, 2010). Where access to cotton leaf worm after graduating from aellata (eggs) from the first larval stage until the fourth larval stage in the agricultural research national institute of Zagazig, East-Sharkia. tween 80 used to prepare the different concentrations for each of the essential oils which were fed for worm larvae cotton leaf on castor paper to provide year-round after treated with various concentrations of the oils, and use Houdan at a temperature of (26°C ± 1) for the treatment of three days feeding. Treatment castor paper with different concentrations, 10% and 25% 50% were conducted for four replications with a control not treated with oil - in each duplicate three jars - and each jar contained (10) larvae of larval quarter of cotton leaf worm (Spodoptera littoralis) to be in duplicate each (30) larval then placed in jars Houdan at a degree of (26°C ± 1) for a period of three days of feeding.

RESULTS AND DISCUSSION Physico-chemical properties of essential oils:

The physico-chemical properties of cedar wood, rose wood and sandal wood essential

oils were determined and the results are shown in Table (2). For optical rotation, cedar wood was distinguished by the highest dextro rotation (+43°). While rose wood was the lowest dextro rotation (+15°). Sandal wood essential oil was characterized by levo rotation of (-0.20°). The specific gravity of sandal wood essential oil (1.0158) was higher than rose wood and cedar wood which recorded 0.928 and 0.925, respectively. The refractive index also showed the same trend in which sandal wood had high value (1.507) while rose wood and cedar wood recorded 1.471 and 1.462 respectively. The solubility of sandal wood essential oil in 80% ethanol was found to be 1:1 vol / vol ratio while this ratio was (1:1.5 vol/vol) in rose wood but cedar wood essential oil was 1: 1.7 vol/vol. The acid value of essential oils under investigation was 1.73 for rose wood, while cedar wood was 1.96 and sandal wood 2.45. The higher values indicated the presence of high amounts of organic acids. As for ester value, data in Table (2) showed that cedar wood essential oil was found to have the highest value (187.54), followed by sandal wood essential oil which recorded 184.75, while rose wood oil was having the lowest ester value (176.97).

Table (2): Phsico-chemical properties of cedar wood, rose wood and sandal wood essential oils.

Property	Cedar wood	Rose wood	Sandal wood
Optical rotation	+43°	+15°	-0.20°
Specific gravity	0.925	0.928	1.0158
Refractive index	1.462	1.471	1.507
Solubility in 80%Ethanol	1:1.7	1:1.5	1:1
Acid value	1.96	1.73	2.45
Saponification number	189.5	178.7	187.2
Ester value	187.54	176.97	184.75

Chemical composition of essential oils:

The major compound found in cedar wood essential oil, was thugopsene (27.5%), α-cedrene (25%), cedrol (14.8%), coumaranone (8.196%), β-cedrene (7.5%) while flavone, 2, 5, 6, 6-tetramethoxy (4.301%), 5-hydroxy-7-methoxy (3.188%), were presented in amounts less than (10%). the minor compounds in such oil were α tocopherol (1.933%), vicidrol (1.5%), 5,7,4trimethoxykaempferol (1.277%), the other identified compounds occurred as trace materials such as apignin 7-glucoside 3-oxolutein (0.401%), and α -(0.779%),tocopherol, o acetyl (0.391%), 5-hydroxy-7methoxyflavone (0.383%), (-) -galbulin (0.247%), coumarin,4,5,7-trimethoxy - 3 -(p-methoxyphenyl) (0.228%), nabilone β -carotene - 4,4 - dione (0.227%),(0.103%), isoflavone,3,4,6,7-tetramethoxy-(0.048%), and genistin (0.0003%).

The rose wood essential oil contains mainly linalool (68.174%), flavone, 2, 5, 6, 6tetramethoxy- (11.981%), in the mean while α-terpineol (4.33%), genkwanin (3.633%), isocoumarine, 3,4- dihydro- 4,8-dihydroxy-3menthyl-, (-) - (1.776 %), sinapic acid (1.355%) and cannabinol (1.317%), presented in amount less than (10%), the other compounds occurred as trace materials such as α- tocopherol (0.832%),α-selinene (0.7%), β-cymene (0.7%),nerol (0.6), sabinene (0.48), thugene (0.34%), hydrocinnamic (0.079%).

The major compounds in sandal wood essential oil were α -santalol in relative concentration of (50.3%), β -santalol in relative concentration of (27.8%), and dihydroxymaleic acid (13.2%). Data showed also that (-)- isolongifolol (1.203%), were in relative concentration of 3,4-dihydro- α -ionone (1.147%) and β -santalene (1.14), Cis -13,16- docasadienoic acid (1.026%), α -

santalene (0.7), conifery alcohol (0.635%), respectively, it as also clear from such data that the following compounds were presented as trace substances: 4-(2,6,6-trimethyl-1-cycolohexen-1-yl)-2-butanone (0.544%), isocarveol (0.416%), humulene (0.396%) and 5-hydroxy-2,3,3-trimethyl-2-(3-methy1-buta-1,3-dieny1)-cyclohexanone (0.373%).

Antimicrobial activity of essential oils:

Although there are several chemicals that can be used as antimicrobial agents, most of them cannot be added to our food to prevent the growth of microorganisms because of their hazardous effect on human health. Spices are widely used through out the world to add flavours to foods. Various spices essential oils were tested for their inhibitory activity towards the growth of some microorganisms. It was proved by several investigators that spices posses antimicrobial activities and are safe for food.

The three spices essential oils were tested against (5) strains of Gram-positive Bacteria , (1) strain of Gram- negative Bacteria, fungi and yeast. The chosen microorganisms are either pathogens to human and plant or food spoilage organisms.

Results shown in tables (3,4) showed the effect of the essential oils of cedar wood, rose wood and sandal wood as diameter of inhibition zone in (mm) on (bacteria), (fungi), and the liner growth of (yeast).

Generally, increasing the cedar wood and sandal wood essential oils to 100% caused doubling of inhibition zone for most of the studied bacteria, except (*Escherichia coli*). Mean while increasing rose wood essential oil to (100%) caused an increment of inhibition zone for (*Citrobacter* sp.), (*Klebsiella* sp.) and (*Sallmonilla* sp.).

Table (3): diameter of inhibition zone of (bacteria) resulted from application of cedar wood rose wood and sandal wood essential oils (mm):

wood, rose wood and sandar wood essential ons (min).								
Essential oils			Diameters of the inhibition zones (mm)					
Concentration		Citrobacter	Klebsiella	Sallmonlla	Protus	Lacto.bacillus	Escherichia.coli	
		sp.	sp.	sp.	sp.			
Cedar	40	7	7`	9	9	0	0	
wood oil	70	10	8	10	10	0	0	
	100	15	10	12	12	10	0	
Rose	40	7	7	8	0	0	0	
wood oil	70	8	10	9	0	0	0	
	100	11	15	11	0	0	0	
Sandal	40	21	9	11	7	0	0	
wood oil	70	31	11	22	10	8	0	
	100	45	13	50	13	10	0	

Data in table (4) show that increasing the cedar wood and sandal wood essential oils to 100% caused the highest inhibition zone for all treatments on all studied fungi, mean while increasing the rose wood essential oil to (100%) caused the highest inhibition zone for *Beauveria bassiana*. Increasing the sandal wood essential oil to (100%) caused an increment of inhibition zone for all treatment on all studied yeast, mean while increasing the cedar wood and rose wood and essential oils to (100%) caused an increment of inhibition zone for *Rhodotorula* sp.

It is well established that cytoplasm membrane coagulation, breakdown of protons motive force, electron flux and active trans-port unbalance are critical events responsible for providing the antimicrobial property to phytochemicals. Rodriguez Vaquero *et al.* (2009).

Insecticidal activity of essential oils:

Although there are several chemicals that can be used as insecticidal agenst, most of them cannot be used because of their hazardous effect on human health.

Various spices essential oil were tested for their inhibitory activity towards the growth of some insects. It was proved by several investigators that spices posses insecticidal activities and are safety. Zaki and Abd EL-Raheem (2010).

Results presented in table (5) showed the effect of the essential oils of cedar wood, rose wood and sandal wood on the larval stage iv of the cotton leaf worm. Increases the oils concentrations from (10 to 50%) led to increasing the mortality of larvae to (16.66%), death of the virgin to (12%) and the proportion of altezr to (83.33%) cedar wood oil. Mortality of larvae (20%), death of the virgin was (13.33%) and the proportion of altezr was (80%) for rose wood oil. Mortality of larvae was (23.33%), death of the virgin was (27.77%) and the proportion of altezr was (76.6%) for sandal wood oil.

The activities of some essential oils appear to be the result of effects on the insect nervous system, either by inhibition of acetylcholinesterase or by antagonism of the octopamine receptors. Inhibition of acetylcholinesterase by occupation of

hydrophobic site of the enzyme's active centre. Also toxicity of constituents of essential oil is related to the octopaminergic nervous system of insects. Enan (2001). Toxicity may result from the inhibition of the mitochondrial electron transport system because changes in the concentration of oxygen or carbon dioxide may affect respiration rate of insect, thus eliciting fumigant toxicity effects. Emekci *et al.*

(2004). Insecticidal activity of essential oils, even from the same source, can be inherently variable for many reasons. The chemical composition and broad spectrum of biological activity for essential oils can vary with plant age, plant tissues or organs used in the distillation process, the type of distillation and the species and age of a targeted pest organism. Akhtar *et al.* (2010).

Table (4): Diameter of inhibition zone of fungi and yeast resulted from application of cedar wood, rose wood and sandal wood essential oils (mm):

cedai wood , rose wood and sandai wood essentiai ons (mm).						
Essentia	al oils	Diameters of the inhibition zones (mm)				
Concentration		Fungi			Yeast	
		Pythium.sp.	Rhizobius.sp.	Beauveria bassiana	Saccharomycess .cerviciae	Rhodotorula sp.
Cedar	40	0	0	9	0	7
wood	70	9	7	13	0	11
oil	100	13	10	20	0	15
Rose	40	0	0	8	0	0
wood	70	0	0	15	0	7
oil	100	0	0	30	0	10
Sandal	40	20	7	15	9	7
wood	70	30	10	20	13	9
oil	100	40	20	40	20	13

Table (5): Effect of cedar wood, rose wood and sandal wood essential oils on larval mortality%, pupal mortality% and pupation%.

Essential oils concentration		Larval mortality %	Pupal mortality %	Pupation %
Cedar	10	6.66	7.14	93.3
wood oil	25	10	7.4	90
	50	16.66	12	83.33
Rose	10	6.66	6.66	93.33
wood oil	25	10	10	90
	50	20	13.33	80
Sandal	10	6.66	3.70	93
wood oil	25	13.33	13.04	86.6
	50	23.33	27.77	76.6

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تقييم نشاط ثلاثة من الزيوت الطبية والعطرية كمضادات ميكر وبية وعوامل مضادة للحشرات

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الملخص العربي

يهدف هذا البحث إلى دراسة الخصائص الفيزيائية والكيميائية لثلاثة زيوت أساسية وهي : زيت خشب السدر وزيت خشب الورد وزيت خشب الورد وزيت خشب الصندل.

وتعتبر هذه النباتات نباتات طبية وعطرية تجود زراعتها في الأراضي المصرية المستصلحة حديثا . تم تقييم نشاط هذه الزيوت كمضادات للميكروبات ضد (البكتيريا الموجبة والسالبة لجرام والفطريات والخميرة وكذلك تقييم نشاط هذه الزيوت كمضادات للحشرات ضد الطور اليرقي الرابع لدودة ورق القطن .

ومن خلال النتائج المتحصل عليها وجد أن زيت خشب السدر يتميز بأعلى قيمة للدوران الضوئي (+43) في حين أن الكثافة النوعية لزيت خشب الصندل كانت أعلى من خشب السدر وخشب الورد. وسجل زيت خشب الصندل أعلى قيمة لرقم الحموضة (2.45). تم دراسة التركيب الكيميائي لزيت خشب السدر ووجد أنه يحتوي على 20 مركب وكان المركب الرئيسي به هو (-2.45) هو المركب الرئيسي في زيت به هو (-2.45) هو المركب الرئيسي في زيت خشب الورد أما بالنسبة لزيت خشب الصندل فالمكون الرئيسي له هو -2.45 بنسبة -2.45 بنسبة -2.45

زيادة تركيز الزيوت الأساسية إلى 100٪ تسبب في اكبر زيادة في منطقة التثبيط لكل المعاملات علي سلالات الميكروبات والفطريات والخمائر.

أيضا زيادة تركيز الزيوت الأساسية من 10 الى 50٪ زاد من نسبة موت اليرقات، ونسبة موت العذراء ونسبة التعذر بدرجة كبيرة.