Research Article

Antiviral – Antimicrobial and Schistosomicidal Activities of *Eucalyptus camaldulensis* Essential Oils

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ABSTRACT

The investigation was designed to determine the antiviral, antimicrobial and schistosomicidal, effects of the leaf essential oil of *Eucalyptus camaldulensis*. Rotavirus Wa strain, Coxsackievirus B4, and herpes virus type 1 were affected by essential oil with percentage of reduction 50%, 53.3%, and 90% respectively. On the other hand, no effect was found at all on adenovirus type 7. Regarding antimicrobial effect, essential oil has high effects against gram positive and negative bacteria with inhibition zones ranged from 9.3 to 12.5 Mm. The same effect was observed on yeast (21% inhibition) and fungi (10% inhibition). The Scanning Electron Microscope observation showed that IC_{90} of essential oil produced sever damage in schistosoma worm's typography. Therefore, the essential oils from *E. camaldulensis* are active candidates and could be used as RNA antivirus, antimicrobial and shistosomicidal agents in new drugs preparation for therapy of infectious diseases.

Keywords: Antimicrobial activity, Antivirus, Essential oil, Schistosomicidal.

INTRODUCTION

Aromatic plants have great importance for food, cosmetics and pharmaceutical industries. Their use has taken place since ancient times, and despite many of them were substituted by synthetic ones and the demand for natural products is increasing. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production.[1] Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties.[2,3] Some oils have been used in cancer treatment; some other oils have been used in food preservation, aromatherapy and fragrance industries.[4-6,1] Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential.[7] In addition, considerable scientific evidence suggested that under situations of oxidative stress, reactive oxygen species (ROS) such as superoxide, hydroxyl, and peroxyl radicals are generated and the balance between anti-oxidation and oxidation is believed to be a crucial concept for maintaining a healthy biological system.[8]

Schistosomiasis is increasing in incidence despite concerted efforts to control and contain the disease in the endemic areas. It is estimated that 200 million people are infected by trematode of the genus *Schistosoma*.[9] While a multipronged method of control using health education and snail control has been used, chemotherapy plays the crucial role in preventing the transmission of the disease. A significant advance in the control of schistosomiasis chemotherapy occurred by the introduction of the relatively safe, effective, and broad-spectrum oral Anthelmintic agent, Praziquantel (PZQ). Unfortunately, reports suggest that partial resistance to PZQ may be developing. [10] New drug discovery technologies are making natural products a more exploitable source of chemical diversity for novel compounds.

Eucalyptus is one of the diverse genus of flowering plants in the world belongs to the family Myrtaceae (subfamily Myrtoideae) and comprises about 800 species.[11] Eucalyptus has been used in folk medicine throughout the world as anti-inflammatory, analgesic and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion. Eucalyptus trees are among the most important hard wood forestry crops worldwide and provide a major source of pulp wood for high quality paper production.

Moreover, Eucalyptus has been prized a rich source of essential oils. Essential oils of various species have been used in the pharmaceutical, cosmetics and medicinal purposes.[12-14] The minimum 1,8-cineole content of pharmaceutical-grade Eucalyptus essential oil as defined in most standards is 70%.[15] In the cosmetics industry, Eucalyptus essential oil is used in detergents, toiletries and little employed in perfumes and as a flavoring agent

in food. In addition, the essential oils of Eucalyptus species possesses important biological activities including diaphoretic, disinfectant, antimalarial, antiseptic, analgesic, anti inflammatory, antibacterial and antioxidant properties.[13,16,17] So the present study aimed to evaluate the antiviral - antischistosomicidal and antibacterial activities of *Eucalyptus camaldulensis* essential oil.

MATERIALS AND METHODS

Plant materials

The leaves of *E. camaldulensis* were collected in March 2014 from one farm in Qalyubia Governorate, Egypt. The plant was botanically identified by the staff at the herbarium of the Botanical Garden of the Orman, Giza, Egypt. Voucher specimen was deposited at the herbarium of Orman garden, Cairo, Egypt (ASU-ECM2007).

Isolation of essential oils

Fresh adult leaves (200 g) were washed and submitted to hydrodistillation for 5 h using a Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (1997), two replicate extractions were done. The essential oil was dried over anhydrous sodium sulfate. The oil was stored at 4°C until further analysis.

Antiviral activity

Cytotoxicity test

It was done according to previous literature.[18,19] Ten-fold dilutions were done to 100 µL of original sample. 100 µL of original sample and 100 µL of each dilution were inoculated in Hep-2, MA104, BGM, and Vero cell lines (obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt) previously cultured in 96 multi well plates (Greiner-Bio one, Germany) to estimate the non toxic dose of the tested samples. Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying trypan blue dye exclusion method.

Cell morphology evaluation by inverted light microscopy

Hep-2, MA104, BGM, and Vero cell cultures (2×105 cells/mL) were prepared in 96- well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation at 37 ◦C in a humidified 5% (v/v) CO2 atmosphere cell monolayers were confluent, the medium was removed from each well and replenished with 100 µL of original and ten-fold dilutions of the sample prepared in DMEM (GIBCO BRL). For cell controls 100 µL of DMEM without samples was added. All cultures were incubated at 37 ◦C in a humidified 5% (v/v) CO2 atmosphere for 72 h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, and cytoplasm granulation and vacuolization. Morphological changes were scored.[18]

Cell viability assay

It was done by trypan blue dye exclusion method.[19] HEp-2, MA104, BGM, and Vero cell cultures (2×105 cells/mL) were grown in 12-well tissue culture plates (Greiner-Bio one, Germany). After 24 h incubation, the same assay described above for tested samples cytotoxicity was followed by applying 100 µL of tested samples dilutions (bifold dilutions) per well. After 72 h the medium was removed, cells were trypsinized and an equal volume of 0.4% (w/v). Trypan blue dye aqueous solution was added to cell suspension. Viable cells were counted under the phase contrast microscope.

Determination of adenovirus type 7, rotavirus Wa strain, and Coxsackievirus B4

Titers using plaque assay

Non toxic dilutions were mixed (100µl) with 100µl of different doses (1X105, 1X106, 1X107 PFU/ml) of adenovirus type 7, rotavirus Wa strain Coxsackievirus B4, and Herpes virus type 1. The infectivities of the rotavirus stocks were activated with 10 µg/ml trypsin for 30 min at 37°C. The mixture was incubated for 1/2 hr in 37°C. The inoculation of (100µl) 10 fold dilutions of treated and untreated Adenovirus type 7, rotavirus Wa strain, Coxsackievirus B4, and Herpes virus type 1 was carried out into Hep 2, MA104, BGM, and Vero cell lines respectively in 12 multi well- plates. After 1 hr of incubation for adsorption at 37°C in a 5% CO2-water vapor atmosphere without constant rocking. The plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of 2X media (Dulbecco's Modified Eagle Medium, Gibco- BRL (DMEM) plus 1ml 1% agarose was added to each well, 0.5 µg/ml was added to the media-agarose mixture in the case of rotavirus Wa strainand the plates were incubated at 37°C in a 5% CO2-water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formaline fixation, and the number of plaques counted. The viral titers were then calculated, and expressed as plaque-forming units per milliliter (pfu/mL).

Antimicrobial activity

Microbial strains (MIC)

The microorganisms were obtained from the American type culture collection (ATCC; Rockville, MD, USA) as well as the culture collection of the Agricultural Microbiology Dept., National Research Centre (NRC), Egypt. The Gram-positive bacteria; *Streptococcus faecalis* (ATCC- 47077), *Bacillus subtilis* (ATCC- 12228), *Listeria monocytogenes* (ATCC- 35152), Gram-negative bacteria; *Escherichia coli* (ATCC- 25922), *Salmonella typhi, Pseudomonas aeruginosa* strain OS4, two yeasts; *Saccharomyces cerevisiae* (ATCC- 9763), *Candida albicans* (ATCC- 10231) and one fungi; *Aspergillus niger* were used in the antimicrobial assays.

Culture medium and inoculums

The stock cultures of microorganisms used in this study were maintained on plate count agar slants at 4°C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and was incubated at 37°C for 24 h. About 60 µl of bacterial suspensions adjusted to 10^6-10^7 colony-forming units (CFU)/ml were taken and poured into Petri plates containing 6 ml sterilized nutrient agar medium. Bacterial suspensions were spread to get a uniform lawn culture.

Antimicrobial activity assay

The agar-well diffusion method was applied to detect antimicrobial activity.[20] Wells of 6mm diameter were dug on the inoculated nutrient agar medium and 60 µl of the essential oil, dissolved in dimethylsulfoxide (DMSO) at concentration (500 µg/ml), were added in each well. The wells introduced with 60 µl of DMSO were used as a negative control. The plates were allowed to stand at 4°C for 2 h before incubation to prevent evaporation of tested samples. The plates were incubated at 37°C overnight and examined for the inhibition zone. The diameter of the inhibition zone was measured in mm. An extract was classified as active when the diameter of the inhibition was equal to or larger than 6 mm. All the assays were performed in triplicate and expressed as average values ± SD.

Minimum inhibitory concentration (MIC)

A bacterial suspension (10^6-10^7 CFU/ml) of each tested microorganism was spread on the nutrient agar plate. The wells (6 mm diameter) were dug on the agar plate, and 60 µl of the essential oil, dissolved in DMSO at different concentrations (25, 50, 100, 150, 200 and 300 µg/ml) were delivered into them. The plates were allowed to stand at 4°C for 2 h before incubation to prevent evaporation of tested samples. The plates were incubated at 37°C for 24 h under aerobic conditions then followed by the measurement of the diameter of the inhibition zone expressed in millimeter. MIC was taken from the concentration of the lowest dosed well visually showing no growth after 24h.

In vitro schistosomicidal activity

Parasite material

Faeces-free intestines excised from infected hamsters 8-10 weeks after exposure to S. mansonicercariae were the source of schistosome eggs. Tissues were cut into pieces and placed into one liter stainless steel container with 100 ml of 0.85% saline. The container was placed on a Waring blender and homogenized for 5-10 sec at a very low speed (30 volts). The suspension was poured into a tiered column of sieves arranged in descending order of mesh pores (425, 180, 105 and 45 µm). Eggs were washed through to the bottom sieve with 1000 ml of 0.85% saline. A volume of 100 ml cold water was poured into the sieve column to rinse eggs from saline. Eggs were cleaned by manual centrifugation of the resulting suspension in Petri dishes. Eggs were exposed to illumination, fresh water was added and miracidia started hatching after 15 min. To obtain cercariae, 50 lab-bred Biomphalaria alexandrina snails (3-5 mm shell diameter) were exposed en masse to miracidia in doses of 5-10 miracidia per snail. The water temperature at exposure was 24-26°C. After exposure, snails were placed as usual into plastic trays containing 1.5 liters of water supplied with preboiled lettuce, blue green algae and mud. One month after exposure to miracidia, snails were tested for cercarial shedding. Shedding snails (not less than 40 snails) were used to obtain a stock of cercarial suspension. This was adjusted so as to get the cercarial dose needed for each mouse or hamster. Adult schistosomes were recovered by perfusion technique from hamsters previously infected percutaneous with cercariae 6-7 weeks earlier. They were cleaned and washed by specific culture medium (RPMI 1640) containing antibiotics. Most of the worm pairs were separated during this procedure. If the worms were recovered from more than one hamster, they were combined in a single Petri dish before in vitro distribution.

In vitro Schistosomicidal bioassay and determination of LC90

A stock solution (10 mg/mL) of essential oil was prepared in DMSO, diluted with RPMI to produce 3mL test solution of 100 mg/mL final concentration in a 10-mL vial for the screening. Three replicates were used for each concentration (50, 30, 10, 7, 5 and 3 mg/mL), and three pairs of worms, males and females equally represented, were placed in each vial using sterilized tissue forceps. Incubation was maintained at 37°C. Positive (praziquantel, at 0.1 mg/mL) and negative (DMSO) controls were similarly used. Examination for worm viability was done after 24 h using a stereomicroscope. Worms showing no signs of motility for 1 min were considered dead. The activity of oil measured by calculating the number of dead worms relative to the total number of worms. The results were used to calculate the LC90 of the oil using probit analysis and utilizing the SPSS computer program (SPSS for Windows version 9=1989; SPSS Inc., Chicago, IL, USA).

Scanning Electron Microscopy

The tegument, suckers and gynaecophoric canal of adult worms were examined by scanning electron microscopy (SEM) to investigate the effect after exposure to solutions containing the LC90 concentration of active fractions. Control and experimental groups of worms were thus fixed at 4°C in 4% gluteraldehyde in sodium cacodylate buffer for 2 h, and then washed in the same buffer (pH 7.4). They were then passed into ascending concentrations of acetone (30%, 40% and 50%), each for 15 min. Worms were kept in 70% acetone until the time of examination (Bricker et al., 1983). Before examination, worms were washed for three times, the first and second were for 30 min in 80% and 90% acetone, respectively; the last wash was for 1 h in 100% acetone. Worms were then mounted on stainless steel holder and subjected to

sputter coat of gold, and examined under a scanning electron microscope (Joel JSM-480).[21]

RESULTS AND DISCUSSION

Antiviral activity

The results of antiviral activity indicated that 1/10 dilution has no toxicity on all cell line under tested, so this concentration was sued in all antiviral assay.

Table 1: Anti rotavirus Wa strain activity of non toxic doses from tested materials

Tested Material	Initial Viral titre (PFU/ml)	Final viral titre (PFU/ml)	% of reduction	Mean % of reduction
Oil sample	$1X10^5$	$5X10^4$	50%	50%
	$1X10^6$	$5X10^5$	50%	
	$1X10^7$	$5X10^6$	50%	

Table 2: Anti adenovirus type 7 activity of non toxic doses from tested materials

Tested Material	Initial Viral titre (PFU/ml)	Final viral titre (PFU/ml)	% of reduction	Mean % of reduction
Oil sample	$1X10^5$	$5X10^5$	0%	0%
	$1X10^6$	$5X10^6$	0%	
	$1X10^7$	$5X10^7$	0%	

Table 3: Anti Coxsackievirus B4 activity of non toxic doses from tested materials

Tested Material	Initial Viral titre (PFU/ml)	Final viral titre (PFU/ml)	% of reduction	Mean % of reduction
Oil sample	$1X10^5$	$4X10^4$	60%	53.3%
	$1X10^6$	$5X10^5$	50%	
	$1X10^7$	$8X10^6$	50%	

Table 4: Anti Herpes Simplex Virus Type 1 activity of non toxic doses from tested materials

Tested Material	Initial Viral titre (PFU/ml)	Final viral titre (PFU/ml)	% of reduction	Mean % of reduction
Oil sample	$1X10^5$	$1X10^4$	90%	90%
	$1X10^6$	$1X10^5$	90%	
	$1X10^7$	$1X10^6$	90%	

Oil sample was promising against rotavirus Wa strain, Coxsackievirus B4, and herpes virus type 1 with percentage of reduction 50% (Table 1), 53.3% (Table 3), and 90% (Table 4), respectively. There was no effect at all on adenovirus type 7 (Table 2). It may indicate promising antiviral effect of the oil against RNA viruses while no effect was observed against DNA virus. In case of Herpes rkvirus type 1, 90% reduction was observed using 1/10 dilution of 100 µL of the oil. The 50% inhibitory concentration for plaque formation of herpes virus type 1 (IC50) which was defined as the concentration at which the plaque number decreased to half of that in cells cultured without the addition of acyclovir antiviral drug was 0.85±0.15 µg/ml.[22]

Antimicrobial activity

Essential oils, derived from aromatic and medicinal plants have been reported to be active against Gram-positive and Gram-negative bacteria as well as against yeasts, fungi, and viruses. They are mixtures of different lipophilic and volatile substances, such as monoterpenes, sesquiterpenes.[23] The antimicrobial and antibacterial activities have been reported on the methanolic extract of these plants.[24] The essential oils of *E. camaldulensis* are used traditionally for the treatment of malaria and typhoid fever.[25]

The results of antimicrobial activities of EO of *E. camaldulensis* against both Gram-positive and Gram-negative bacteria as well as two yeasts were presented in Table (5).

Table 5: Antimicrobial activity of *E. camaldulensis* leaves essential oil at 500µg/ml concentration by agar well diffusion method

Microorganisms	Inhibition zone (mm)[a] at 500µg/ml
Gram-positive	
Streptococcus faecalis	10 ± 0.5
Bacillus subtilis	9.3 ± 0.4
Listeria monocytogenes	10.3 ± 0.5
Gram-negative	
Escherichia coli	12.5 ± 0.8
Salmonella typhi	11 ± 0.3
Pseudomonas aeruginosa	12.5 ± 0.4
Yeast	
Saccharomyces cerevisiae	10.5 ± 0.2
Candida albicans	21 ± 1.2
Fungi	
Aspergillus niger	10 ± 0.5

[a] Values are mean inhibition zone (mm) ±SD of three replicates. The diameter of the well (6mm) is included.

The results of MIC values obtained from antimicrobial tests ranged from 25 to 150 µg/ml were presented in Table 6.

The zones of inhibition representing the antimicrobial activity of *E. camaldulensis* at 500 µg/µl against both Gram positive and Gram negative bacteria as well as two yeasts and one fungus are presented in Table 5. Results showed that the essential oil of *E. camaldulensis possessed antibacterial activity* and it is more effective against *L. monocytogenes, E. coli* and *Candida albicans*. The antimicrobial effect of *E. camaldulensis* due to the presence of α-pinene and eucalyptol substance in Eucalyptus oil.[26] Some other studies showed that

Eucalyptus oil has widespread antimicrobial effects against different type of bacteria.[27,28] There is some evidence implying that functional groups in plant materials such as alcohol, phenols, terpenes and ketones are associated with their antimicrobial characteristics.[29-30] The essential oil of *E. camaldulensis* possessed antibacterial activity and it is more effective against *Staphylococcus aureus* than *E. coli*.[31] The same authors concluded that, antibacterial activity of the *E. camaldulensis* essential oils suggested it's clinically useful potentials, although further studies are required.

The obtained results of antimicrobial assay and MIC experiments in the present study showed that the essential oil of *E. camaldulensis* can strongly prevent the growth of *S. faecalis, E. coli* and *Candida albicans.* The MIC of *E. camaldulensis* was determined in order to assess its antimicrobial activity; it is ranged from 50-25. Therefore, essential oil of *E. camaldulensis* could be recommended as a source of pharmaceutical materials required for the preparation of new antimicrobial agent.

Table 6: Minimal Inhibitory Concentration (MIC) of *E. camaldulensis* leaves essential oil

Microorganisms	MIC (µg/ml)
Gram-positive	
Streptococcus faecalis	25
Bacillus subtilis	100
Listeria monocytogenes	25
Gram-negative	
Escherichia coli	50
Salmonella typhi	50
Pseudomonas aeruginosa	200
Yeast	
Saccharomyces cerevisiae	250
Candida albicans	150
Fungi	
Aspergillus niger	300

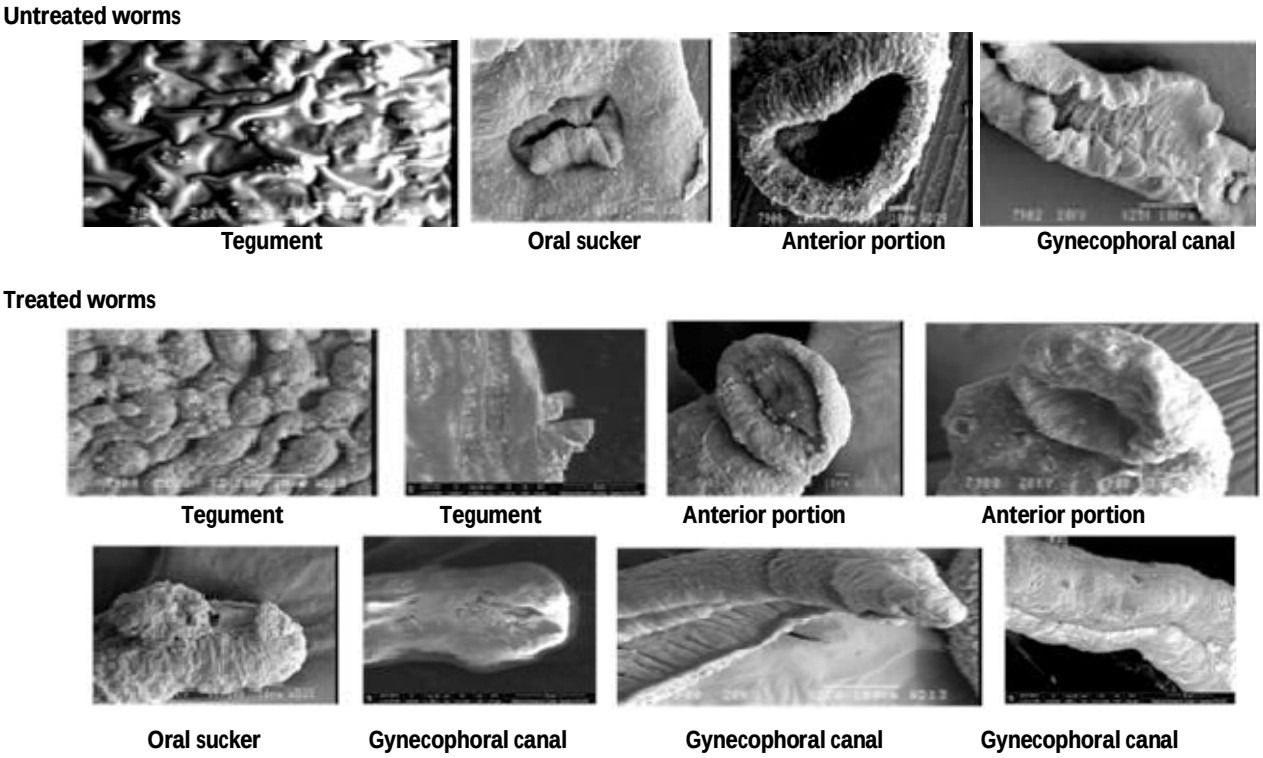

Figure 1: Scanning Electron Microscope Observation

Schistosomicidal activity

LC_{90} of *Eucalyptus camaldulensis* essential oil

The results of the essential oils showed that the LC_{90} value was 25µg/ml.

Scanning electron microscopy observation

Observations on *Schistosoma mansoni* worms exposed to LC_{90} of the bioactive fractions of essential oil. Twenty-four hours after *in vitro* exposure, to separate solutions containing the LC_{90} concentration of essential oil, all male and female *S. mansoni* adult worms examined showed evident changes in schistosoma worm typography. These included intensive contraction and swelling, bending of worm body posterior to the acetabulum. Tegumental damage was observed in all treated worms expressed by swollen tubercles with smooth surface, shortened spines and emergence of vesicles around the tubercles. The tegument also showed peeling, erosion and vesiculation in the anterior section. These effects impair the function of the tegument and its muscles destroying the defense system of the worm, so that it could easily be attacked by

the host's immune system, which ultimately result in the death of the parasite. Vacuolization of the tegument has been ascribed to the direct effect of Praziquantel on *S. mansoni*.[32] However, the mechanism by which these vacuoles form has not been clearly established. The same authors suggested that they arise from dilation of the basal membrane, and that they enlarge because of the water and ion imbalance.

Most of the treated worms showed extensive deformation and severe swelling of both suckers. This would lead to a loss of ability to adhere to the host's blood vessels and to ingest nutrients from the blood. The above results are in harmony with.[33] The authors studied alteration in the tegument of 21-day-old *S. mansoni*, caused by Artermether administered to infected mice.

The gynaecophoric canal relaxed and deformed in the treated groups. These results are best represented in Figs. and their legends. Relaxation and deformation of gynaecophoric canal in male worms caused by essential oil was expected to decrease the chance of conjugation between male and female worm (Figure 1).

CONCLUSION

Rotavirus Wa strain, Coxsackievirus B4, and herpes virus type 1 were affected by essential oil with percentage of reduction 50%, 53.3%, and 90% respectively. Essential oil has high effects against gram positive and negative bacteria with inhibition zones ranged from 9.3 to 12.5**.** The essential oils from *E. camaldulensis* are active candidates and could be used as RNA antivirus, antimicrobial and shistosomicidal agents in new drugs preparation for therapy of infectious diseases.

REFERENCES

1. Van de Braak SA, Leijten GC, Essential Oils and Oleoresins: A Survey in the Netherlands and other major markets in the European union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, 1999, 116.
2. Burt SA, Essential oils: their antibacterial properties and potential applications in foods: A review, International Journal of Food Microbiology, 94, 2004, 223–253.
3. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A, Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum* and *Artemisia spicigera* essential oils, Journal of Agricultural and Food Chemistry, 53, 2005b, 9452–9458.
4. Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J, Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe, Journal of Ethnopharmacology, 103, 2006, 99–102.
5. Faid M, Bakhy K, Anchad M, Tantaoui-Elaraki A, Alomondpaste, Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon, Journal of Food Protection, 58, 1995, 547–550.
6. Buttner MP, Willeke K, Grinshpun SA, Sampling and analysis of airborne microorganisms. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD, Walter MV, editor. Manual of Environmental Microbiology. ASM Press: Washington, DC, 1996, 629–640.
7. Delaquis PJ, Stanich K, Girard B, Mazza G, Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, International Journal of Food Microbiology, 74, 2012, 101–109.
8. Davies KJA, Oxidative stress, antioxidant defences, and damage, removal, repair and replacement systems, International Union of Biochemistry and Molecular Biology, 50, 2000, 279-289.
9. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J, Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk, Lancet Infectious Diseases, 6, 2006, 411–425.
10. Caffrey CR, Chemotherapy of schistosomiasis: present and future, Current Opinion in Chemical Biology, 11, 2007, 433–439.
11. Gil L, Tadesse W, Tolosana E, López R. Eucalyptus species management, history, status and trends in Ethiopia. Addis Ababa, Ethiopia: Ethiopian Institute of Agricultural Research, 2010.
12. Tsiri D, Kretsi O, Chinou IB, Spyropoulos CG, Composition of fruit volatiles and annual changes in the volatiles of leaves *Eucalyptus camaldulensis* dehn. growing in Greece, Flavour and Fragrance Journal, 18, 2003, 244-247.
13. Sefidkon F, Assareh MH, Abravesh Z, Barazandeh MM, Chemical composition of the essential oils of four cultivated Eucalyptus species in Iran as medicinal plants (*E. microtheca, E.spathulata, E. largiflorens* and *E. torquata*), Iranian Journal of Pharmaceutical Research, 6, 2007, 135-140.
14. Giamakis A, Kretsi O, Chinou I, Spyropoulos CG, *Eucalyptus Camaldulensis*: Volatiles from immature flowers and high production of 1.8-cineole and β-pinene by *in vitro* cultures, Phytochemistry, 58, 2001, 351-335.
15. Barton AFM, Tjandra J, Nicholas PG, Chemical evaluation of volatile oils in Eucalyptus species, Journal of Agricultural and Food Chemistry, 37, 1989, 1253-1257.
16. Ahmad NR, Hanif MA, Rashid U, Chemical compositional and intra provenance variation for content of essential oil in *Eucalyptus crebra*, Asian Plant Science, 4, 2005, 519-523.
17. Lee KG, Shibamoto T, Antioxidant activities of volatile components isolated from Eucalyptus species, Journal of the Science of Food and Agriculture, 81, 2001, 1573-1597.
18. Simoes CMO, Amoros M, Girre L, Mechanism of antiviral activity of triterpenoid saponins, Phytoth Res, 21, 1999, 317-325.
19. Walum E, Strenberg K, Jenssen D, Understanding cell toxicology: Principles and pratice. Ellis Howood, NewYork, 1990, 97-111.
20. Albayrak S, Aksoy A, Sagdic O, Hamzaoglu E, Compositions, antioxidant and antimicrobial activities of Helichrysum (Asteraceae) species collected from Turkey, Food Chemistry, 119, 2010, 114-122.
21. Lanfredi RM, De Souza W, Gomes DC, Comparative study of four species of *Trichuris* Roederer, 1761 (Nematoda,

Trichurinae) by scannig electron microscopy, Memórias do Instituto Oswaldo Cruz Rio de Janeiro, 90, 1995, 489-496.

22. Suzuki M, Kasai K, Saeki Y, Plasmid DNA sequences present in conventional herpes simplex virus amplicon vectors cause rapid transgene silencing by forming inactive chromatin, Journal of Virology, 80, 2006, 3293-3300.
23. Reichling J, Plant-microbe interaction and secondary metabolites with antiviral, antibacterial and antifungal properties, in functions of plant secondary metabolites and their exploitation in biotechnology, M. Wink, Ed., Annual Plant Review, Sheffield Academic Press, Sheffield, UK, 3, 1999, 187–273.
24. Mehraban F, Nasim OT, Fereshteh J, Antidermatophyte activities of *Eucalyptus camaldulensis* in comparison with Griseofulvin, Iran Journal of Pharmacolgy and Theraputics, 4, 2005, 80-83.
25. Udeh MU, Agbaji AS, Williams ISP, Ehinmidu P, Ekpa E, Dakare M, Screening for the antimicrobial potentials of *Azadirachta indica* seed oil and essential oils from *Cymbopogon ciratus* and *Eucalyptus citriodora* leaves, Nigerian Journal of Biochemistry and Molecular Biology, 16, 2001, 189-192.
26. Pasi S, Harvala E, Kretsi O, Chinou E, Chinou I, *In vitro* antimicrobial activity of natural products from Eucalyptus growing in Greece and mechanism of action. Clinical Microbiology and Infection, 7, 2001, 391-394.
27. Akin M, Aktumsek A, Nostro A, Antibacterial activity and composition of the essential oils of Eucalyptus *camaldulensis* Dehn. and Myrtus communis L. growing in Northern Cyprus, African Journal of Biotechnology, 9, 2010, 531-535.
28. Ghalem B, Mohamed B, Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis,* African Journal of Pharmacy and Pharmacology, 2, 2008, 211-215.
29. Braca A, Siciliano T, Arrigo M, Germano MP, Chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil, Fitoterapia, 79, 2008, 123-125.
30. Mohammadreza VR, Chemical composition and antimicrobial activity of *Pimpinella affinis* Ledeb essential oil growing in Iran, International Journal of Green Pharmacy, 2, 2008, 138-140.
31. Lima LM, Babakhanib B, BOLDAJI SAH, Asadi M, Boldaji RM, Essential oils composition and antibacterial activities of *Eucalyptus camaldulensis* Dehn, International Journal of Medicinal and Aromatic Plants, 3, 2013, 214-219.
32. Shaw MK, Erasmus DA, *Schistosoma mansoni*: the effects of a subcurative dose of Praziquantel on the ultra structure of worms *in vitro*. Zeitschrift fur Parasitenkunde, 69, 1983, 73-90.
33. Shuhua X, Hotez PJ, Tanner M, Artemether, an effective new agent for chemoprophylaxis against shistosomiasis in China: its *in vivo* effect on the biochemical metabolism of the Asian schistosome, Southeast Asian Journal of Tropical Medicine and Public Health, 31, 2000, 724-732.

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