Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2019, Vol. 35, No.(2):
Pg. 738-743

Effect of Drought Condition of North Region of Saudi Arabia on Accumulation of Chemical Compounds, Antimicrobial and Larvicidal Activities of *Thuja Orientalis*

EMAN R. ELSHARKAWY[1,2] and AHMED M.H.ALI[3,4]

[1]Department of Chemistry, Faculty of Science, Northern Border University, Saudi Arabia.
[2]Department of Ecophysiology, Desert Research Center, Mathaf El-Mataria, 15753. Egypt.
[3]Department of Laboratory Sciences, College of Sciences and Arts, Qassim University, Al-Rass, Saudi Arabia.
[4]Department of Zoology and Entomology, Faculty of Science, Assiut University, Egypt.
*Corresponding author E-mail: elsharqawyeman2017@gmail.com

http://dx.doi.org/10.13005/ojc/350233

(Received: December 17, 2018; Accepted: April 01, 2019)

ABSTRACT

Drought stress and drastic condition of the Northern region of Saudi Arabia especial high temperature in summer season affect widely on the chemical component of plant and its biological actively plant grow in these condition adapt to this stress by accumulation different compound, *T. orientalis* it is tree grow in garden and farms, when it grows in desert habitat show different content, Gc-Ms analysis of the terpenoid fraction of the plant where it is best fraction given high antimicrobial and Larvicidal activity was done and show the presence of many promising diterpene compounds, Ferruginol, Torulosol, dihydro, 18-Oxo-kauran-17-yl acetate and Andrographolide. The antibacterial testing showed that, the tested *gram-positive* bacteria exhibited good susceptibility to the terpenoid fraction of methanol extract, which was *Staphylococcus epidermidis* and *Staphylococcus aureus*, recorded 13.0±0.7 and 11.0±0.0 mm inhibition zones, respectively. Indicating promising antibacterial activity against the *gram-positive* pathogens. While all the tested *gram-negative* bacteria recorded weak or no susceptibility. The larvicidal activity of plant was studied on Spodoptera littoralis, The lethal concentration was 27.63 % of terpenoid fraction of *Thuja orientalis*. The plant can considered as a good herbal insecticide instead of synthetic insect side after further studies where the IC_{50}: Botanical pesticides are highly effective, and ecologically acceptable.

Keywords: *Thuja orientalis*, larvicidal activity, *Spodoptera littoralis*, Antimicrobial.

INTRODUCTION

Desert plants are in general did not find the required international interest for many reasons; they are not a potential source for food due to their low density in this harsh conditions, its location in arid zones high in temperature and drought which decrease the interest on them, they low diversity among the vegetation compared with other environments rich in all growth parameters such as water, soil fertility and good climate. However, regarding drugs, desert plants could be unexploded

source for bioactive compounds of medicinal importance, this is because desert plants are rich in this bioactive compounds to protect them from this harsh environmental conditions, challenge and competition from soil microorganisms, herbivores and insects which feed on them, also competition between different plant species in desert and tolerance against ecological conditions, drought and high temperature. All these factors lead to production of unique compounds helps in survival of these arid environment.

Spodoptera littoralis (Lepidoptera: Noctuidae), which infests a wide range of economic crops causes critical injuries and loss to cotton, soybean, groundnut, chilli, tobacco, caster, bhendy, tomato and potato[1]. This pest is widely distributed in Middle East countries and temperate zones in Asia and Africa[2].

Several methods have been performed to control S. *littoralis* chemically using synthetic chemical pesticides. Due to the residual toxic effect of these chemicals in the environment, the need for the development of products that not hazardous to the environment can be an alternative strategy for the control of S. *littoralis*[3,4]. Botanical pesticides hat not hazardous to the environment, are highly effective, and ecologically acceptable[5].

Thuja orientalis, Cupressaceae family is an evergreen and monoecious tree or shrub evergreen tree, up to 10-20 m high which grows in south and east of Europe, west part of Asia including various parts of Iran. *Thuja orientalis* has been used in the different activity that is, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic[6]. (Yeung, 1985). The phyto-constituents of *T. orientalis* such as flavonoids and terpenoids, coumarine showed high biological activities[7].

Comparative study between *Thuja orientalis* plant growing in KSA and Egypt and evaluated its antioxidant and cytotoxic activity, against HCT116, MCF7, PC3, A549 and Hep-G2 cell lines. The essential oil extract from Saudi plant exhibited higher antioxidant activity and cytotoxic activity against deferent cell lines. than the Egyptian plant oil extracts, which is correlated with its high content of some compounds which found in Saudi plant and absence in Egyptian plant[8].

Thuja occidentalis cones extract contain high levels of bioactive phenolics, flavonoids and other free radical scavengers that can help to control lipid oxidation. This study showed that extract had effective antioxidant activity in raw ground chicken meat during refrigerated storage because use of these extracts inhibited the formation of lipid peroxide and thiobarbituric acid reactive substances in ground chicken meat[9].

Traditional Plants considered a good source for novel drugs, Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial and insecticidal activity from botanicals source are the main goal for many researchers. This study aimed to select some species form these plants *(Thuja orientalis*), dominated in the Northern Region according to plant's information and literature, then study It through chemical analyzing and knowing some of its antimicrobial properties and the the bioactivity of plant extract against the larva of S. *littoralis*. Which provide basic necessary information to explore the properties of this plant in this area, which are not studied adequately, and give a picture about the role of environment on the accumulation of chemical compounds to adapt the desert condition.

MATERIAL AND METHODS

Plant Material

Plant *Thuja orientalis* were collected from wild population from Arar region, Northern Region, Saudi Arabia in March 2018 the Authentic sample was identified and deposited in Faculty of science girl section.

Extraction

The air-dried powdered leaves (500 g) of *Thuja orientalis* were subjected to successive extraction with $CHCl_3$ using a Soxhlet apparatus. The $CHCl_3$ extract (38.00 g) was dissolved in a suitable amount of hot distilled H_2O–MeOH (95:5 v/v, 200 mL), then partitioned between petroleum ether and $CHCl_3$. The petroleum ether (20.50 g){C1) and $CHCl_3$ (17.10 g, C2) fractions were individually concentrated under reduced pressure and kept for biological analysis.

Fractionation of the $CHCl_3$ Fraction

The $CHCl_3$ fraction was subjected to silica gel column chromatography (550 g, mesh size 0.063–0.200 mm, Merck) (150 cm × 3 cm i.d.) eluted with n-hexane, gradually increasing the polarity with EtOAc, which resulted in the isolation of terpenoid fraction (C2)which were subjected to analyzed with GC-MS.

GC-MS analysis

The compounds were analyzed using a Thermo GC-Trace ultra system (Thermo Co. USA), they were separated on 30m X0.25 mm X 0.25 µm Elite-5MS column (Thermo Scientific GC Column). The column temperature was increased from 40°C to 220°C at a rate of 4°C/ min; injector temperature, 250°C; injection volume, 1 µl; helium carrier gas flow rate 20ml/min; transfer temperature, 280°C. MS parameters were as follows: EI mode, with ionization voltage 70 ev, ion source temperature, 180°C; scan range, 50-600 Da. The peaks were tentatively identified based on library search using NIST and Wiley Registry 8 Edition .

Insects

laboratory strain of S. *littoralis* was reared in the laboratory at 26± 2°C and 65±5 % R.H., with 8:16 L:D h photoperiod. Larvae were fed on fresh castor leaves, Ricinus communius. The experiments were performed on the 4th instar larvae.

Insecticidal Bioassay

The determination of the lethal concentration values (LC30; LC50; LC90) of the *Thuja orentalis* against the 4th larval instar of S. *littoralis* was evaluated by leaf dipping technique method. Five concentrations (10, 15, 20, 30, and 40%) of the *Thuja orentalis* were used. Equal discs of fresh castor bean leaves were dipped in each tested concentrations of the extract for a while and left to dry. Ten starved larvae were transferred into each cup and allowed to feed on the treated and untreated leaves. Five replicates for each concentration were performed. Mortality counts were evaluated 24 h post-treatment and corrected according to Abbott (1925)[10].

Antibacterial activity

The antibacterial potential of the leaves of *Thuja orientalis* was evaluated using the disc diffusion method as mentioned in,(Elsharkawy *et al.*,2018)[8] with slit modifications. Dr. Fiaz Ahmed generously provided pathogenic bacterial strains (2 *gram-positive* and 3 *gram-negative* bacteria) from the pathology lab, Al-Rass Hospital, Saudi Arabia. Bacterial strains were sub-cultured in blood agar plates for 24 h and 37°C. the plant extract was reconstituted in absolute methanol (not lethal for bacteria) to get a concentration 500 mg/ml. For disc diffusion method, a single colony was transferred to a sterile tube containing 100 µl normal saline (0.9%) and diluted to get a turbidity equivalent to 0.5 McFarland standard (1 × 108 cfu/mL). 100 µl of that suspension was spread over previously prepared Mueller Hinton agar plate, then, covered and left for a while. Sterile paper discs (size 6 mm) were cut from Whatman filter paper (No.1) and immersed in the reconstituted extract and then loaded over the inoculated plates. Another paper disc saturated with chloramphenicol solution (5mg/ml) was loaded on the plate to act as a positive control. All plates were incubated at 37°C for up to 24 hour. The diameter of inhibition zones (in mm) was measured and the mean was calculated from two replicates.

RESULTS AND DISCUSSION

GC-MS analysis of chloroform fraction of plant Thuja orientalis revealed the existence of diterpene compounds, Ferruginol, Andrographolide, Torulosol, dihydro, 18-Oxo-kauran-17-yl acetate and of ent-16-Hydroxy-17-acetoxy-19-kauranal, Other non terpenoid compound also detected in chloroform fraction, Cymarin and K-Strophanthin, cardic glycoside type. the results are shown in Table 1.

The antibacterial of active diterpene types isolated from Chamaecyparis lawsoninana have modulator activity against multi drug resistance of *Staphylococcus aureus*, *S. aureus* strains and two epidemic methicillin-resistant, clinical isolates. ferruginol, pisiferol and its epimer 5-epipisiferol, formosanoxide, trans-communic acid and torulosal, Some of these compounds also exhibited modulatory activity in potentiating antibiotic activity against effluxing strains and ferruginol, used at a sub-inhibitory concentration, resulted in an 80-fold potentiation of oxacillin activity against strain EMRSA-15. An efflux inhibition assay using an *S. aureus* strain possessing the MDR NorA efflux pump resulted in 40% inhibition of ethidium bromide efflux at 10 microM ferruginol (2.86 microg/ml)[11]. (Smith *et al.,* 2007), this come in harmony with

current study where ferruginol and torusolal are detected in diterpene fraction of the plant in high amount.

Table 1: Major compounds of chloroform fraction of leaves of *Thuja orientalis*

	Compounds	%	RI	Molecular Formula
1	Andrographolide	1.6	1005	$C_{20}H_{30}O_5$
2	ent-16α-hydroxy-17-acetoxy-19-kauranal	1.4	1934	$C_{22}H_{34}O_4$
3	3α-Acetyloxy-5α-pregnan-20-one	0.15	1050	$C_{23}H_{36}O_3$
4	alpha.-D-Glucopyranose	0.24	1404	$C_6H_{12}O_6$
5	18-Oxo-kauran-17-yl acetate	0.35	1980	$C_{22}H_{34}O_3$
6	Torulosol, dihydro	2.5	1200	$C_{20}H_{36}O_2$
7	Ferruginol	5.5	2225	$C_{20}H_{30}O$
8	Benzoic acid	5.2	1697	$C_7H_6O_2$
9	Dihydro-coumarone	0.3	1036	C_8H_8O
10	Chromone-hydroxy6,7,8-trimethoxy-2,3-dimethyl	0.11	2250	$C_{14}H_{16}O_6$
11	Card-20(22)-enolide, 3-[(2,6-dideoxy-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxy]-5, 14-dihydroxy-19-oxo-, [Cymarin]	26.94	4191	$C_{30}H_{44}O_9$
12	Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-.beta.-D-glucopyranosyl-3-O-methyl-.beta.-D-ribo-hexopyranosyl)oxy]-5, 14-dihydroxy-19-oxo-[K-Strophanthin]	2.25	5558	$C_{36}H_{54}O_{14}$

Antibacterial properties

The evaluation of the antibacterial activity of the chloroform fraction of methanolic extract of *Thuja orental* leaves are represented in Fig. 1,2 and Table 2. As shown from the results, only the *gram-positive* were susceptible to the plant extract while the *gram-negative* bacteria showed very weak or no susceptibility against the extract. The tested *gram-positive* bacteria recorded 13.5±0.7mm for *Staphylococcus epidermidis* and 11.0±0.0 mm for *Staphylococcus aureus*, respectively. Where as, the tested *gram-negative* bacteria recorded 6.5±0.7mm for *Klebsiella pneumoniae* and *Escherichia coli* and 6.0±0.0mm for Acinitobacter baumannii, putting in consideration that the blank disc diameter is 6.0 mm. The antibiotic, chloramphenicol (5mg/ml) was used as a positive control. The noticeable antibacterial activity of the methanol extract of the leaves of Thuja orental against *Staphylococcus epidermidis* and *Staphylococcus aurerus* in the current study is attributed to some bioactive phytochemical constituents present in this aromatic plant. Plants are rich source of alternative antibacterial copounds able to combat the growing phenomenon of antibiotics resistant bacteria[12]. The finding of the current study is in agreement with previous studies on the antibacterial activity, in general. Duhan *et al.,* 2013[13], cited that the methanol, acetone and ethyl acetate extracts of *Thuja orental* leaves were found effective against various *gram-negative* and *gram-positive* bacteria (i.e. *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Klebsiella pneumoniae*) and were mostly competitor to some antibiotics. Kshirsagar *et al.,*[14], mentioned that, the acetone, ethyl acetate and methanol extracts of *Thuja orentalis* leaves where tested against different bacterial strains. Only methanol and acetone extracts recorded highest antibacterial efficacy, particularly against Bacillus subtilis. Moreover, Jain and Grag 1997[15] reported significant antibacterial activity of the essential oils of *Thuja orental* against all tested *gram-positive* and *gram-negative* bacteria, especially *S. typhi*. Accordingly, more future studies should be conducted on this plant particularly its aromatic compounds, which could be used in the formulation of new natural antibacterial agents.

Table 2: Antibacterial activity of chloroform fraction of *Thuja orentalis* leaves compared with the antibiotic (Chloramphenicol)

Tested	Zone of Inhibition (mm)				
	Sa	Se	Ab	Kp	Ec
Extract (500mg/ml)	11.0±0.0	13.5±0.7	6.0±0.0	6.5±0.7	6.5±0.7
Chloramphenicol (5mg/ml)	25.5±0.7	27.0±0.0	21.0±1.4	26.5±0.7	29.5±0.7

Sa=*Staphylococcus aureus*, Se= *Staphylococcus epidermidis*, Ab=*Acinitobacter baumannii*, Ec=*Escherichia coli*, Kp=*Klebsiella pneumonia* Zone diameter equal 6 mm= no inhibition, mean ± standard deviation.

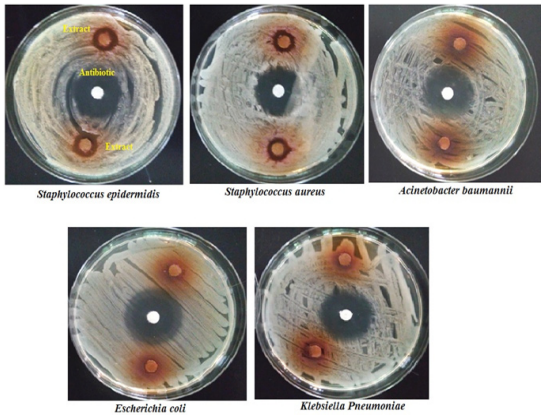

Fig. 1. Representative photo showing susceptibility of *S. epidermidis* and *S. aureus* to the chloroform fraction

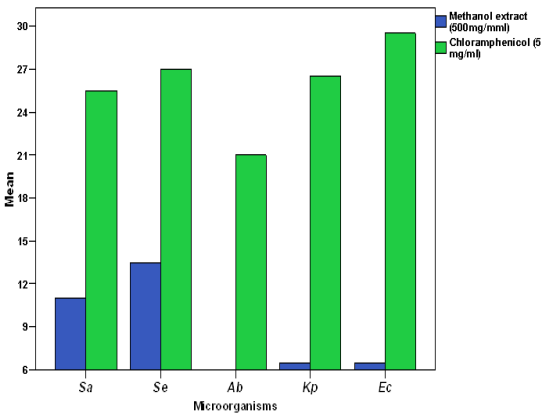

Fig. 2. Antibacterial activity of the chloroform fraction of *Thuja orentalis* leaves using disc diffusion test

Insecticidal activity

Insecticidal activity of *Thuja orentalis* was calculated based on larval mortality after treatment. According to the LC_{50} values of the fraction, the present results indicated that *Thuja orentalis* have larvicidal activity against 4th instar larvae of *S. littoralis* as was shown in Table 3 .

Table 3: Susceptibility of the 4th instar larvae of *S. littoralis* to *Thuja orientalis*

Plant	Lethal concentrations (%)		95 % confidence limits for concentrations	
			Lower	Upper
Thuja orentalis	LC_{30}	22.74	21.37	24.04
	LC_{50}	27.63	26.17	29.26
	LC_{90}	44.48	40.74	49.86

LC_{30} - Lethal concentration that kills 30% of the exposed larvae,
LC_{50} - Lethal concentration that kills 50% of the exposed larvae,
LC_{90} - Lethal concentration that kills 90% of the exposed larvae

Little know about the effects of plant metabolites on the biology of *S. littoralis*. terpenoid and cardiac glycoside, considered as secondary metabolites synthesized by desert plants with physical and biochemical properties. Hexane extract of was found to have antimicrobial and larvicidal properties against Aedes aegypti larva, chromatographic separation of the extract leading to the isolation of five Kaurane diterpene as the major compounds, this agree with present study, where the diterpene fraction contain 18-Oxo-kauran-17-yl acetate and kuran 19-ol-acetate, two type of kauran[16].

Andrographolide compound show antifedent activity against fourth instar larvae of Papilo demoleus at concentration of 200 ppm and it was recommended to use as a pest control against Papilo demoleus[17]. The use of plants as larvicidals agents are very useful and can be used as substitute against pesticides.

CONCLUSION

Plant *Thuja orentalis* is medicinal plant used for antipyretic, antitussive, astringent, diuretic current study was aimed to study the role of plant as a herbal insecticide and bactericide instead of synthetic drugs, and role of chemical composition of the plant on these biological activity, Gc-Ms analysis reflect the presence of diterpene compounds as major compound in chloroform fraction, many promising compound, which have antimicrobial and larvicidal activity was detected, Ferruginol, Torulosol, dihydro, 18-Oxo-kauran-17-yl acetate and Andrographolide. Insecticidal activity of *Thuja orentalis* was calculated based on larval mortality after treatment. According to the LC_{50} values of the tested products, the present results indicated that Thuja orentalis have larvicidal activity against 4th instar larvae of *S. littoralis*, also have good antimicrobial activity against *gram-positive* bacteria while all the tested *gram-negative* bacteria recorded weak or no susceptibility. we can concluded that plant Thuja orentalis have a potent activity as a larvicidal and microbial activity.

ACKNOWLEDGMENT

The author gratefully acknowledgment the approval and support of this research by the grant no: SCI- 2017-1-8-F-7417 from the deanship of Scientific Research of Northern Border University, Arar, Saudi Arabia.

Conflict of Interest

The authors declare any conflict of interest

REFERENCES

1. Ferry N, Edwards MG, Gatehouse AMR. Plant-insect interaction: Molecular approaches to insect resistance. In: *Biotechnology (Eds.: T. Sasaki and P. Christou)*., **2004**, *15*, 155-161.
2. Salama, H. S.; Salem, S. A.; Zaki, F. N. and Shams EL-din, A. (1990): Comparative effectiveness of Bacillus thuringiensis and lannate against Spodoptera littoralis. *J. Islam. Acad. Sci.*, **19903**, *4*, 325-329.
3. Nakata, H.; Hirakawa, Y.; Kawazo, M.; Nakabo, T.; Arizono, K.; ABE, S. I.; Kitano, T.; Shimada, H.; Watanabe, I.; LI, W.; Ding, X. Concentrations and compositions of Organochlorine contaminants in sediments, soils, crustaceans, fishes and birds collected from Lake Tai, Hangzhou Bay and Shangai city region. *China Environmental Poly.,* **2005**, *133*, 415-429.
4. Pavela, R.; Vrchotova, N.; ERA, B. Growth inhibitory effect of extracts from Reynoutria sp. plants against Spodoptera littoralis larvae. *Agrociencia (Montecillo).,* **2008**, *42*(5), 573-584.
5. Senthil Nathan, S. and Kalaivani, K. Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on Spodoptera litura Fabricius (Lepidoptera :Noctuidae). *Biological Control*., **2005**, *34*, 93-98.
6. Young. Him-Ch. Handbook of Chinese herbs and formulas Instute of Chinese Medicine., Los Angeles., **1985**.
7. Sridhar S. R., Rajagopal R. V., Rajavel R., Masilamani S., and Narasimhan S. Antifungal activity of some es sential oils. *J. Agric. Food Chem.*, **2003**, *51*, 7596D 7599.
8. Elsharkawy ER, Ed-dra A, Abdallah EM, Ali AM. Antioxidant, antimicrobial and antifeedant activity of phenolic compounds accumulated in Hyoscyamus muticus L. *African Journal of Biotechnology.*, **2018**, *17*(10), 311-321.
9. Yogesh K. and Jamshed A. Antioxidant potential of (*Thuja occidentalis*) cones and peach (Prunus persia) seeds in raw chicken ground meat during refrigerated (4 ± 1°C) storage, *Food Sci Technol.*, **2014**, *51*(8), 1547–1553.
10. Abbott, W. S. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology.,* **1925**, *18*, 265–267.
11. Smith ECJ, Williamson EM, Wareham N, Kaatz GW, Gibbons S. Antibacterials and modulators of bacterial resistance from the immature cones of Chamaecyparis lawsoniana. *Phytochem.*, **2007**, *68*(2), 210-217.
12. Abdallah EM. Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science.*, **2011**, *1*(6), 16-20.
13. Duhan JS, Saharan P, Surekha A. Phytochemical analysis and antimicrobial potential of leaf extracts of *Thuja orientalis*. *Asian Journal of Pharmaceutical and Clinical Research.,* **2013**, *6*(2), 291-294.
14. Kshirsagar S, Malode S, Bansode S. Pharmacological activities of Thuja orientalis Linn. *International Journal of Pharmacognecy.,* **2018**, *5*(6), 331-336.
15. Jain RK, Grag SC . Antimicrobial activity of the essential oil of *Thuja orental* L. *Ancient science of Life.*, **1997**, *I3*, 186 – 189.
16. Dayanne MS, Emmanoel VC, Paulo C. L, Nogueira Valéria R. , Souza M. ent-Kaurane diterpenoids and other constituents from the stem of Xylopia laevigata (Annonaceae), *Quim. Nova.*, **2012**, *35*(8), 1570-1576.
17. Vattikonda, S. R. Effect of andrographolide on feeding behaviour of Papilio demoleus L., (Lepidoptera: papilionidae) larvae. *Asian J. Bio. Sci.*, **2015**, *10*(1), 65-70.