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EFFECT OF *EUCALYPTUS MICROTHECA* F. MUELL EXTRACTS ON MORPHOLOGICAL DEFORMATIONS WHEN TREATED FORTH INSTAR LARVAE OF MOSQUITOES *CULEX MOLESTUS* FORSKAL

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ABSTRACT : The effect of aqueous cold, boiling and chloroform of roots, stems and leaves extracts of *Eucalyptus* plant *Eucalyptus microtheca* was studies by using concentrations (1, 5 and 10 mg/ml) of aqueous extracts and (0.25, 0.5 and 1 mg/ml) of chloroform extract on some biological aspects of *Culex molestus* by treated fourth instar larvae of the insect.

The results of the study showed that the above mentioned extracts have an effect in inhibition of emergence and that effect is positively correlated with the concentrations used. The most of these effects were demonstrated by the using the chloroform extract of the leaves, as for the effect of the mortality of the fourth instar larvae and pupae, we note that the highest percentage of mortality occurred when using aqueous cold, boiled and chloroform for leaves also noted many morphological deformations during the experiments.

Key words : Mosquito, active compounds in plants, *Eucalyptus microtheca*.

INTRODUCTION

Mosquitoes one of the insect that feeding blood by sucking,return to Diptera (Al-Rahimy and Al-Essa, 2019), medically and veterinary importance because transmits for many dangerous pathogens (Kassim *et al*, 2012). One of the most important parasites transmitted by mosquitoes is malaria parasites and ring viruses such as Dengue fever virus, yellow fever virus and West Nile virus (Abul-hab, 1979 and Service, 2012). As a result of the rapid proliferation of this insect and the production of large numbers of eggs, a wide spread of these diseases has been observed, causing an epidemic, so attention has been paid to developing programs to combat and reduce their spread (Al-Rahimy and Al-Essa, 2019). Mosquitoes come back to the Culicidae family wich contain 3601species and subspecies (Wilkerson *et al*, 2015), which the *Culex* genera is found in tropical and temperate regions with favorable environmental conditions for the transmission of many viral diseases (Al-Rahimy and Al-Essa, 2019). *Culex molestus* Forskel mosquitoes are the most prevalent in the human environment (Alahmed *et al*, 2010 and Fodeal, 2014). However, the use of chemical pesticides is the fastest method among many control methods (Al-Rahimy and Al-Essa, 2019), but this method of control pull faces many criticisms and warning, in order to preserve the environment and non-target organisims, researchers began to use other methods of control, including control by using secondary metabolites of plants. Plant extracts have toxic effects to larvae, pupae and adult mosquitoes (Al-Rahimy and Al-Essa, 2019). In general, *Eucalyptus microtheca* to the oral family myrtaceae has important functions from the medical point of view and insect repellent as mosquitoes (Habiba, 2010) and given the importance of combating some of the water stages to control the adult stages. The current research aims to study the effect of water and chloroform extracts of Eucalyptus in some of the of biological aspects mosquitoes *Cx. molestus* through:

1. Studying the effect of plant extracts on the mortality percentage of the fourth instar larvae that treated.

2. The effect of these substances in causing deformations to individuals treated and individuals resulting from them.

Diagnosis of active compounds using FT-IR teqnique.

MATERIALS AND METHODS

Collect and diagnose plant samples

Samples of Eucalyptus plant were collected from the orchards of the province of Kerbala in December 2016

and separated into leaves, stems and roots, and after drying and grinding separately, put in glass bottles and the name of each vegetable section was recorded and stored in the refrigerator until use. The diagnose of plant in Department of Biology, College of Sciences, University of Babylon.

Preparation of plant extracts

The modified method (Al-Mansour, 1995) was adopted for Harborne (1973) in the preparation of water extracts. In the preparation of organic solvent extracts, the organic solvent chloroform was used in the preparation of organic plant extracts. The method of Samurai (1983) and the modified by Harborne (1973) were adopted in the extraction process for roots, stems and leaves of peppermint.

Collection and diagnosis of mosquito samples

Egg samples of mosquitoes *Cx. molestus* Forskal were collected from one of the exposed sewage areas of open heavy water measuring (2 × 2 m) and containing its limbs on reed plants *Typha* sp. In the province of Karbala district of Ramadan, the role of stone on 20/3/ 2017. The boats were placed in laboratory conditions for the purpose of hatching and were reared until they reached the fourth larval instar (Al-Rahimy and Al-Essa, 2019). To diagnose mosquitoes within the study area where samples were taken from the fourth instar larvae and identified using the taxonomic key (Abul-Hab and Kassal, 1989). In order to confirm the diagnosis, it was based on a vital phenomenon, namely Autogeny (Al-Rahimy and Al-Essa, 2019).

Mosquitoes breading

The colony was purified for three generations before the experiments were started (Al-Rahimy and Al-Essa, 2019). The egg boats collected from the site were isolated and each boat was placed in a 400 ml. plastic container containing tap water. After hatching the eggs to larvae, they were fed with bread crumbs and when they were turned into pupae, the pupae were transferred to the breeding cage (30 × 30 × 30 cm) was enclosed by a metal wire on four sides and a fifth-line tulle. The adults were fed on a 10% glucose solution and don't fed to the blood meal. The colony was purified for three generations before the experiments began. On fourth-generation larvae and adults used for diagnosis and Conduct experiments. This method of breeding was used for the purpose of obtaining adults and the fourth larval instar of diagnosis and conducting the necessary experiments (Al-Rahimy and Al-Essa, 2019).

Effect of water extracts and chloroform of roots, stalks and leaves of peppermint plant in the biological aspects of the insect

Total (10) larvae/replicate of fourth instar larvae and (3) replicates per concentration were transferred to 100ml plastic containers containing the above mentioned extracts (1,5and 10) mg/ml for the water extract and 0.25, 0.5 and 1 mg/ml for the chloroformed extract with bread crumbs for the purpose of feeding them used distilled water and solvent in control experiments, recorded. The percentage of mortality in the fourth instar larval and the pupal stage (produced after treatment of the fourth instar larval) and until it reached the adult stage. The percentages of mortality are calculated according to equation (Abbott, 1925).

% of mortality corrected = (mortality % in treatment – mortality % in control) / (100- mortality % in control) × 100

The deformations that occurred during the experiment to the treated individuals and they were produced were observed.

Determination of active groups of plant extracts

Using Foureir Transform Infrared Spectrophotometer FT-IR teqnique, according to Al-Rahimy (2017).

The results of the study experiments were analyzed according to the model of global experiments and with full randomization design, the loss ratios were adjusted according to equation (Al-Rawi and Khalfallah, 2000) and the corrected values were converted to angle values for inclusion in the statistical analysis.

RESULTS AND DISCUSSION

Effect the concentrations of cold and boiling water extracts of the Eucalyptus plant *Eucalyptus microtheca* in the percentage of cumulative mortality of the immature stages of the *Cx. molestus*

Table 1 shows the effect of concentrations of eucalyptus extract on the mortality of immature stages of the insect. It is clear that the increased concentration of the extract led to an increase in the mortality rates of the immature stages of the treated insect. A positive relationship was observed between the concentration of the extract and the percentage of destruction. The percentage of cumulative mortality of immature stages in the cold and boiling water extract of the roots and stems and leaves of eucalyptus was 90% in the concentrations (5, 10 mg/ml) and when used the concentration of 1 mg / ml was noted the effect of root extract were higher than the rest of the treatments the mortality rate was 90% compared with (72.2,84.5%)

when treated with cold water extract of stem, leaves respectively and (833, 33%). When treated with boiling water extract for the stem and leaves respectively. In control treatment the cumulative mortality in cold water extract were (6.8, 5.2, 4.6%) and in boiling water were (2.3, 8.7, 3.6%) in root, stem and leaves treatments, respectively.

The same table shows that the cold and boiling water extract had a high effect on the percentage of cumulative mortality. The cold water extract was more effective than the boiling water extract. The results of the statistical analysis indicated the differences in the results obtained. This may be due to the effect of these extracts in the insect stages because they contain phenol compounds, toxic substances and other effective compounds that act as anti-feeding, causing the insect mortality. The deadly cause of the insect is caused by the nervous and digestive system of the insect by contact these extracts to insect body and enterance through the spiracles (Halawa *et al*, 1998 and Saleh *et al*, 2010). The reason for the existence of high cumulative loss rates in the plant under study for larvae is an indication of the presence of poisoning and accumulation of active substances in the studied plant in the digestive canal of larvae and the appearance of many deformities, the cause may be the effect of these substances on the digestive enzymes of the food in the gut. Wigglesworth (1972) reported that a group of enzymatic microsomal oxidase enzymes in the gastrointestinal tract of insects is important in removing the toxic effect of plant natural compounds, any effect on these enzymes by these compounds can cause gastrointestinal tract toxicity of the insect.

A study of Saleh *et al* (2010) confirms the superiority of the water extract of leaves of the Nerium oleander plant in the biological efficiency of the cumulative mortality of mosquito larvae *Cx. quinquefasciatus* given a concentration of 3000 ppm mortality of 100% killing.

Effect the chloroform extracts of *E. microtheca* in the percentage of cumulative mortality of immature stages in *Cx. molestus*

The results of Table 2 shows that the cumulative mortality rates on immature stages in the chloroform extract increased with the increase in concentrations used. When using the root extract and the stems, the percentage of mortality was 80%, 54% respectively at 0.25 mg /ml and increased to 90% at the concentration of 0.5 and 1 mg/ml either in the extract of leaves, the rate of the percentage of mortality was 33.3% and 70.8% when treated with concentrations 0.25 and 0.5 respectively and increased to 90% at the concentration of 1 mg/ml, while in control treatment the mortality percentage was 5.4% in all treatments, the results of the statistical analysis showed the significance of the differences in results.

It is noticed through the present study that the greater the concentration and duration time, the percentage of cumulative mortality of immature stages was increased, this may be due to the effect of these extracts in insects stages because they contain toxic substances or other effective compounds acting as antifeeders causing the insect to die. The reason of the insect may be affected by the nervous system and the digestive system of the insect by contact with these extracts of the surface of the body of the insect or the entry of these substances through the respiratory spiracles (Halawa *et al*, 1998) or may explain the destruction of the fourth larvae to starvation (Frankel, 1969). These compounds effective on epithelial cells of the digestive tract and causes cases of insect poisoning,or combined with fatty substances in the digestive system and causing larval death as lipids are removed without benefit (Pederson *et al*, 1976 and Metspalu *et al*, 2001).

Al-Gazali (1999) noted the effect of turbent extract of *C.citrinus* when treating *Cx. pipiens* Mosquitoes produced the cumulative mortality of 100% immature stages for all concentrations. Al-Shukri (2000) noted the extract of the crude turbent compounds of the leaves of the *L. lutea* showed a cumulative mortality on immature stages of *Cx. pipiens* mosquito. by (38 and 100%) in concentrations (0.25 and 1.5 mg/ml), respectively. Mehdi (2001) found that by continuing to expose the immature stages of the Anopheles mosquito to the extract of the turbent compounds of *M. azedarach*, it resulted in a 100% cumulative mortality at concentrations (100 and 200 ppm). Al-Khafagi (2003) confirmed that the treatment of immature stages of mosquitoes *Cx. pipiens* with crude turbent compounds of *Schangina aegyptica* cause a cumulative mortality (37.2% and 100%) at concentrations of 0.5 and 2 mg/ml. Thomas *et al* (2004) confirmed the volatile oils of *Ipomoea cairicalinn* caused cumulative mortality of mosquito species *Ae. aegypti* and *An. stephensi* and *Cx. quinquefasciatus* in 100% for concentrations (100, 120 and 170 ppm). Mahmoud (2007) found the continue exposure to immature stages of mosquitoes *An. pulcharrhimus* forterbenic compounds of leaves *Datura inoxia* caused cumulative mortality 74.2% in the concentration 20 mg/ml. Al-Khafagi (2010) reported the continuous exposure the immature stages of mosquitoes *Cx. pipiens* to the terbinoid leaves extracts of *Ricinus communis* caused cumulative mortality 100% in all concentrations. The results of Al-Khafagi (2010)

Table 1 : Effect the the water extracts of *E. microtheca* in the percentage of cumulative mortality of immature stages in mosquitoes *Cx. molestus*.

Concentrations (mg/ml)	% Mortality cumulative of the immature stages					
	Cold aqueous extracts			Boiled aqueous extracts		
	Stems	Leaves	Stems	Leaves	Stems	Leaves
Control	6.8	5.2	4.6	2.3	8.7	3.6
1	90	84.5	72.2	90	83	33.3
5	90	90	90	90	90	90
10	90	90	90	90	90	90

L.S.D. interaction between effect of difference of the type of extract and type of plant part in the percentage mortality cumulative of the immature stages 12.733

Table 2 : Effect the interaction of the chlorophoram extract of *E. microtheca* in the percentage of cumulative mortality of immature stages of mosquitoes *Cx. molestus*.

Concentrations (mg/ml)	% mortality cumulative of the immature stages		
	Roots	Stems	Leaves
Control	5.4	5.4	5.4
0.25	80	54	33.3
0.5	90	90	70.8
1	90	90	90

L.S.D. interaction between effect of difference of the type of extract and type of plant part in the percentage mortality of cumulative of immature stages 11.241

showed that the extract of turbine compounds for the leaves and roots of *G. glabra* caused cumulative mortality to the immature stages of mosquitoes *Cx. pipiens* 100% for all concentrations. The results of AL-Fetlawy (2014) confirmed cumulative mortality of the immature stages of mosquitoes *Cx. pipiens* by 100% when treated with turbinet extract of *Tamarix ramsissima* in concentrations (2.5, 5, 10 and 20 mg/ml).

Effect the water extracts of euocalyptus in morphological deformations in insect stages

The results of the present study showed the appearance of some morphological abnormalities in the form of the fourth larval stage, pupae and adult insect observed. Inflammation the fourth stage larvae were treated with cold water extract of the leaves of the eucalyptus plant at the concentration of 5 mg/ml by 100%. The concentration of 10 mg/ml was dwarfed in the fourth stage larvae by 100% compared to the control treatment with a percentage of deformities was 0%.

In the boiling water extract of leaves of eucalyptus, a partial ecdysis of the insect pupae was observed in the concentration of 1 mg/ml by 60.60% and a partial emergence of 30.30% compared to the control treatment. the percentage of deformities in the control treatment was 0%.

Table 3 : Types and frequency of active groups in cold and boiling water extract and chloroform for the roots of eucalyptus plant using FT-IR technique.

The Type/ Eucalyptus roots	Package Frequency	Type of active groups
Cold Aqueous extract	868.00	Bending C-H aromatic
	1452.45, 1516.10	The structural stretching of benzene ring
	1618.33	Stretching C-C alkene
	1734.06	Stretching C=O carbonyl
	2929.97	Stretching C=H aliphatic
	3217.37	Stretching alcoholic O-H
Boiled Aqueous extract	773.48, 821.70	Bending C-H aromatic
	1444.73, 1525.74	The structural stretching of benzene ring
	1608.69	Stretching C=O+C=C (coupling vibration)
	2931.90	Stretching C=H aliphatic
	3385.18	Stretching alcoholic O-H
Chloroform extract	729.12, 821.70, 883.43	Bending C-H aromatic
	1464.02, 1604.83	The structural stretching of benzene ring
	1732.13, 1687.77	Stretching C=O carbonil
	2852.81, 2924.18	Stretching C=H aliphatic
	3408.33	Stretching alcoholic O-H

Fig. 1 : Male of *Cx. molestus* treated with disstilled water (control) (4X).

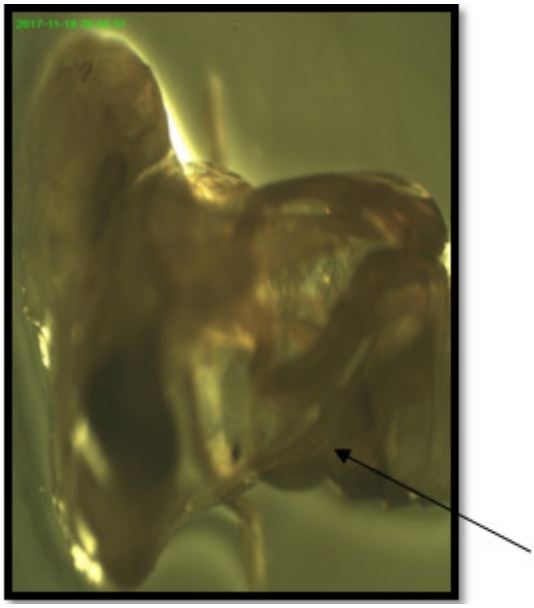

Fig. 2 : Fusion the head and thorax in *Cx. molests* treated with chloroform root extract of *E. microthec*a in conc. 0.25mg/ml (power zoom 4X).

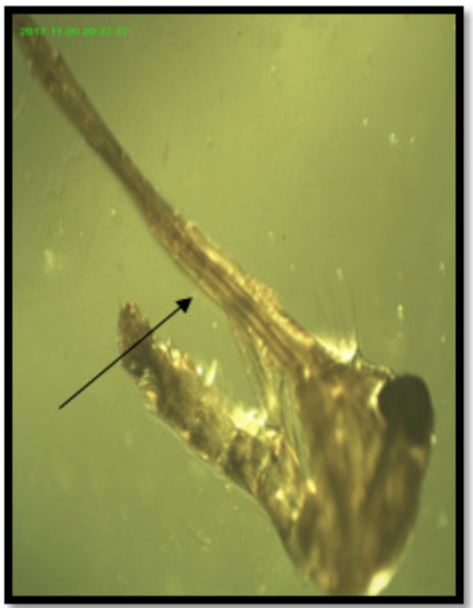

Fig. 3 : Adhesion the wings and legsin *Cx. molests* treated with chloroform extract of *E. microtheca* in conc.0.25mg/ml. (power zoom 4X) stem.

Fig. 4 : Enlarge the head and atrophy of the rest of the body parts in *Cx. molests* treated with chloroformal stem extract of *E. microtheca* in conc.1mg/ml (power zoom 4X).

It is clear from the foregoing that the type of deformation shown by the treatment of plant extracts is associated with the type of plant extract as well as the concentration used. on the other hand, the appearance of phenotypic deformities in the treatment is related to the nature of the effect of the material. The appearance of deformities was associated with the insect growth regulators. This may give the impression that the effect of these substances is similar to the effects insect growth regulators.

Mahmoud (2007) showed that there is a clear effect of the aquatic and organic extracts of albizia plants and the leaves of *Mytrus* against the white fly insect on the cucurbitae. These were the appearance of abnormalities in the immature stages and the intermediate stage between the larva and the pupae, in addition to the failure of some adult in emergence.

Basak (2001) indicated the larvae and nymph of insect that treated with Azadirachtin extracted from Neem plant *Azadirachta indica* caused mortality 70-

60% because Azadirachtin inhibits the secretion of Ecdysone, which inhibits the process of larvae and nymphs moulting to complete their life cycle and transfer to complete insect.

Effect the chloroform extracts of eucalyptusin morphological deformations in insect stages

The results of the treatment of the fourth instar larvae with chloroform extracts showed that the adults resulting from the treatment were weak and could not fly and remained on the surface of the water and died within a few hours of their emergence and extracts of roots, stems and leaves of eucalyptus. The percentage of deformities was 100% in concentration 0.25 mg/ml and in conc. 0.5 mg/ml for stem and leaves extracts it was similar to the control treatment, which had a percentage of malformations (0%). Most cases of deformity were caused by the merger of the head with the chest in the root extract (Fig. 9) and the adhesion of the wings with the legs in the adults resulting from the treatment with the stem extract. Fig. 10 as well as the size of the head and the atrophy of the rest of the parts of the body (Fig. 11) and also can be observed severe curvature in the abdomen (Fig. 12) or shift the larva to the dark color (Fig. 13). As well as the emergence of partial dissolution of the insect pupae in the chloroform extract of the roots of the eucalyptus plant at a concentration of 0.25 mg/ml 22.22% and the emergence of partial emergence of insect pupae by 22.22% compared to the treatment of control as the percentage of abnormalities 0%, as in Fig. 13.

The results of the study conducted by Saleh *et al* (2010), confirmed the use of organic extracts of eucalyptus *E. microtheca* and *Datura innoxia*. The petroleum extract had an effect on the appearance of deformities of *Cx. quinquefasciatus* larvae treated with these extracts.

Janite (1996) showed that the treatment of *Ipsilon agrotis* eggs with extracts of black pepper *Nigrum piper* and *Chmomilla matericaria* led to the failure of a large number of pupae resulting from eggs that treated and deformation in the wings that become crusty and the insect can separate it.

Pointed out that the extracts of the *Indica azadirachta* caused a deformation in the winges of *Clavigralla scutellaris* (Hemiptera: Heteroptera) in each concentration that used, Coreidae, also there was a positive correlation between deformation and concentration, where at 0.2% the deformity was in the two wings and at 0.1% concentration there were deformities in one wing. The concentration of 0.25% and 0.5% resulted in slight deformation in the wing (Mitchell *et al*, 2004).

Fig. 5 : The curved shape in larvae *Cx. molests* treated with chloroformal stem extract of *E. microtheca* in conc.1mg/ml. (power zoom 4X).

Fig. 6 : Transfer the colour larvae to dark in *Cx. molests* treated with chloroformal stem extract of *E. microtheca* in conc. 0.5mg/ml. (power zoom 4X).

Detection of active chemical groups in cold water, boiling water and chloroform for the roots of eucalyptus *E. microtheca* using FT-IR technology

The FT-IR spectroscopy technique showed a number of characteristic active groups found in the roots of the eucalyptus plant. They were shown in Table (3), which showed the most effective groups that appeared in the cold water extract. For the benzene ring at frequencies

Table 4 : Type and frequency of active groups in cold water, boiling water and chloroform extracts of eucalyptus *E. microtheca* using FT-IR technique.

The Type/ Eucalyptus stem	Package Frequency	Type of active groups
Cold Aqueous extract	1452.45, 1512.24, 1545.03	The structural stretching of benzene ring
	1620.26	Stretching C-C alkene
	1716.70	Stretching C=O carbonyl
	2926.11	Stretching C=H aliphatic
	3400.62	Stretching alcoholic O-H
Boiled Aqueous extract	723.33	Bending C-H aromatic
	1462.09, 1514.17	The structural stretching of benzene ring
	1631.83	Stretching C-C alkene
	1689.70	Stretching C=O carbonyl
	2931.90, 2922.25	Stretching C=H aliphatic
	3394.83	Stretching alcoholic O-H
Chloroform extract	883.43	Bending C-H aliphatic
	1417.73, 1514.17	The structural stretching of benzene ring
	1631.83	Stretching C-C alkene
	1689.70	Stretching C=O carbonyl
	2922.25	Stretching C=H aliphatic
	3394.83	Stretching alcoholic O-H

Table 5 : Type and frequency of active groups in cold water, boiling water and chloroform for leaves of eucalyptus *E. microtheca* using FT-IR technique.

The Type/ Eucalyptus leaves	Package Frequency	Type of active groups
Cold Aqueous extract	1516.10	The structural stretching of benzene ring
	1624.12	Stretching C-C alkene
	1716.70	Stretching C=O carbonyl
	2935.76	Stretching C=H aliphatic
	3227.02	Stretching alcoholic O-H
Boiled Aqueous extract	760.56	Bending C-H aromatic
	1460.45, 1514.17	The structural stretching of benzene ring
	1610.61	Stretching C-C alkene
	1714.77	Stretching C=O carbonyl
	2924.18	Stretching C=H aliphatic
	3356.18	Stretching alcoholic O-H
Chloroform extract	771.55	Bending C-H aromatic
	1514.17	The structural stretching of benzene ring
	1631.83	Stretching C-C alkene
	1730.21, 1689.70	Stretching C=O carbonyl
	2850.88	Stretching C=H aliphatic
	3414.12	Stretching alcoholic O-H

1452.45 and 1516.10, for the alkene range C = C at 1618.33, for the carbonyl C = O at 1734.06, CH for aliphatic at CH 2929.97, for O-H at 3217.37.

The boiling water extract showed a CH aromatic group at frequencies 773.48 and 821.70, the structural group of the benzene ring at frequencies 1444.73 and 1525.74, and two sets of C = O and C = C alkene at 1608.69 and CH At 2931.90 and OH at 3385.18.

In the chloroform extract, the active groups were aromatic at frequencies 729.12 and 821.70, the structural sequence of the benzene ring at frequencies 1464.02 and 1604.83, the group C = O at frequencies 1732.13 1687.77, and the CH group at the frequencies 2852.81 and 2924.18 and the O-H group at 3408.33.

Detection of active groups in cold water, boiling water and chloroform for *E. microtheca* plant using FT-IR technique

The infrared spectroscopy technique FT-IR showed

a number of active groups found in the Eucalyptus stem. In Table (4), which showed the most active groups that appeared in the cold water extract. The frequencies of 1452.45, 1512.24 and 1545.03, the alkene range C=C at the frequency of 1620.26, the carbonyl group C= O at 1716.70, the C-H aliphatic at 2926.11 and the O-H at 3400.62.

The boiling water extract was observed as C-H aromatic at 723.33, the structural scale of the benzene ring at the frequencies 1462.09 and 1514.17, the alkene range C = C at frequency 1631.83, the carbonyl group C = O at frequency 1689.70, and the C-H aliphatic array at frequencies 2931.90 and 2922. 25, and the O-H alcohol set at 3349.83.

In the chloroform extract, the active groups were C = C in alkenes at 1616.40, carbonyl C = O at 1689.70, C-H at 2922.25, and O-H at 3394.83.

Detection of active groups in cold water, boiling water and chloroform for *E.microtheca* leaves using FT-IR technique

The FT-IR spectroscopy technique showed a number of active groups present in the eucalyptus leaves, where they were shown in Table (5), which showed the most effective groups that appeared in the cold water extract, the structural structure of the gasoline ring at 1516.10, alkene at 1624.12 and the carbonyl group C = O (dehydrate or ketone) at 1716.70, CH at 2935.76 and OH at 3227.02.

In the boiling water extract, the presence of CH aromatic at 760.56, the structural sequence of the benzene ring at the following frequencies (1460.45 and 1514.17), C = C at 1610.61, C = O at 1714.77, CH at frequency 2924.18, the OH alcohol is at 3365.90.

In the chloroform extract, the active groups were aromatical at 771.55, the structural matrix of the aromatic benzene ring at 1514.17, C = C at 1631.83, and C = O at 1689.70 and 1730.21, CH at 2850.88, OH electrolytes at frequency 3414.12.

CONCLUSION

The above mentioned extracts have an effect in inhibition of emergence and that effect is positively correlated with the concentration.

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