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A New Dammarane Triterpenoid Compound Isolated From Ixora Finlaysoniana

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Abstract: *The air-dried leaves of Ixora finlaysoniana Wall. ex. Don (family Rubiaceae) afforded a new dammarane triterpenoid named: 17β-dammara-1-one -20 ene-3β-3', 8'-dimethyloctanoate* ***(1)*** *together with two known saturated fatty acids capric* ***(2)*** *and palmitic* ***(3)*** *from the chloroform extract as well as three phenolic acids: gallic* ***(4)****, caffeic* ***(5)****, chlorogenic* ***(6)****, six flavonols: Kaempferol 3-O-β-D-arabinopyranoside* ***(7)****, afzelin* ***(8)****, Kaempferol-3-O-β-glucopyranosyl-7-O-glucuropyranoside****(9)****, Kaempferol-3-O-α-L-rhamnopyranosyl (1'''→6'')(4''-trans-p-coumaroyl)β-D-glucopyranoside-7-O-α-L-rhamnopyranoside* ***(10)****, Kaempferol-3-O-α-L-rhamnopyranosyl(1'''→2'')(4''-trans-p-coumaroyl)β-D-arabinopyranosid-7-O-α-L-rhamnopyranoside* ***(11)*** *and kaempferol* ***(12)*** *in addition to two isoflavones: Biochanin A* ***(13)****, Biochanin A-7-O-β-D-rutinoside* ***(14)*** *were isolated from methanol extract . All these structures were isolated for the first time from Ixora finlaysoniana leaves and were elucidated by different chromatographic and UV, extensive 1D and 2D NMR spectroscopy and MS.*

Keywords: *Ixora finlaysoniana, Rubiaceae, dammarane triterpenoid, kaempferol derivatives, isoflavones*

I. Introduction

Medicinal plants have been of long age remedies for human diseases because they contain components of therapeutic value and used in modern medicine where they occupy a very significant place as raw material for economically important drugs [1]. *Ixora* is a genus of flowering plants (family Rubiaceae) of Asian origin and was introduced into Egypt as an ornamental. The plant is traditionally found to be useful for many ailments like hepatic disorder, cancer, microbial infection, antioxidant, pain, inflammation and has been documented for various medicinal properties. Red *Ixora* is the most popular one commonly used in Indian folk medicine and it is known as *Ixora coccinea* [2]. *Ixora finlaysoniana* Wall.ex.G.Don. is a handsome woody shrub with showy flowers in clusters and evergreen foliage. The dried entire plant of *Ixora finlaysoniana* is used in Thailand as a strength medicine, while the ethanol extract of the plant was proved to have estrogenic effect. Reviewing the current literature for the chemical composition of *Ixora finlaysoniana* recovered only the isolation of apigenin-4`-*O*-β-**D**-glucopyranoside and 11-hydroxy-dodec-5-en-2-one. The botanical study of the stem and leaf of *Ixora finlaysoniana* cultivated in Egypt was previously carried out [3]. Although there are several reported studies on *Ixora* species, little was carried out on *Ixora finlaysoniana* Wall. ex G. Don., (Rubiaceae) relative to other species. Therefore, it was deemed of interest to continue our work [4] for isolation the phytoconstituents from biologically active extracts.

II. Materials And Equipments

2.1. *Plant material*

The fresh leaves of *Ixora finlaysoniana* Wall. ex. G. Don. were collected from plants cultivated in the El-Zohria Botanical Garden, Giza, Egypt, during the spring (2011). The identity of the plant was kindly confirmed by Dr. Mohamed El Gebaly, Lecturer of Taxonomy and Prof. Dr. Wafaa M. Amer, Department of Botany, Faculty of Science, Cairo University for whom the authors are thankful. The leaves were air-dried and kept in tightly closed container while the flowers were used immediately for preparing the essential oil from it. Voucher specimens [IF-1] are kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University until extraction process.

2.2. *General equipments and chemicals*

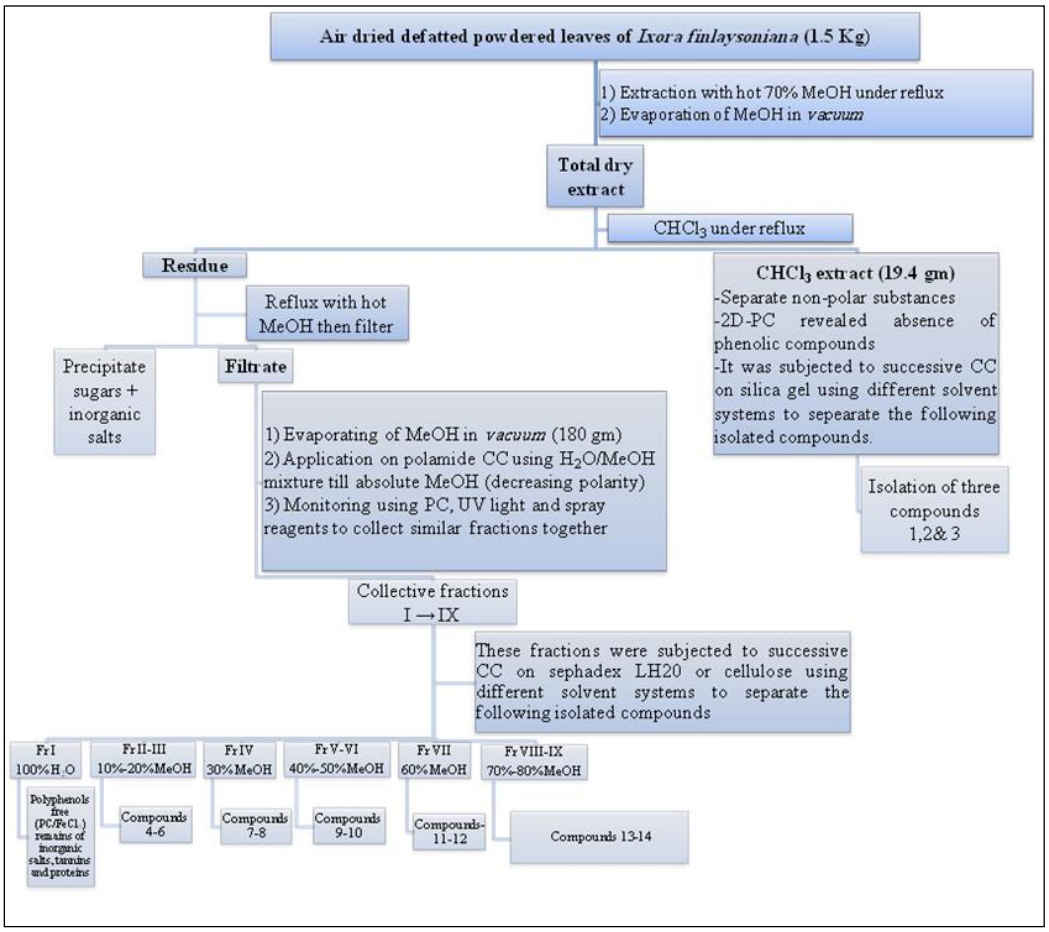
NMR analyses were measured on Bruker Avance III operating at (400 MHz for ^{1}H and 100 MHz for ^{13}C) (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet. Data were analyzed using Topspin 3.1 Software. All samples have been prepared in DMSO-d_6 with TMS as internal reference, with the chemical shifts expressed in δ and coupling constants (*J*) in Hertz.

Electrospray (ESI)-MS was carried out using Thermo Finnigan LCQ Advantage MAX (ion trap) instrument (Finnigan, Bremen, Germany). Samples dissolved in 10 µl of 50% methanol. EI Mass spectrometer: (Chro N_{29} M_y 5526) Ver.1 on UIC 22. UV Spectrophotometer Shimadzu UV 240 (P/N 240-58000) used for recording different UV spectra. UV spectra of pure samples were recorded, separately, in MeOH using different diagnostic UV shift reagents in case of flavonoid [5]. For column chromatography (CC), for isolation of phenolic compounds was carried out on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia), Uppsala, Sweden), silica gel (Sigma, 28-200 mesh) was used for terpenoids CC, and F254 for TLC (Merck, Germany). Plates were visualized by spraying with EtOH/H_2SO_4 and heating at 120°C. PC (descending) Whatman No. 1 and 3 MM papers phenolic and sugar , using solvent systems 15% HOAc (H_2O–HOAc 85:15), BAW (*n*-BuOH: HOAc: H_2O 4:1:5, upper layer). Complete acid hydrolysis for *O*-glycosides was carried out & followed by CO-chromatograph with authentic samples to identify the aglycone and sugar moieties.

III. Methods

3.1. Extraction and isolation

The methodology of the extraction and isolation of *Ixora finlaysoniana* Wall. Ex. G. Don. leaves was summarized in Fig. (1). Identification of the structures of the isolated compounds was carried out using the chemical and physical methods of analysis as well as spectroscopic analysis such as UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR and 2D NMR.



Flgure (1): The methodology of the extraction, isolation and purification of *Ixora finlaysoniana* Wall. ex.

G. Don. Leaves

3.2. Biological studies

3.2.1. Preparation of plants extracts:

The biological evaluation as a continuation of our work [4] was performed on different extracts of *Ixora finlaysoniana* Wall. ex. G. Don. leaves (alcohol extract, petroleum ether fraction, chloroform fraction, ethyl acetate fraction, butanol fraction of leaves) and aqueous decoction as taken in folk medicine was obtained by boiling (100 gm) of the powdered aerial parts of *Ixora finlaysoniana* in 200 ml distilled water. Water was

evaporated under vaccum to give 5.4 gm residue. A solution of extract (in bi-distilled water) containing few drops of Tween 80 was prepared in different dilutions. For the pharmacological activity, 2.5 gm was taken from each fraction. All fractions were dissolved in 1 % aqueous Tween 80.

3.2.2. Experimental animals

Adult male and female albino rats (25-30 g), in addition to male albino rats of Sprague Dawely Strain (130-150 g) were used. Animals were obtained from the animal house, of the National Research Center, Dokki, Giza, Egypt. The animals were fed on standard laboratory diet composed of vitamin mix (1 %), mineral mix (4 %), corn oil (10 %), sucrose (20 %), cellulose (0.2 %), casein (10.5 %) and starch (54.3 %).

3.2.3. Reference drugs and kits

Indomethacin (Epico, Egyptian Int. Pharmaceutical Industries Co) for anti-ulcer activity, Carbamazepin (Novartis Pharma, S.A.E., Egypt; under license of CIBA-GEIGY limited, Basle, Switerland) for anticonvulsant activity, Cidophage (CID Co., Egypt) Glutathione Kit (Wak, company-Germany) for antioxidant activity, Bio-Merieux kit for hypoglycemic activity, carbon tetrachloride (analar), Transaminase kits (Bio-Merieux Co.) for

hepatoprotective activity, Alloxan (Sigma Co.), and Vitamin E (Pharco Pharmaceutical Co. Egypt) inform of soft gelatin capsule, were used in the biological screening.

3.3. Biological Experiments

3.3.1. Toxicity study:

The LD_{50} of both alcohol extract of the leaves and aqueous extracts of the aerial parts of *Ixora finlaysoniana*was determined according to Karber, 1931[6] procedure

3.3.2. Anti-ulcer activity

Acute ulcerogenic activity or gastric mucosa eroding of tested extracts was evaluated and compared with that of indomethacin as a standard using the method described by Corell *et al*., 1979[7].

3.3.3. Anticonvulsant activity

The anticonvulsant activity of tested extracts was evaluated and compared with that of carbamazepinas a standard, as described by Alberto *et al*., 1982 [8].

3.3.4. Antioxidant activity

The antioxidant activity of the tested samples of *Ixora finlaysoniana* was calculated by the determination of glutathione in blood of alloxan – induced diabetic rats adopting the method of Beutler *et al*., 1963 [9]. and using Vitamin E as a positive control

3.3.5. Chronic hypoglycemic activity

The tested samples were evaluated and compared by adopting the method described by Trinder method [10] and using Metformin (150 mg / kg b.wt.) as standard. Glucose levels were measured in blood samples collected at zero time (G_0) i.e. prior treatment and after 4 and 6 hrs intervals from dose administration in case of treated animals (G_t). The percentage change in blood glucose level from initial glycaemia was, in each case, calculated according to the following equation: % of change = $(G_0–G_t) \times 100 / G_0$

3.3.6. Hepatoprotective activity

The tested samples were evaluated by measuring serum AST, ALT [11] and serum ALP [12] using Silymarin (25 mg/Kg b. wt.) as a standard. Male albino rats of Sprague Dawely Strain (130- 150 g) were injected (i.p.) with 25 % Carbon tetrachloride in liquid paraffin (5 ml/Kg b.wt.) to induce liver damage [13]. After oral administration of the tested samples and standard; serum ALT, AST and ALP were collected and the percentages of change in function of liver were calculated after 72 hours of administrating carbon tetrachloride and after 1 month. Serum ALT, AST and ALP were measured according to the method of Thewfweld, 1974[11] and serum ALP according to method [12] at zero time (L_0) and after treatment (L_t). The percentage of change in function of liver was calculated according to the following equation: % of change = $(L_0–L_t) \times 100 / L_0$.

3.4. Statistical analysis

Results are expressed as mean ± S.E., the significance of difference between test and control groups was established by the paired student's t test [14] P values of 0.05 or less were considered as criteria for significance.

IV. Experimental Data Of Isolated New Compound

Compound 1(17β-dammara-1-one -20 ene-3β-3', 8'-dimethyloctanoate) is obtained as white needle crystals; (74.7 mg) gave positive Libermman test, pink color after spraying with $EtOH/H_2SO_4$ reagent indicating terpenoid nature. NMR spectroscopic data for new compound (300 *MHz* for ^1H and 100 *MHz* for ^{13}C, $CDCl_3$) summarized in Table 1.

V. Result And Discussion

The chloroform extract of air-dried *Ixora finlaysoniana* Wall. ex. Don leaves (family Rubiaceae) afforded a new dammarane triterpenoid obtained as white needle crystals; (74.7 mg) gave positive Liebermann–Burchard test, pink color after spraying with $EtOH/H_2SO_4$ reagent indicating terpenoid nature.

IR spectrum revealed the presence of C=O of ester at 1715 cm^{-1} CH stretching at 2927 cm^{-1} and 2857, >C=C< at 1675 cm^{-1} with geminal methyls at 1380 cm^{-1}[13]; whereas the terminal methylene showed vibration at 915 cm^{-1} in $CDCl_3$ [16]. ^{13}C NMR Table (1) showed 40 carbon signals. The Attached Proton Test (APT) spectrum exhibited 10 methyls, 15 methylenes, 8 methines, and 7 quaternary carbons. Seven signals of the dammara moiety were assigned to methyl carbons at [C-18 (δ_c 14.768), C-19 (δ_c 16.351), C-26 (δ_c 15.58), C-27 (δ_c 17.57), C-28 (δ_c 28.267), C-29 (δ_c 18.199), and C-30 (δ_c 18.199)]. Two oxygen-substituted carbons were observed at C-1 (δ_c 215) and C-3 (δ_c 79.045). In addition, an olefinic carbon was detected at C-20 (δ_c 151.24). This data, in combination with the ^1H- NMR signals spectrum of compound Table (1), showed 7 methyl groups at [δ 0.949 (3H, *s*), 0.90 (3H, *s*), 0.886 (3H, *s*), 0. 833 (3H, *s*), 0.793 (3H, *s*), 0.77 (3H, *s*), 1.265 (3H, *s*) and one doublet of terminal olefinic protons at δ 4.57 (*d*, J_{gem} =2, H-21a) and δ 4.69 (*d*, J_{gem} =2, H-21b) [17]. A one oxymethine proton resonates in doublet of doublet at δ 3.18 (H-3a) with a coupling constant 11 *Hz* and 5.5 Hz which indicated that **C** exhibited β-oriented hydroxyl group [18] with absence of signal at δ 5.39 of olefinic bond at C-12 which prove 12-13 dihydro dammarane type triterpene and two olefinic proton at H-21 (δ_H 4.57, 1H, *brs*-H-21a and δ_H 4.69 , 1H, *brs*-H-21b), suggested that it was a dammarane-type triterpene[19,20].

^{13}C NMR spectrum was similar to that of triterpenoid dammarane type; but downfield shifts for C-1 (δ = 215) featured an additional one oxygenated quaternary carbon atom (δ = 215 for C- 1) and absence of H-1.These results confirm the position of the carbonyl group at C-1. Allocation of this proton was clearly established by HSQC experiments, which showed correlations of H-21a H-21b (δ = 4.57 *brs*, 4.69 *brs*) with carbon signal at δ = 109.503 (C-21). The ^{13}C NMR and APT spectra revealed an olefinic methine carbon resonance at δ = 151.240 (C-20), whereas seven quaternary carbon resonances at 215 ,41.045, 43.064, 37.405, 43.238, 151.240 and 192.0 ppm could be assigned to C-1, C-4, C-8, C-10, C-14, C-20 and C-1`, respectively Table (1). The $^1H$ and $^{13}C$ signals were fully assigned according to heteronuclear single quantum correlation (HSQC) spectra Table (1). The NMR data of the compound skeleton were compared with related structure to those of ixorene 3- isovalarate and ixorene 3`, 8`-dimethyloctanoate [21] that show certain similarity with two exception firstly; the absence δ_c 121,142 of double bond between C-12, 13 and the second difference presence of downfield shift of carbon resonance signal of C-1 (δ_c 215) which confirm other oxygen substituted carbon.
The side chain containing gem methyls group appear in IR spectrum at 1380 cm^{-1} and showing two doublet at δ 0.85 (*J*=6.5, H-26) and δ 0.82 (*J*=6.5, H-27) in NMR with the one bond correlations with δ_c 22.70 and δ_c 19.75 in HSQC plot. The spectral data for ring A, B, C and D completely agree with those reported dammarane teriterpene[15,22]. The 17β-substituted configuration of the side chain is based on downfield chemical shift at δ 2.35 *m* (H-17) [15, 22-24]. All of above data led to fully identify the compound as 17β-dammara 20 ene-3β-ol [14].
The side chain at C-3; methylene alpha to the ester group is at 2.4 ppm and the remaining CH_2 group protons have similar resonance frequencies and overlap in the range of 1.2–1.6 ppm. The integral of this region is proportional to the total number of these CH_2 protons [25]. Four aliphatic methylenes in a single signal, two methine and two terminal methyl groups; integration of these signals indicated that the substituent at position 1'. The ^{13}C NMR data showed ten signals for the 10 carbon atoms arising from aliphatic carbons. The assignment of the protonated carbons, C-2', C-3', C-4', C-5', C-6', C-7', C-8' and C-9', 10' was made from a $^{13}C/^1H$ chemical shift correlation experiment and one due to a methylene dioxy group at δ_c 192.0. Therefore the δ_c from C-2' to C-10' suggest that ^{13}C NMR resonances at 48.3, 38.472, 29.684, 49.238, 40.02, 38.098, 38.098, 19.743 and 18.199 respectively. Table(1) all data of the side chain similar to the reported data of 17β-dammara-12, 20 diene-3β-3', 8' -dimethyloctanoate previously isolated from *Ixora coccinea*[21]. Finally this new structure was identified as 17β-dammara-1-one -20 ene-3β-3', 8'-dimethyloctanoate or [12, 13 dihydroixoroid-1-one-3β-3', 8'-dimethyloctanoate] **(1)** Fig. (2).

Also, isolation of two known saturated fatty acids capric **(2)** [26] & palmitic **(3)** [26], from the chloroform extract as well as three phenolic acids: Gallic **(4)** [27], Caffeic **(5)** [28,29], Chlorogenic **(6)** [27,30], six flavonols: Kaempferol 3-*O*-β-D-arabinopyranoside **(7)** [31], afzelin **(8)** [32,33], Kaempferol-3-*O*-β-glucopyranosyl-7-*O*-glucuropyranoside **(9)** [31], Kaempferol-3-*O*-α-L-rhamnopyranosyl(1'''→6'')(4''-*trans*-p-coumaroyl)β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside **(10)** [34], Kaempferol-3-*O*-α-L-rhamnopyranosyl (1'''→2'')(4''-*trans*-p-coumaroyl) β-D-arabinopyranosid-7-*O*-α-L-rhamnopyranoside **(11)** [34], and Kaempferol **(12)** [35]. Also, two isoflavones: Biochanin A **(13)** [36], Biochanin A-7-*O*-β-D-rutinoside **(14)** [37], were isolated from methanol extract. All these compounds were isolated for the first time from *Ixora finlaysoniana* leaves and were elucidated by different chromatographic and spectroscopic techniques; UV, extensive 1D and 2D NMR and MS in comparison with previous published data [26-37]. LD_{50} of both leaves and aqueous extracts of the aerial parts were (8.4 gm/Kg b.wt.) and (7.8 gm/Kg b.wt.), respectively. Therefore, all tested samples are safe in the range of used doses. *In vivo* studies of *Ixora finlaysoniana* Wall. ex G. Don exhibited different biological activities of the tested samples which is useful in treating certain disease as peptic ulcer, seizures, diabetes and liver diseases.

Anti-ulcer activity; results in Table (2) and Fig. (3), obviously showed potent effect where all tested samples are significantly considered safe on gastric mucosa, where ulcer number was negligible in comparison with ulcerogenic standard indomethacin .

Anticonvulsant activity; results in Table (3) and Fig. (4) revealed variable and significant efficacy where all tested samples at pre-mentioned doses significantly increased the voltage needed to induce an electric shock after treatment when compared with Carbamazepin standard. Both leaves alcohol extract and the aqueous extract of aerial parts of *Ixora finlaysoniana* Wall. ex G. Don showed higher activity in increasing the voltage needed to induce an electric shock so, exhibited anticonvulsant activity, and by more clinical trials it may be involved in many antiepileptic drugs in the future.

Antihyperglycemic and antioxidant activity; From Table (4, 5) and Fig. (5, 6) it could be concluded that, Cidophage at a dose (150 mg/Kg b. wt.) significantly reduced the blood glucose level of induced diabetic rats after two and four weeks by (43.47% and 66.1%), respectively. Tested samples significantly reduced the blood glucose level of induced diabetic rats by (35 %, 33.7 %, 20 %, 16.3 %, 32.7 %, 25.5 %, 28.8 % and 22.9 % after two weeks), (51.5 %, 47.2 %, 26.6 %, 24.4 %, 43.9 %, 42.3 % after four weeks), respectively. Both leaves alcohol extract and the aqueous extract of aerial parts of *Ixora finlaysoniana* Wall. ex G. Don showed potent antidiabetic activity. *Ixora* showed strong antihyperglycemic activity and restored blood glutathione level in diabetic rats due to their strong antioxidant effect, this study prove claim of use it in Indian folk medicine. Finally, it is concluded that the total alcoholic extract of *Ixora finlaysoniana* leaves possesses antidiabetic activity but there is a need of evaluation with proven clinical trials.

Animals treated with Vitamin E (7.5mg/Kg b. wt.) restored the level of blood glutathione in diabetic rats. The level of blood glutathione in diabetic rats was restored after the oral administration of the tested samples. All tested samples possessed a powerful antioxidant activity. Effect may be due to effect of phenolics compounds in all samples. Both leaves alcohol extract and the aqueous extract of aerial parts of *Ixora finlaysoniana* Wall. ex G. Don showed higher antioxidant activity.

Hepatoprotective activity; Chronic liver diseases commonly result in liver fibrosis and oxidative stress is the common mechanism contributing in the initiation and progression of hepatic damage in a variety of liver disorders. Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver fibrosis. CCl_4 is a potent hepatotoxin producing centrilobular necrosis, which causes liver injury [38]. Since the changes associated with CCl_4-induced liver damage are similar to those of acute viral hepatitis [39]. CCl_4-mediated hepatotoxicity was chosen as the experimental model. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects [40].

Liver damage induced by CCl_4 involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue [41]. The toxic effects of CCl_4 on liver have been extensively studied. Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases [42]. During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the blood flow. Their quantifications in plasma are useful biomarkers of the extent and type of hepatocellular damage so; increased levels of ALT, AST, and ALP are conventional indicators of liver injury. The prevention of this phenomenon can be considered as hepatoprotective activity [43]. The results are illustrated in Table (6, 7 and 8) and Fig. (7, 8 and 9).It could be concluded that, silymarin at a dose (25 mg/Kg b. wt.) as a standard significantly reduced the serum AST, ALP and ALT after 72 hrs after administration of carbon tetrachloride by (57.4%, 60.5% and 56.2%) and then after 1 week (78.3%, 88.7% and 75.7%), respectively. After oral administration of the tested samples of the plant under investigation and standard, serum AST, ALP and ALT were collected and the percentage of change in function of liver were calculated after 72 hrs of administrating carbon tetrachloride were recorded and compared to standard silymarin. Total leaves alcohol extract of *Ixora finlaysoniana* Wall. ex G. Don showed higher hepatoprotective activity.

For the therapeutic strategies of liver injury and disease, it is important to find antioxidant compounds that are able to block liver injuries through free radicals generated due to toxic chemicals. Therefore, the present study speculated that the total alcoholic extracts of *Ixora finlaysoniana* leaves protect against diseases that are caused by reactive oxygen species (ROS) because it has radical scavenging ability based on its antioxidant activity against CCl_4 in mice which developed significant hepatic damage as manifested by a significant increase in activities of AST, ALT and ALP concentration that are indicators of hepatocyte damage and loss of functional integrity [44]. The metabolic transformation of CCl_4 by the NADPH-cytochrome P-450 system to form the trichloromethyl radical which is further converted to a peroxy radical. These free radicals readily react with polyunsaturated fatty acids of the endoplasmic reticulum and other hepatocellular membranes to initiate the formation of organic lipid peroxides. In the presence of cellular O_2, these organic peroxy radicals in turn can react with other polyunsaturated fatty acids to perpetuate a series of self-propagating chain reactions, known as "propagation of lipid peroxidation". In addition, it has been shown that CCl_4-induced toxicity may stimulate endogenous reactive oxygen and nitrogen species that have also been suggested to play an important role in the

pathogenesis of hepatotoxicity. Enhanced lipid peroxidation may be associated with depletion of the antioxidant; GSH that found in the heart tissues of CCl_4 treated mice which are a characteristic observation in CCl_4-intoxicated mice. GSH is important in detoxification of the reactive metabolites of cells; tissue necrosis is initiated when the reserves of GSH are markedly depleted. Thus, the reduced (relative to normal) levels of GSH observed in the heart tissues of CCl_4 intoxicated mice might be a reflection of increased oxidative damage [44, 45]. Antioxidant enzymes activities in liver of CCl_4-treated group mice were significantly lower than those in normal control group. Antioxidant enzymes SOD (enzymes that catalyze the conversion of two superoxides into oxygen and hydrogen peroxide which substantially less toxic than superoxide) and CAT (enzymes which degrade hydrogen peroxide to water and oxygen, and hence finish the detoxification reaction started by SOD.) represent one of the protection mechanisms against oxidative tissue-damage.

The administration of the total alcoholic extract of *Ixora finlaysoniana* leaves caused an elevation of the levels of GSH in the liver in serum suggesting that it can restore these antioxidant enzymes and/or re-activate enzyme after the damage caused by CCl_4. In conclusion, this study showed that the total alcoholic extract of *Ixora finlaysoniana* leaves has a remarkable protective effect against CCl_4-induced liver injury in mice and its mechanism is related, at least in part, to its free radical scavenging and antioxidant activities.

VI. Conclusion

Dammarane is a group of triterpenoids isolated from various plants and exhibited valuable biological activities such as antitumor, anti-inflammatory, immunosuppressive and antitubercular [4, 21]; so further studies are needed in order to establish the effect and action mechanisms of the isolated new compound responsible for these biological effects to obtain additional information on the structural factors needed for the potent activity. After illustrating the activities done on *Ixora finlaysoniana*; it could be concluded that the total alcohol extract, the total aqueous extract (as given in folk medicine) has highest biological activities compared to other fractions give *Ixora finlaysoniana* great potential and supporting to use it as a source of natural raw material for phytopharmaceuticals preparations as the new coming treatment since it is an effective safe as hepatoprotective, antiulcer, anticonvulsant, antioxidant and anti-diabetic herbal drug.

This article abstracted from PhD of Asmaa I. Ali, Pharmacognosy Department, Faculty of Pharmacy, Misr International University, Cairo, Egypt

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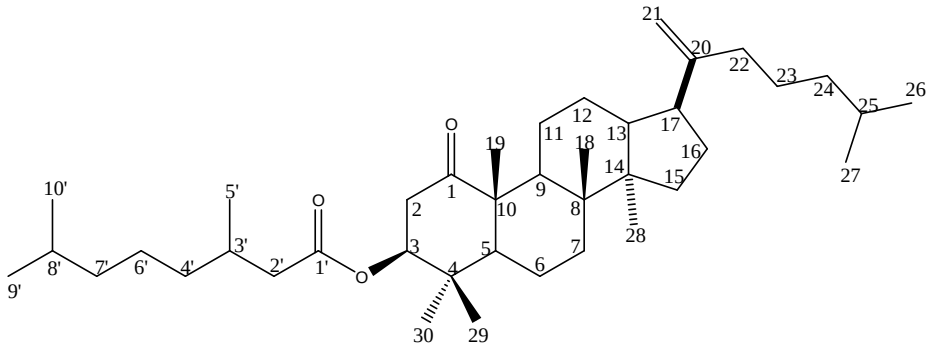

Figure (2): Dammarane Triterpenoid (17β-dammara-1-one-20ene-3β-3', 8' dimethyl octanoate) **OR** (12, 13 dihydroixoroid-1-one-3β-3', 8' dimethyl octanoate)

Table (1): NMR spectroscopic data for new compound (300 *MHz* for ^{1}H and 100 *MHz* for ^{13}C, $CDCl_3$)

Ποσιτιον	δH	δX	Ποσιτιον	δH	δX
1	–	215	21	4.57 $\beta\rho\sigma$H–21α 4.69 $\beta\rho\sigma$H–21β	109.503
2	1.689 $\delta\delta$	28.267	22	1.3 μ	29.68
3	3.18 $\delta\delta$ (ϑ = 5.5, 11 $H\zeta$)	79.045	23	1.208 μ	29.68
4	–	41.064	24	1.26 μ	29.68
5	0.74 σ	55.473	25	1.26 σ	34.5
6	1.302 μ	21.201	26	0.886 σ	15.58
7	1.26 μ	34.502	27	0.833 σ	15.57

8	–	43.064	28	0.793 σ	28.267
9	1.26 μ	50.547	29	0.74 σ	18.199
10	–	37.405	30	1.265 σ	18.199
11	1.62 μ	27.63	1ə	–	192
12	1.26 μ	28.819	2ə	2.43 $\delta\delta(\vartheta = 7.5, 15\ H\zeta)$	48.3
13	1.175 μ	35.819	3ə	1.63 μ	38.472
14	–	43.238	4ə	0.973 σ	29.684
15	1.654 μ	39.098	5ə	1.854 σ	49.238
16	1.2 σ	34.5	6ə	1.89 μ	40.02
17	2.35 μ	48.238	7ə	1.918 μ	38.098
18	0.949 σ	14.768	8ə	1.963 μ	38.098
19	0.900 σ	16.351	9ə	0.973 $\delta(\vartheta = 6.5\ H\zeta)$	19.743
20	–	151.240	10ə	1.003 $\delta(\vartheta = 6.5\ H\zeta)$	18.199

Table (2): Anti-ulcer activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.:

Groups (mg/kg b.wt)	**Number of gastric ulcers (Mean±S.E**	**% protection**
Indomethacin + Total alcoholic extract of leaves (100 mg/kg)	6.4 ±0.1*	57
Indomethacin + Butanol fraction (100 mg/kg)	6.7±0.1*	55
Indomethacin +Total aqueous extract of aerial parts(100 mg/kg)	7.2±0.2*	51.7
Indomethacin + Ethyl acetate fraction (100 mg/kg)	8.2±0.3*	45
Indomethacin + Petroleum ether fraction (100 mg/kg)	11.3±0.4	24.2
Indomethacin + Chloroform fraction (100 mg/kg)	13.6±0.5	**8.7**
Indomethacin (20 mg/kg)	14.9±0.6	0

*****

Statistically significant from the control at p < 0.01.

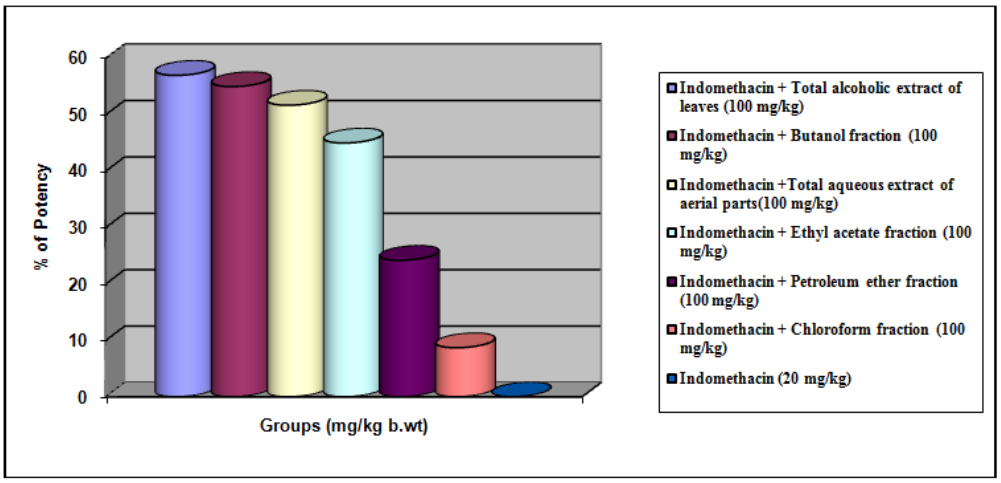

Figure (3): Anti-ulcer activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.

Table (3): Anticonvulsant activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.:

Groups (mg/kg b.wt)	**Volts induced seizures Mean ± S.E.**	**% change**	**% of potency**
Carbamazepin(100mg/kg)	11.7±0.9*	387.5	100
Total alcoholic extract of leaves (100mg/kg)	8.3±0.4*	245.8	**63.4**
Total aqueous extract of aerial parts (100 mg/kg)	7.9±0.3*	229.2	59.1
Ethyl acetate fraction (100 mg/kg)	7.4±0.2*	208.3	53.8
Butanol fraction (100 mg/kg)	5.9±0.3*	145.8	37.6
Chloroform fraction (100 mg/kg)	3.4±0.1*	41.7	10.8
Petroleum ether fraction (100 mg/kg)	3.3±0.1*	37.5	**9.7**
Control (1 ml saline)	2.4± 0.1	----------	----------

*** Statistically significant from the control at p < 0.01.**

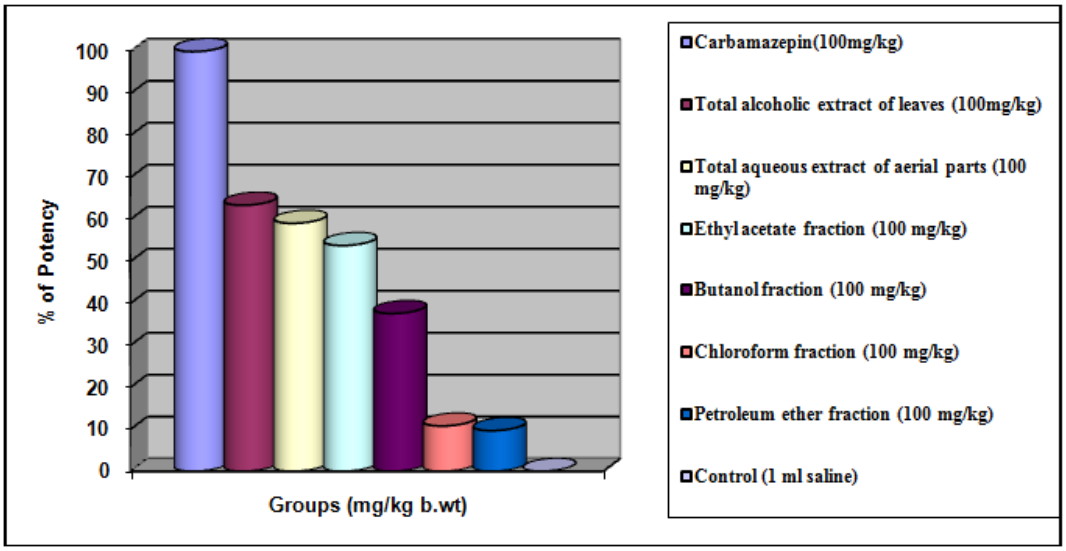

Figure (4): Anticonvulsant activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.

Table (4): Antioxidant activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.:

Animal Group (n=6)	**Blood glutathione (mg %)**		
	Mean±S.E.	**% of change**	**% of potency**
Diabetic + Vitamin E (7.5 mg/kg)	35.9±1.1	67.8	100
Diabetic + Total alcoholic extract of leaves (100 mg/kg)	35.3 +0.9	65	**95.9**
Diabetic + Total aqueous extract of aerial parts (100 mg/kg)	34.6±1.2	61.7	91
Diabetic + Ethyl acetate fraction (100 mg/kg)	34.3±1.2*	60.3	89
Diabetic + Butanol fraction (100 mg/kg)	31.8±0.6*	48.6	71.7
Diabetic + Petroleum ether fraction (100 mg/kg)	28.4±0.6*	32.7	48.3
Diabetic + Chloroform fraction. (100 mg/kg)	26.9±0.5*	25.7	**37.9**
Control (1 ml saline)	36.3±1.4	----------	----------
Diabetic	21.4±0.3*	0	----------

*** Statistically significant from the control at p < 0.01.**

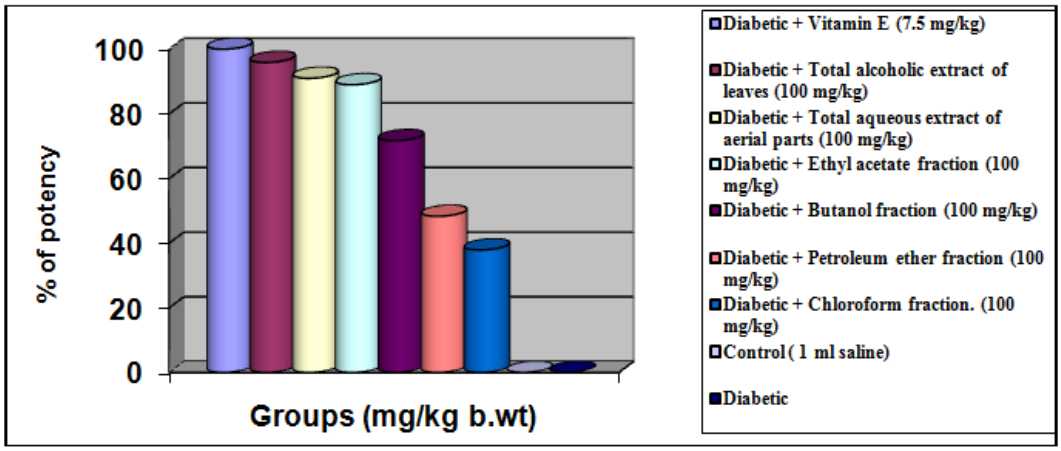

Figure (5): Antioxidant activity of the tested samples of *Ixora finlaysoniana* Wall. ex G

Table (5): Chronic hypoglycemic activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.:

Animal Group (n=6)	Blood glucose level before the treatments (G_0)	After 2 weeks of treatment			After 4 weeks of treatment		
		Blood glucose level (G_t)	% Change	% Potency	Blood glucose level (G_t)	% Change	% Potency
Control (1 ml saline)	83.2±1.9	85.4±2.3	------	------	81.7±2.1	-------	-------
Diabetic non treated	251.6±8.4	259.3±8.1	-----	------	266.2±9.7	-------	-------
Metformin (150 mg/kg)	263.4±7.9	148.9±9.4*	43.47	100	89.3±2.6*	66.1	100
Total alcoholic extract of leaves (100 mg/kg)	251.3±7.8	163.4±5.2*	35	**80.5**	121.9±4.7*	51.5	**77.9**
Total aqueous extract of aerial parts (100 mg/kg)	261.4±8.3	173.2±7.5*	33.7	77.6	137.9±5.4*	47.2	71.5
Petroleum ether fraction (100 mg/kg)	254.6±9.2	203.7±8.5*	20	46	186.8±6.3*	26.6	40.3
Chloroform fraction (100 mg/kg)	258.9±8.1	216.7±6.4*	16.3	**37.5**	195.8±6.1*	24.4	**36.9**
Ethyl acetate fraction (100 mg/kg)	251.6±8.4	169.3± 5.9*	32.7	75.2	141.2±4.3*	43.9	66.4
Butanol fraction (100 mg/kg)	263.8±9.2	196.4±6.5*	25.5	58.8	152.3±4.7*	42.3	63.9

*** Statistically significant from the control at p < 0.01.**

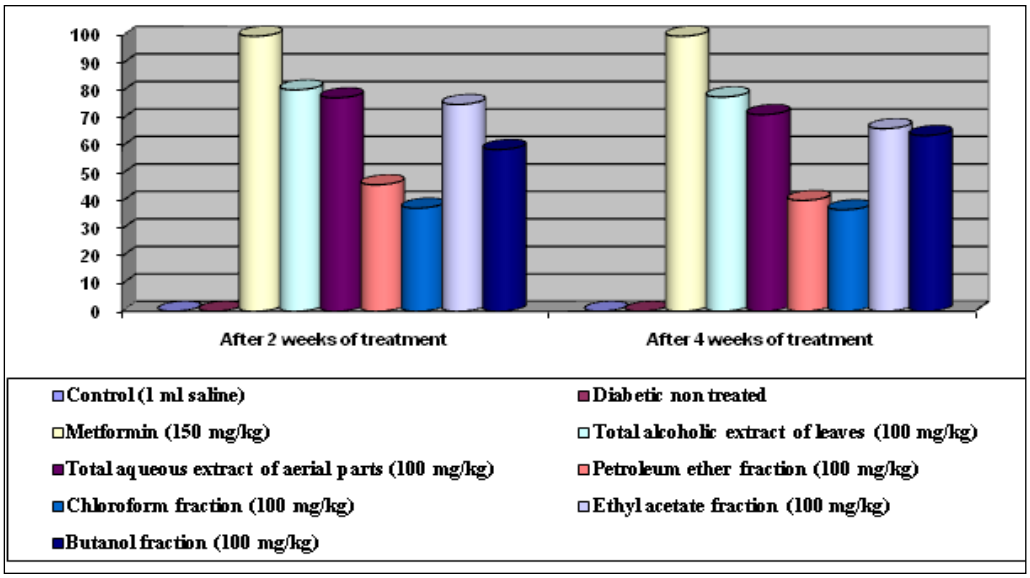

Figure (6): Chronic hypoglycemic activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.:

Table (6): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Aspartate transaminase [AST (m/L)])

Group	Before		% of change and potency after damaging liver 72 hrs			% of change and potency after damaging liver 7 days		
	zero	7d	72h	% of change	% of potency	7d	% of change	% of potency
Control (1 ml saline)	41.8±1.3	42.9±1.8	131.8±4.6*	-------	--------	161.2±3.9□*	-------	-------
Silymarin (25 mg/kg)	38.6±1.4	38.8±1.7	56.2±1.4*	57.4	100	34.9±1.1□	78.3	100
Total alcoholic extract of leaves (100 mg/kg)	38.9±1.3	38.1±1.2	71.4±2.9*	45.8	**79.9**	49.6±2.1□*	69.2	**88.4**
Total aqueous extract of aerial parts (100 mg/kg)	42.6±1.7	41.5±1.4	82.8±3.9*	37.2	64.8	55.1±3.1	65.8	84
Petroleum ether fraction (100 mg/kg)	41.2±1.4	40.1±4.7	105.5±3.6*	20	34.8	94.7±2.6	41.3	52.7
Chloroform fraction (100 mg/kg)	39.5±.12	39.4±1.2	114.9±4.8*	12.8	**22.4**	101.3±4.1	37.2	**47.4**
Ethyl acetate fraction (100 mg/kg)	39.1±0.8	39.8±0.6	78.4±2.3	40.5	70.6	69.6±1.2	56.8	72.5
Butanol fraction (100 mg/kg)	44.1±1.3	43.6±1.4	89.4±3.9*	32.2	56.1	78.5±2.4	51.3	65.5

*** Statistically significant from zero time at p < 0.01**
•Statistically significant from 72h after CCl_4 at p < 0.01.

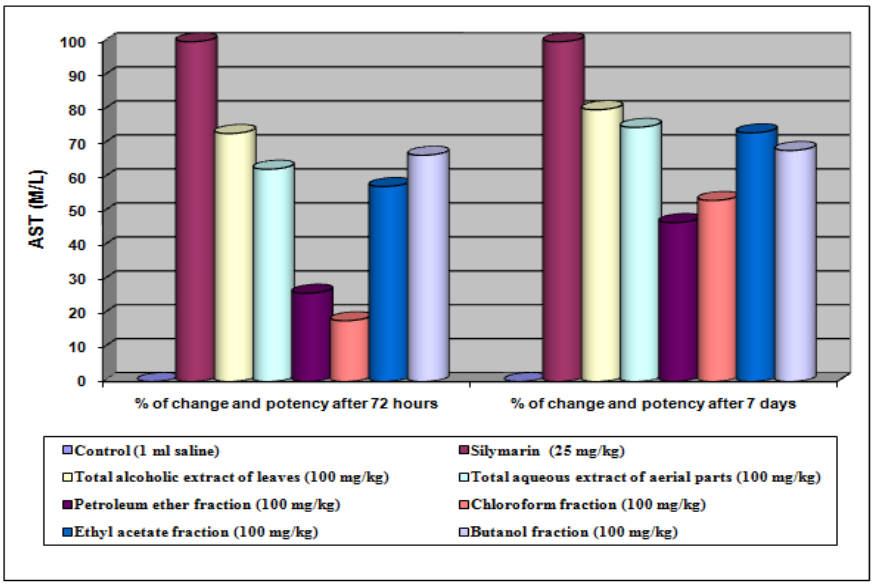

Figure (7): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Aspartate transaminase [AST (m/L)])

Table (7): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Alkaline phosphatase [ALP(m/L)])

Group	Before		% of change and potency after damaging liver 72 hrs			% of change and potency after damaging liver 7 days		
	zero	7d	72h	% of change	% of potency	7d	% of change	% of potency
Control (1 ml saline)	7.1±0.1	6.9±0.1	48.9±1.8*	-------	-------	66.2±2.1□*	-------	-------
Silymarin (25 mg/kg)	7.2±0.1	7.3±0.1	19.3±0.4*	60.5	100	7.5±0.1*	88.7	100
Total alcoholic extract of leaves (100 mg/kg)	7.1±0.1	7.2±0.1	27.3±0.7*	44.2	**73**	19.2±0.4□*	71	**80**
Total aqueous extract of aerial parts (100 mg/kg)	7.5±0.1	7.4±0.1	30.4±1.2*	37.8	62.5	22.3±0.9*□	66.3	74.8
Petroleum ether fraction (100 mg/kg)	7.2±0.1	7.3±0.1	41.2±1.4*	15.7	26	38.7±1.2*	41.5	**46.8**
Chloroform fraction (100 mg/kg)	7.4±0.1	7.5±0.1	43.6±1.7*	10.8	**17.9**	34.9±1.3*	47.3	53.3
Ethyl acetate fraction (100 mg/kg)	7.4±0.1	7.3±0.1	31.9±0.8*	34.8	57.4	23.2±0.7*□	65	73.2
Butanol fraction (100 mg/kg)	7.1±0.1	7.2±0.1	29.2±0.6*	40.3	66.6	26.3±0.6*□	60.3	68

*** Statistically significant from zero time at p < 0.01**
•Statistically significant from 72h after CCl_4 at p < 0.01.

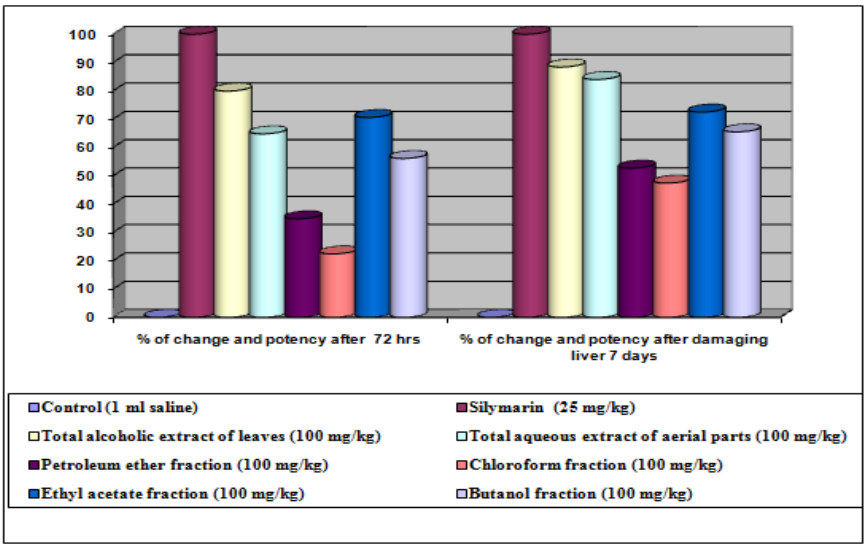

Figure (8): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Alkaline phosphatase [ALP(m/L)])

Table(8): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Alanine transaminase [ALT(m/L)])

Group	Before		% of change and potency after damaging liver 72 hrs			% of change and potency after damaging liver 7 days		
	zero	7d	72h	% of change	% of potency	7d	% of change	% of potency
Control (1 ml saline)	36.8±1.2	37.1±1.4	141.3±4.2*	-------	---------	156.9±4.3•*	-------	--------
Silymarin (25 mg/kg)	39.1±1.3	38.4±1.2	61.9±2.4*	56.2	100.0	38.2±1.1•*	75.7	100.0
Total alcoholic extract of leaves (100 mg/kg)	40.5±1.7	39.2±1.3	51.6±3.5*	63.5	**113.0**	46.8±3.1*•	70.2	**92.8**
Total aqueous extract of aerial parts (100 mg/kg)	37.8±1.4	37.5±1.1	78.3±4.2*	44.6	79.3	53.5±3.9*•	65.9	87.1
Petroleum ether fraction (100 mg/kg)	38.5±1.3	38.6±1.2	118.7±5.1	16.0	28.5	101.3±4.8	35.4	46.8
Chloroform fraction (100 mg/kg)	41.9±1.7	41.7±1.3	124.3±5.28	12.0	**21.4**	109.6±4.7	30.1	**39.8**
Ethyl acetate fraction (100 mg/kg)	33.9±1.1	31.5±0.9	83.6±2.7	40.8	72.7	61.3±1.6	60.9	80.5
Butanol fraction (100 mg/kg)	42.5±1.6	42.3±1.7	81.1±4.9*	42.6	75.8	69.7±3.4*	55.6	73.5

*** Statistically significant from zero time at p < 0.01**
•Statistically significant from 72h after CCl_4 at p < 0.01.

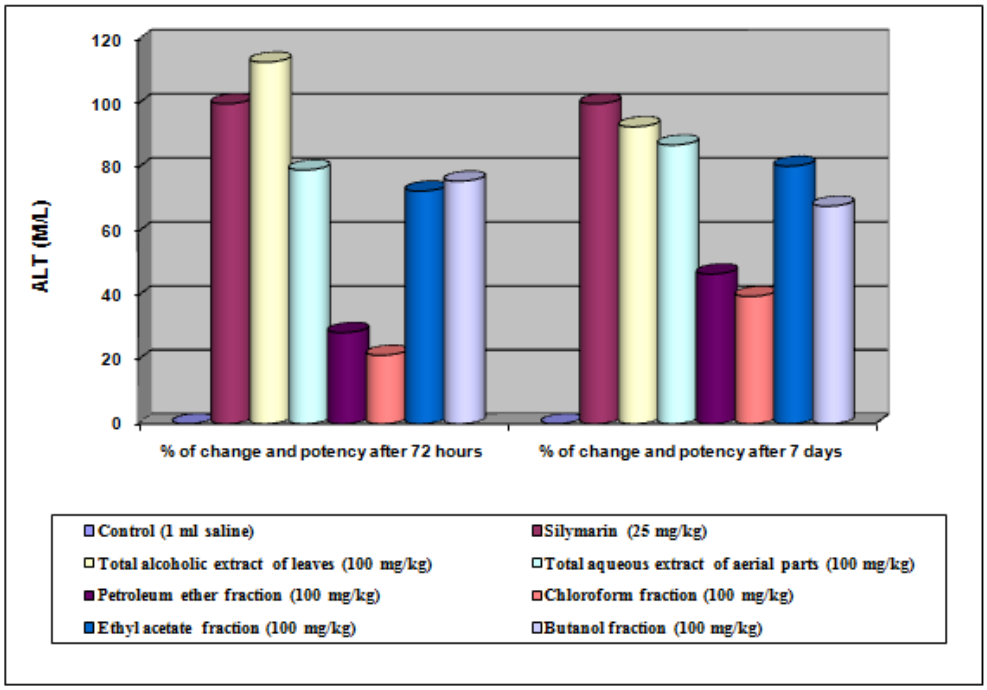

Figure (9): Hepatoprotective activity of the tested samples of *Ixora finlaysoniana* Wall. ex G. Don.: (Alanine transaminase [ALT(m/L)])