PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF IXORA FINLAYSONIANA WALL. EX. G. DON. GROWING IN EGYPT.

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Abstract

*Five flavonoid compounds; 5, 5`-dihydroxy-3, 6, 7, 3`, 4`-penta-methoxy-flavone(* Azazieh H *et al., 2010 ); 5-hydroxy-3, 6, 7, 3`, 4`, 5`-hexa-methoxy-flavone (***Balandrin *et al.*, 1993***); 5, 7,4` tri-hydroxy-flavone[Apigenin](* **Mukherjee et al., 2003** *); 5, 7, 4`-tri-hydroxy-6-methoxy-flavone*[6-methoxy-apigenin] *(***Verma & Singh, 2008** *);Apigenin-4`-O-β-D-glucopyranoside (***Metcalfe & Chalk, 1970** *).In addition to threepentacyclic triterpinoids; 3-Lup-20(29)-en-3-ol[Lupeol] (***Baily, 1960** *),3-Lup-20(29)-en-3,28-diol [Betulin] (***Kirtikar & Basu, 1975** *), and ursolic acid-3-O--D-glucopyranoside (***Hortus, 1976***) were isolated from the ethyl acetatesoluble fraction of the 70 % ethanolic extract of Ixora finlaysoniana. The structures of these compounds were determined by extensive use of UV, ESI-MS, and NMR spectroscopy. The Ethyl acetate fraction and its individual components showed significant antiradical activity by bleaching 1,1-diphenyl-2-picrylhydrazyl radical SC% ranging from (81.63 to 52.68 %) which was comparable to ascorbic acid (SC%72.55) and BHT (SC% 58.82).*

Introduction

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products and their derivatives still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing 25% of the total (**Azazieh et al.,2010; Balandrin et al., 1993***)*. Traditional medicine has served as a source of alternative medicine, new pharmaceuticals, and healthcare products. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds **(Mukherjee, 2003)**. The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involved the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs (Verma **&** Singh, 2008). *Ixora* is a genus of flowering plants inthe Rubiaceae family, it is a plant of Asian origin and was introduced into Egypt as an ornamental and used as hedges and for landscaping.There are numerous named cultivars differing in flower colour (yellow, pink, orange) and plant size. Leaves arecoriaceous, up to 10 cm long, sessile orsubsessile, oblong, obtuse. Flowers arenumerous and found to grow in clusters. Theyare bright scarlet, odorous, in sessile, corymbiform, dense-flowered cymes. Fruits are globose, fleshy, size of a pea and have 2-seeded berry seeds plano- convex. The plant flowers usually in April- May and fruits in May-June (**Baily, 1965; Metcalfe &**

Chalk, 1970; Kirtikar & Basu, 1975; Hortus, 1976; Sunitha et al; 2015). The genus *Ixora* has been used in the Ayurvedic system of medicine for a variety of ailments e.g., leaves in diarrhea, antimicrobial and anti-inflammatory; roots in hiccough, fever, scores, ulcers and skin diseases; flowers in catarrhal bronchitis, dysentery, cytotoxic and antitumor principles. The aerial parts were used as antioxidative, antibacterial, gastroprotective, hepatoprotective, anti-diarrhoeal, anti-nociceptive, anti-mutagenic, anti-neoplastic and chemopreventive effects, hypothermic, semen coagulant activity and ameliorate some metabolic disorders in *schistosomal mansoni* infected mice, thus lending scientific support to the plant's ethnomedicinal uses. (**Hortus, 1976; Kharat et al., 2013 ; Sunitha et al., 2015; Usha, 2016).** Genus *Ixora* consists of tropicalevergreen trees and shrubs and holds around500 species, three of which were cultivated in Egypt;*Ixora coccinea, Ixora finlaysoniana* and *Ixora undulata* (**Hortus, 1976**). *Ixora finlaysoniana* Wall.ex.G.Don. is a handsome woody shrub with showy flowers in clusters and evergreen foliage (**Baily, 1965; Hortus, 1976; Kirtikar & Basu, 1975; Metcalfe &Chalk, 1970**). *Ixora finlaysoniana* wood is febrifuge and the root is aperient, diuretic and deobstruent. The dried entire plant of *I. finlaysoniana* is used in Thailand as a strength medicine (**Baily, 1965; Metcalfe & Chalk, 1970; Kirtikar & Basu, 1975; Hortus, 1976**). while the ethanolic extract of the plant was proved to have estrogenic, abortifacient and anti-implantation effects (**Baily, 1965; Metcalfe & Chalk, 1970; Kirtikar &Basu, 1975; Hortus, 1976**).

Phytochemical studies of some species of genus *Ixora*like *I. coccinea, I. chinensis, I. parviflora, I. undulate, I. amplexicaulis, I. lutea, I. javanica, I. pavetta,I. finlaysoniana,I. philippinensis* and *I. arborea*indicated the presence of important phytochemicals (**Shapna et al., 2009; Kharat et al., 2013; Magdy et al., 2013; Asmaa et al., 2015; Consolacion et al., 2015 ; Saleha et al., 2015; Sunitha et al., 2015; Sunitha et al., 2015; Usha et al., 2016**). such as: **Flavonoids**: (rutin, leucocyanadin glycoside, cyanadin-3-*O*-rutinoside, delphinidin monoglycoside, kaempferol-7-O-α-L-rhamnoside, kaempferol-3-*O*-α-L-rhamnoside, kaempferol-7-*O*-glucoside, kaempferol-3,7-*O*-α-L-dirhamnoside,kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→6)-(4"-*trans*-p-coumaroyl)-β-D-galactopyranoside, kaempferol 3-*O*-robinobioside, kaempferol-7-*O*-methyl ether, kaempferitrin, quercetin,quercitrin, quercetin-3-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-β-D-galactopyranoside, quercetin 3-*O*-robinobioside,quercetin-3-rutinoside, quercetin-3- *O*-glucoside,apigenin, apigenin-7-*O*-β-D-glucopyranoside,apigenin-4`-*O*-β-D-glucopyranoside,apigenin-5-O-β-D-galactopyranoside icariside,Chrysin-5-O-β-D-xylopyranoside,formononetin,5,7-dihydroxyflavone-5-O-β-D-xylopyranoside, epicatechin, and procyanidin A2). Phenolic: (5-*O*-caffeoylquinic acid, chlorogenic acid, 3-methoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside, variabiloside E, and acteoside, 3,3'-bis-(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran), protocatechuric acid, ferulic acid, caffeic acid, gallic acid, (3*R*,5*R*)-3-(β-glucopyranosyloxy)-5-hexanolide (parasorboside) and 1-(*R*)-phenylethanol-*O*-β-gentiobioside, 2-methyl-phenyl-methanol-*O*-β-gentiobioside, 3,4-dimethylphenol-*O*-β-gentiobioside, 2,6-dimethoxyphenol, and 3,4,5-trimethoxyphenol,).Lignans: pinoresinol syringaresinol, and variabiloside E).Coumarin: isoscopoletin).Tannins: ixoratannin A-2 and cinnamtannin B-1). Iridoid: ixoroside, ixoside (7,8-dehydroforsythide), mussaenosidic acid and geniposidic acid). Sesquiterpenes volatile oils: (β-sesquiphellandrene, curcumene, E-α-bergamotene, α-zingiberene, caryophyllene oxide and δ-nerolido).Triterpenoidal compounds:(maslinic acid,ursolic acid,oleanolic acid, 21,23-Epoxy-tirucall-7-en-3β-ol, ixoroid (stigmast-5-

en-3-*O*-β-D-glucoside), cycloartenol esters,lupeol, lupeol fatty ester, β- sitosterol,β-sitosterol-3-*O*-β-D-glycoside, stigmasterol, 3-*O*-β-D-glucopyranosyl-2α,19α-dihydroxyurs-12-en-28-oic acid, 2α,3β,19α-trihydroxyurs-12-en-28-oic acid, α-amyrin, β-amyrin, squalene, 17β-dammara-12,20-diene-3β-ol also known as (ixorene), (24R)-6β-hydroxy-24-ethyl-cholest-4-en-3-one and 7β-hydroxy-sitosterol). Fatty acid composition:(capric, lauric, myristic, palmitic, stearic, oleic, linoleic, arachidic, behenic, crepenynic, ixoric, octadecadienoic acids and methyl esters of palmatic, oleic, stearic and linolic acid, hexadecanoic acid, 11-hydroxy-5-dodecen-2-one,hydroxyhexan-5-olide,9-octadecenoic acid methyl ester, 13-docosenamide, 4,8,12,16-tetramethyl heptadecan-4-olide, 9,12-octadecadienoic acid, di-*n*-octyl phthalate, 1,5-cyclooctadiene, (10*E*)-9-oxooctadec-10-en-12-ynoic acid, azelaic acid, dihydromasticadienolic acid, 8,10,12,14-octadecatetra-enoicacid, 3-butyn-2-ol, 3-butyn-1-ol, 2-octyn-1-ol, 1,9-decadiyne and buglyoxylate).A hydrocarbon alcohol, nonacosanol.Alkaloid:7-[(β-D-glucopyranosyl)oxy]-6-hydroxy-2-methoxy-6,7-dihydro-1,3-thiazepine, also known as rubiothiazepine, in addition to sugar alcholsgalactitol (dulcitol) and D-1-*O*-methyl-myo-inositol.

The present study is aiming to investigate the main chemical constituents of the ethyl acetate soluble fraction of the 70% ethanolic extract and its antioxidant activity of the leavesand stems of of *Ixora finlaysoniana*growing in Egypt.

Materials and Methods

1) Plant Material:

The leavesand stems of *Ixora finlaysoniana* Wall. ex. G.Don. were collected in spring 2014 from El-Zohria Botanical Garden, Giza, Egypt. The identity of the plant was kindly confirmed by Dr. Mohamed El Gebaly (Plant Taxonomy and Egyptian Flora Department, National Research Center, Doki, Giza, Egypt).

2) General Experimental Procedures:

UV spectra were determined with a Hitachi 340 spectrophotometer. The ^{1}H- and ^{13}C-NMR measurements were obtained with a Bruker NM spectrometer operating at 500 MHz (for ^{1}H) and 100 MHz (for ^{13}C) in DMSO-$d_6$or $CDCl_3$solution, and chemical shifts were expressed in □ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{1}H-^{13}C HMBC NMR experiments were carried out using a Jeol AMX-500 high field spectrometer equipped with software Master nova version 5.1.1-3092 program for NMR. ESI-MS (positive ion acquisition mode) was carried out on a TSQ700 triple quadruple instrument (Finnegan, Santos, CA, USA). TLC was performed on pre-coated TLC plates with silica gel 60 F254 (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany). Column chromatography was carried out using Silica gel 60 (Merck, 40-63 and 63-200 µm) and Sephadex LH-20 (Sigma, 25-100 µm). Developed chromatgrams were visualized by spraying with 1% vanillin-H_2SO_4reagent, followed by heating at 100^{o} for 5 min.

3) Extraction and Isolation:

3.a.Extraction and fractionation of the leaves and stems of Ixora finlaysoniana

Powdered air-dried (leaves and stems) of *Ixora finlaysoniana*, (1Kg), were exhaustively extracted at room temperature with 70% ethanol (3 x 5 L). The combined ethanolic extracts were concentrated in *vacuo* at 40 °C to a brown residue (175 g). The

concentrated extract was suspended in distilled H_2O (750 mL) and filtered through filter paper. The filtrate and washings were combined and defatted with petroleum ether(3 x 2 L). The concentrated defatted crude extract (98 g) was partitioned successively (5 x 2 L) with ethyl acetate and *n*-Butanol. Each fraction was pooled and concentrated under *vacuo* at a temperature not exceeding 40°C to afford (32 and 28 g); respectively.

3.b. Isolation and Characterization of Compounds from ethyl acetate solublefraction of

Ixora finlaysoniana

Part of the ethyl acetatesoluble fractionof *Ixora finlaysoniana*(25 g) was chromatographed over a silica gel column (100 × 5 cm i.d., 450 g), eluting with *n*-hexane-ethyl acetate (90:10 → 20:80) to yield 35 fractions (each 75 ml), which were combined into four main fractions; **A** (1.75 mg), **B** (6.90 g), **C** (5.70 g) and **D** (2.68 g) according to their thin layer chromatographic pattern [CH_2Cl_2: MeOH (80:20) or *n*-hexane-ethyl acetate (50:50)]. Fractions **B** and **C**were separately rechromatographed over several Si gel columns eluted with gradual increasing amount of EtOAc in *n*-hexane solvent systems; or with CH_2Cl_2 initially, with increasing concentrations of MeOH solvent systems for further purification.Final purification using sephadex LH-20 column chromatography with CH_2Cl_2−MeOH (70:30) as eluent afforded compounds [**1** (37 mg), **2** (55 mg) and **4** (48 mg), respectively; from Fraction B] and compounds [**3** (42 mg), **5** (23 mg), **6** (30 mg), **7** (25 mg) and **8** (40mg), respectively.

Compound-1(5,3`-dihydroxy-3,6,7,4`,5'-penta-methoxy-flavone):

UV α_{max} (MeOH) 338, 273 and 212 nm; (NaOMe) 379, 317 and 282 nm; ($AlCl_3$) 359, 278 and 215 nm; ($AlCl_3$/HCl) 359, 284 and 216 nm; (NaOAc) 336 and 266 nm; ESI-MS (m/z 405 $[M + H]^+$, m/z 387 $[M + H - H_2O]^+$ calc. for $C_{20}H_{20}O_9$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.90 (1H, s, H-8), 7.32 (1H, d, *J* = 1.2 Hz, H-2`), 7.55 (1H, d, *J* = 1.2 Hz, H-6`), 3.80 (3H, s,OCH_3-3), 3.75 (3H, s,OCH_3-6), 3.90 (3H, s,OCH_3-7), 3.85 (3H, s,OCH_3-3`), 3.75 (3H, s,$OCH_3$-4`); 12.65 (s, OH-5); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 152.30 (C-2), 140.13 (C-3), 179.56 (C-4), 153.48 (C-5), 133.70 (C-6), 160.35 (C-7), 92.45 (C-8), 153.15 (C-9), 106.90 (C-10), 124.84 (C-1`), 103.15 (C-2`), 153.68 (C-3`), 138.55 (4`), 156.25 (C-5`), 107.90 (C-6`), 58.78 (OCH_3-3), 59.12 (OCH_3-6), 57.18 (OCH_3-7), 56.70 (OCH_3-3`), 57.18 ($OCH_3$-4`).

Compound-2 (5`-hydroxy-3, 6, 7, 3`, 4`, 5-hexamethoxy-flavone):

UV α_{max}(MeOH) 336, 274 and 212 nm; (NaOMe) 329, 293 and 215 nm; ($AlCl_3$) 354, 278 and 214 nm; ($AlCl_3$/HCl) 354, 284 and 215 nm; (NaOAc) 329, 277 and 219 nm; ESI-MS (m/z 419 $[M +1]^+$ and 401$[M + H - H_2O]^+$calc. for $C_{21}H_{22}O_9$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.76 (1H, s, H-8), 7.40 (2H, s, H-2`, H-6`), 3.86 (3H, s,OCH_3-3), 3.92 (3H, s,OCH_3-6), 3.99 (3H, s,OCH_3-7), 3.95 (3H, s,OCH_3-3`), 3.95 (3H, s,$OCH_3$-4`), 3.95 (3H, s,OCH_3-5`), 12.55 (s, OH-5); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$ 156.15 (C-2), 138.77 (C-3), 178.65 (C-4), 153.95 (C-5), 133.12 (C-6), 157.92 (C-7), 93.12 (C-8), 152.45 (C-9), 106.28 (C-10), 126.15 (C-1`), 105.44 (C-2`), 153.60 (C-3`), 142.24 (C-4`), 153.45 (C-5`), 105.44 (C-6`), 59.10 ($OCH_3$-3), 59.88 ($OCH_3$-6), 58.50 ($OCH_3$-7), 56.75 ($OCH_3$-3`), 56.46 (OCH_3-4`), 56.75 ($OCH_3$-5`).

***Compound-3 (5,7,4`-Tri-hydroxy-flavone or (Apigenin)*:**

UV λ_{max}(MeOH) 340 and 270 nm; (NaOMe) 395, 349 and 270 nm; ($AlCl_3$) 426, 328 and 274 nm; ($AlCl_3$/HCl) 385, 355, 275 and 266 nm; (NaOAc) 384 and 279 nm; ESI-MS (m/z 271 $[M + 1]^+$ and 293 $[M + Na]^+$ calc. for $C_{15}H_{10}O_5$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.83 (1H, s, H-3), 6.62 (1H, d, *J* = 1.8 Hz, H-6), 6.72 (1H, d, *J* = 1.8 Hz, H-8), 7.84 (2H, d, *J* = 9.0 Hz, H-2`, 6`), 7.09 (2H, d, *J* = 9.0 Hz, H-3`, 5`), 13.68 (brs, 5-OH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 164.15 (C-2), 104.30 (C-3), 183.55 (C-4), 163.0 (C-5), 100.30 (C-6), 164.97 (C-7), 95.62 (C-8), 158.70 (C-9), 105.18 (C-10), 122.70 (C-1`), 129.84 (C-2`, 6`), 119.05 (C-3`, 5`), 162.80 (C-4`).

***Compound-4 (Hispidulin (5,7,4`-Tri-hydroxy-6-methoxy-flavone or (6-methoxy-apigenin)*:**

Yellow powder, UV λ_{max}(MeOH) 338, and 276 nm; (NaOMe) 380, and 287 nm; ($AlCl_3$) 340, 300 sh., and 291nm; ($AlCl_3$/HCl) 342, 300 sh.,289nm; (NaOAc) 339and 297 nm, (NaOAc/H_3BO_3) 334 and 277 nm; ESI-MS (m/z 301 $[M + 1]^+$, 323 $[M + Na]^+$and283$[M + 1 – H_2O]^+$, calc. for $C_{16}H_{12}O_6$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.79 (1H, s, H-3), 6.85 (1H, s, H-8), 7.84 (2H, d, *J* = 8.5 Hz, H-2`, 6`), 7.18 (2H, d, *J* = 9.0 Hz, H-3`, 5`), 13.02 (brs, 5-OH), 3.79 (3H, s, OCH_3-6); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 161.54 (C-2), 108.12 (C3), 182.50 (C-4), 152.80 (C-5), 131.45 (C-6), 157.76 (C-7), 94.30 (C-8), 153.05 (C-9), 104.20 (C-10), 122.56 (C-1`), 130.10 (C-2`, 6`), 119.74 (C-3`, 5`), 160.72 (C-4`), 60.05 (OCH_3-6).

Compound-5*:*(5,7,4`-trihydroxy-flavone-4`-O-β-D-glucopyranoside or (Apigenin-4`-O-β-D-glucopyranoside)

yellow powder, UV λ_{max}(MeOH) 342and 268 nm; (NaOMe) 392, 349 and 274 nm; ($AlCl_3$) 411, 328 sh. and 274 nm; ($AlCl_3$/HCl) 388, 275 and 266 nm; (NaOAc) 372 and 276 nm, (NaOAc/H_3BO_3) 344 and 272 nm; ESI-MS (m/z 433 $[M + H]^+$ and 271 $[M + H - glc]^+$ calc. for $C_{21}H_{20}O_{10}$); ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.86 (1H, s, H-3), 6.48 (1H, d, *J* = 1.6 Hz, H-6), 6.81 (1H, d, *J* = 1.6 Hz, H-8), 7.94 (2H, d, *J* = 8.6 Hz, H-2`, 6`), 6.94 (2H, d, *J* = 8.6 Hz, H-3`, 5`), 12.82 (brs, 5-OH), 4.85 (1H, d, *J*= 7.8 Hz, H1``), 3.29 (1H, dd, *J* = 7.8, 9.5 Hz, H-2``), 3.56 (1H, t, *J* = 9.0 Hz, H-3``)3.72 (1H, t, *J* = 9.5 Hz, H-4``), 3.25 (1H, m, H-5``), 3.72 (1H, dd, *J* = 12.5, 5.6 Hz, H-6``A), 3.92 (1H, dd, *J* = 12.5, 2.7 Hz, H-6``B); $^{13}$C-NMR (100 MHz, DMSO-$d_6$): $\delta_C$162.92 (C-2), 103.40 (C-3), 182.50(C-4), 161.65 (C-5), 99.54 (C-6), 166.12 (C-7),94.79 (C-8), 157.15 (C-9), 105.66 (C-10), 121.25 (C-1`), 128.47 (C-2`, 6`), 117.20 (C-3`, 5`), 161.10 (C-4`), 99.95 (C-1``), 74.67 (C-2``), 77.80 (C-3``), 72.54 (C-4``), 77.10 (C-5``), 62.83 (C-6``).

***Compound-6 (Lup-20(29)-en-3β-ol or Lupeol)*:**

White powder; ESI-MS (m/z 427 $[M + H]^+$, 411 $[M – CH_3]^+$, 409 $[M + H – H_2O]$,207 $[M$ – rings A and B$]^+$and other fragmemt ion peaks at 365, 207, 189, 161, 135, 107, 79 and 41 calc. for $C_{30}H_{50}O$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 1.63, 0.99, (2H, m, CH_2-1), 1.95, 1.84 (2H, m, CH_2-2), 3.46 (1H, dd, *J*= 11.2, 5.5 Hz, *H*□-3), 0.74 (1H, d, *J* = 9.8 Hz, H-5), 1.50, 1.44 (2H, m, CH_2-6), 1.57, 1.35 (2H, m, CH_2-7), 1.54 (1H, m, CH-9), 1.62, 1.29 (2H, m, CH_2-11), 1.72, 1.61 (2H, m, CH_2-12), 2.28 (1H, m, CH-13), 1.65 (2H, m, CH_2-15), 2.20, 1.40 (2H, m, CH_2-16),1.59 (1H, m, CH-18), 2.68 (1H, td, *J* = 10.2, 10.5, 6.5 Hz, CH-19), 1.90, 1.47 (2H, m, CH_2-21), 1.80, 1.35 (2H, m, CH_2-22), 1.03 (3H, s, Me-23), 0.81 (3H, s, Me-24), 0.88 (3H, s, Me-25), 0.96 (3H, s, Me-26), 1.12 (3H, s, Me-27), 0.76 (3H, s, Me-28),

4.58 (1H, d, *J*= 0.5 Hz, Ha-29), 4.72 (1H, dq, *J*= 0.5, 0.8 Hz, Hb-29) and 1.65 (3H, s, Me-30); ^{13}C-NMR (100 MHz, DMSO-d_6): δ_C 38.45 (C-1), 27.30(C-2), 79.15 (C-3), 38.90 (C-4), 55.23 (C-5), 18.55 (C-6), 34.13 (C-7), 42.05 (C-8), 51.14 (C-9), 37.18 (C-10), 20.42 (C-11), 26.70 (C-12), 39.0 (C-13), 41.25 (C-14), 27.62 (C-15), 35.55 (C-16), 47.22 (C-17), 48.25 (C-18), 48.12 (C-19), 152.10 (C-20), 30.18 (C-21), 36.78 (C-22), 28.34 (C-23), 16.20 (C-24), 16.58 (C-25), 16.24 (C-26), 14.75 (C-27), 18.20 (C-28), 109.60 (C-29), 19.72 (C-30).

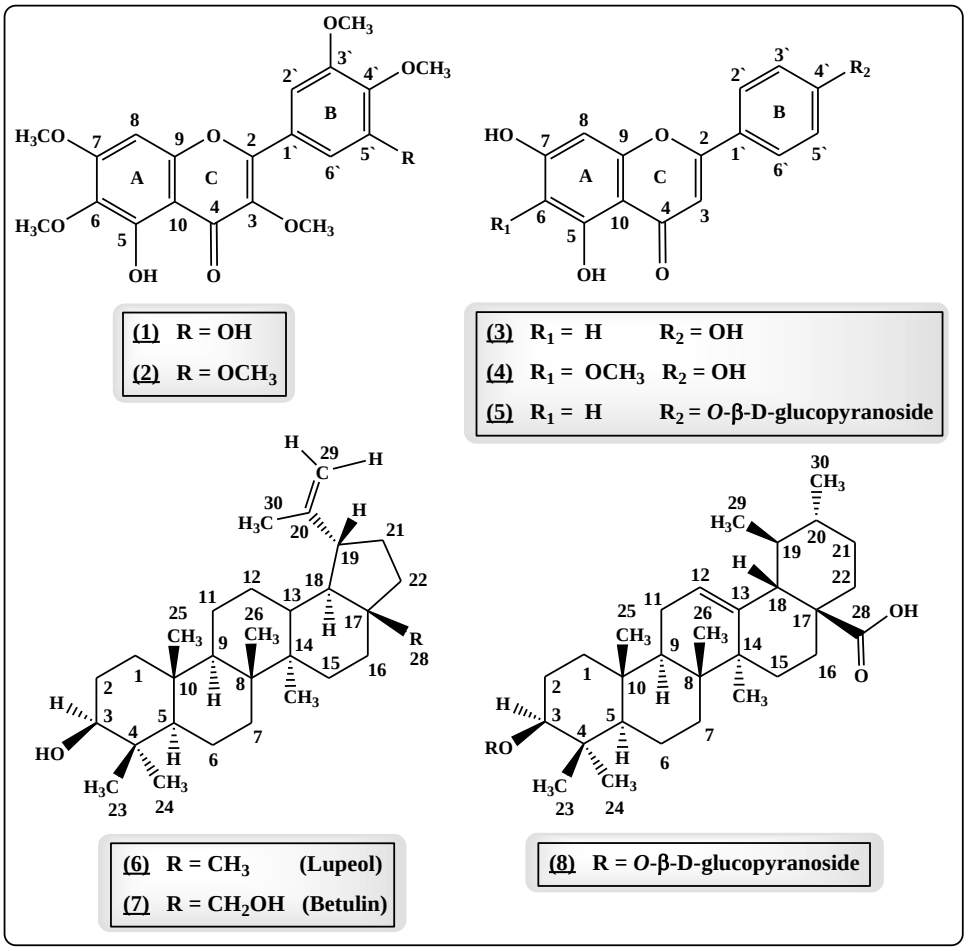
***Compound-7(Lup-20(29)-ene-3, 28-diol or Betulin)*:**

White powder; ESI-MS (m/z 443 $[M + H]^+$, 427 $[M –CH_3]^+$,425 $[M + H – H_2O]$,411 $[M –CH_2OH]^+$,401 $[M – C_3H_5]$,207 $[M$ – rings A and B$]^+$and other fragmemt ion peaks atm/z 208, 163-210, 189, 161, 107, and 79calc. for $C_{30}H_{50}O_2$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 0.92, 1.68 (2H, m, CH_2-1),1.71, 1.59 (2H, m, CH_2-2), 3.68 (1H, dd, *J*= 10.8, 5.6 Hz, *H*□-3),0.67 (1H, brd, *J* = 9.5, H-5), 1.52, 1.39 (2H, m, CH_2-6), 1.40, 1.18 (2H, m, CH_2-7), 1.29(1H, m, CH-9), 1.45, 1.24 (2H, m, CH_2-11), 1.64 (2H, m, CH_2-12), 2.18 (1H, m, CH-13),1.48, 0.98(2H, m, CH_2-15), 1.63, 1.13(2H, m, CH_2-16),1.73(1H, m, CH-18), 2.94(1H, ddd, J = 11, 11, 6.0 Hz, CH-19),1.93, 1.30(2H, m, CH_2-21),1.93, 1.30(2H, m, CH_2-22),0.95 (3H, s, Me-23), 0.77 (3H, s, Me-24), 0.90 (3H, s, Me-25), 0.99 (3H, s, Me-26),1.05 (3H, s, Me-27),3.72, 3.90 (each, 1H, each, d,*J* = 10.8 Hz,CH_2-28), 4.65 (1H, d, *J*= 0.9Hz, Ha-29), 4.81 (1H, d, *J*= 0.9Hz, Hb-29) and 2.05 (3H, s, Me-30);^{13}C-NMR (100 MHz, DMSO-d_6): δ_C38.73 (C-1), 26.94(C-2), 78.90(C-3), 38.72(C-4), 55.48(C-5), 18.32 (C-6), 34.55 (C-7), 40.86 (C-8), 50.66 (C-9), 37.45 (C-10), 20.97 (C-11), 25.81 (C-12), 37.95 (C-13), 42.85 (C-14), 29.10 (C-15), 35.14 (C-16), 48.90 (C-17), 49.80 (C-18), 47.26 (C-19),151.70 (C-20), 29.83 (C-21), 35.93 (C-22),28.90 (C-23), 15.90 (C-24), 15.98 (C-25),16.55 (C-26), 15.10 (C-27), 64.72 (C-28), 109.38 (C-29),19.60 (C-30).

***Compound-8*:*3-β-Hydroxyurs-12-en-28-oic acid-3-glucopyranoside (ursolic acid-3β- glucopyranoside)*:**

White powder; ESI-MS (m/z 619 $[M + H]^+$, 601 $[M + H – H_2O]$,457 $[M + H –$ glc], 439 $[M + H –$ glc $–H_2O]^+$,395 $[M + H –$ glc $–COOH]^+$calc. for $C_{36}H_{58}O_8$;^{1}H-NMR (500 MHz, DMSO-d_6): δ_H1.58, 1.12 (2H, m, CH_2-1),1.88, 1.62(2H, m, CH_2-2), 3.80 (1H, dd, *J*= 11.5, 5.4 Hz, *H*□-3),0.88 (1H, d, *J* = 11.5, H-5), 1.56, 1.39 (2H, m, CH_2-6), 1.66, 1.40(2H, m, CH_2-7), 1.69(1H, m, CH-9), 1.95, 1.15(2H, m, CH_2-11),5.28(1H, t, *J*= 3.8 HzCH-12),2.33, 1.20(2H, m, CH_2-15),2.14, 1.98(2H, m, CH_2-16),2.63(1H, d,*J* = 11.0 Hz, CH-18),1.50(1H, m, CH-19),1.34 (1H, m, H-20),1.58, 1.46(2H, m, CH_2-21),1.90, 1.78(2H, m, CH_2-22), 1.20 (3H, s, Me-23), 0.98 (3H, s, Me-24), 0.92 (3H, s, Me-25), 1.08 (3H, s, Me-26),1.27 (3H, s, Me-27), 0.84 (3H, d, *J* = 6.5 Hz, Me-29), and 0.98 (3H, d, *J*=6.5 Hz, Me-30), 4.55 (1H, d, *J*= 7.5 Hz, H1`-glc), 3.36 (1H, dd, *J* = 7.5, 9.0 Hz, H-2`-glc), 3.62 (1H, t, *J* = 9.0 Hz, H-3`-glc), 3.44 (1H, t, *J* = 9.0 Hz, H-4`-glc), 3.56 (1H, m, H-5`-glc), 3.69 (1H, dd, *J* = 11.5, 5.8 Hz, H-6`A-glc), 3.92 (1H, dd, *J* = 11.5, 3.5 Hz, H-6`B`-glc);^{13}C-NMR (100 MHz, DMSO-d_6): δ_C39.22 (C-1), 28.54(C-2), 81.90(C-3), 39.95(C-4), 55.90(C-5), 18.77 (C-6), 33.84 (C-7), 40.30 (C-8), 48.27 (C-9), 37.55 (C-10), 24.72 (C-11), 126.60 (C-12), 145.12 (C-13), 42.69 (C-14), 29.05 (C-15), 25.44 (C-16), 48.70 (C-17), 53.85 (C-18), 39.64 (C-19),39.78 (C-20), 31.74 (C-21), 37.78 (C-22),28.96 (C-23), 16.50 (C-24), 15.70 (C-25),17.82 (C-26), 24.18 (C-27), 182.30 (C-28), 17.55 (C-29),21.95 (C-30);

99.30 (C-1`-glc), 73.80 (C-2`-glc), 76.18 (C-3`-glc), 71.90 (C-4`-glc), 78.12 (C-5`-glc), 62.75 (C-6`-glc).



(Figure 1) Structure of isolated compounds from*Ixora finlaysoniana*

3.c Evaluation of antioxidant activity by DPPH radical scavenging method (**Brand-Williams et al.,1995; Gordon, 2001; Koleva et al., 2002; Lee et al., 2003**).

The DPPH (1, 1-Diphenyl–2-picrylhydrazyl) assay method is based on thereduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple color). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow color) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenyl picryl hydrazine; non radical) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present) (**Brand-Williams et al., 1995; Gordon, 2001; Koleva et al., 2002; Lee et al., 2003).**

Procedure:

In brief, Dissolve 100 mg of DPPH in 100 ml of ethanol, sonicate for 15 minutes and protected from light by covering the test tubes with aluminum foil and absorbance was taken immediately at 517 nm for control reading (blank) on UV-visible spectrometer Shimadzu. For sample analysis;add 10 µL of sample solution (EtOAc soluble fraction andand its individual components**1-8**(10 mg/ ml)in a 96-well plate to 190 µL of DPPH working solution. The final volume in each is therefore equal to 200 µL. The concentration of DPPH in final solution is approximately 300 µM. Incubate solutions to (30 $\pm$ 2 ^{o}C) for 30 minutes. After incubation, measure the absorbance of each solution at 517 nm. Reference standard compounds being used were ascorbic acid and BHT (butylatedhydroxytoluene). Experiment was done in triplicate. The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical (**Brand-Williams et al.,1995; Gordon, 2001; Koleva et al., 2002; Lee et al., 2003**).

The percent DPPH scavenging effect was calculated by using following equation:

$$\textbf{DPPH scavenging effect (\%) or Percent inhibition} = A_0 - A_1 / A_0 \times 100$$

(Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard samples).The effective concentration of sample required toscavenge DPPH radical by 50% (IC_{50} value) wasobtained by linear regression analysis of doseresponsecurve plotting between % inhibition andconcentrations (**Brand-Williams et al.,1995; Gordon, 2001; Koleva et al., 2002; Lee et al., 2003**).

Preparation of samples

(aDPPH solution was 300 □M in Ethanol.**b)** Extract sample:[EtOAc soluble fraction*and its individual components***1-8**]are 5 mg/ml in DMSO.**c)**Positive samples contains 5mM ascorbic acid (Vitamin C) and BHT (butylatedhydroxytoluene).

Processing

(aThe samples were applied on 96-well microtiter plate.**b)*Blank sample***:10 □l extract sample[EtOAc soluble fraction*and its individual components***1-8**]+ 190 □l Ethanol.**c)*Negative control***: 10 □l DMSO 10% + 190 □l DPPH solution.**d)*Positive control***: 10 □l positive sampls [ascorbic acidand BHT] + 190 □l DPPH solution.**e)**Test samples: 10 □l extract sample [EtOAc soluble fraction*and its individual components***1-8**] + 190 □l DPPH solution.**f)**Plates are covered with aluminum foil to protect the light and incubated at 37^{o}C in 2hrs.and absorbance was taken at 517 nm on UV-visible spectrometer Shimadzu.All the extracts were carried out triple.

Calculate the SC(%) value

From the absorbance value, SC% (Scavenging capacity) was calculated as follows:

$$\textbf{SC\%} = \left[100 - \frac{\textbf{ADS}_{\textbf{(Test sample)}} - \textbf{ADS}_{\textbf{(Blank sample)}}}{\textbf{ADS}_{\textbf{(Negative control)}}} \textbf{ X 100} \right]$$

If the SC% of any samples is much more 50%, they are considered having activemanifestation. Afterward, those samples would test for SC_{50} (substrate concentration to produce 50% reduction of the DPPH (**Brand-Williams et al.,1995; Gordon, 2001; Koleva et al., 2002; Lee et al., 2003**).

Results and Discussion

The 70% ethanolic extract of the air-dried leaves and stems of*Ixora finlaysoniana*was defatted with petroleum ether and partitioned successivelywith ethyl acetate and *n*-Butanol to give the corresponding soluble fractions. A combination of normal phase Si gel, and Sephadex LH 20 column chromatography of the ethyl acetate soluble-fraction led to the isolation of compounds.

***Compounds-1 and 2*:**

were obtained asamorphous yellow powder.Theygave intense yellow color with ammonia vapor and yellowish brown color with vaniline/ H_2SO_4 spraying reagents and heated. UV data of both [**1**] and [**2**] revealed the presence of only a C-5 free hydroxyl group in each compound[Mabry, T. J., et al. 1970]. The ^{1}H- and ^{13}C-NMR showed a singlet proton at ($\square_H$ 6.90, δ_C 92.45 and $\square_H$ 6.76, δ_C 93.12) for [**1**] and [**2**], respectively; assigned for H-8 and two meta coupled protons at ($\square_H$ 7.32, d, *J* = 1.2 Hz, δ_C 103.15 and $\square_H$ 7.55, d, *J* = 1.2 Hz, δ_C 107.90) and ($\square_H$ 7.40, 2H, s, δ_C 105.44) assigned for H-2` and H-6` for compounds [**1**] and [**2**], respectively. Both ^{1}H- and ^{13}C-NMR indicated the presence of five methoxyl groups. A fact was supported by the ESI-MS at m/z 405 $[M + H]^+$ and 427 $[M + Na]^+$ consistent with the molecular formula $C_{20}H_{20}O_9$ for and ESI-MS (m/z 419 $[M + H]^+$ and 441 $[M + Na]^+$ calc. for $C_{21}H_{22}O_9$ for HMBC correlations indicated that [**1**] is 5,3`-dihydroxy-3,6,7,4`,5'-pentamethoxyflavone previously reported from *Gardenia cramerii* and *G. fosbergii*family Rubiaceae **(Gunatilaka et al., 1982)** and *Cleome* species (**Vishal et al., 2012**). In comparison of [**1**] with [**2**] indicated that one of the two free hydroxyls of [**1**] is methoxylated in [**2**]. The additional methoxylation was assigned to position 5` based on the appearance of H-2` and H-6` as a singlet at $\square_H$ 7.40 integrated for two protons and correlated to two overlapped CH at $\delta_C$ 105.44 in the $^{13}$C-NMR. The appearance of signals for three methoxyl carbons at $\delta_C$ 56.46 ($OCH_3$-4`) and δ_C 56.75 (2 X OCH_3-3`, 5`) and for one singlet at $\square_H$ 3.95 integrated for 9 protons, as well as HMBC correlations,further support C-3` methoxylation in [**2**]. From the above discussion and comparison of $^1$H- and $^{13}$C-NMR with the literature, compound [**2**] was identified as 5`-hydroxy-3, 6, 7, 3`, 4`, 5-hexamethoxyflavone; previously reported from *Gardenia cramerii* and *G. fosbergii*and *Cleome* species (**Gunatilaka et al., 1982 ; Vishal et al., 2012**).

***Compound 3*:**

was also obtained as a yellow powder andgave intense yellow color with ammonia vapor. The colored spot appeared as dark purple under UV lamp, turned to yellowish brown color with vaniline/ H_2SO_4 spraying reagents and heated.Its molecular formula was established as $C_{15}H_{10}O_5$ from itsESI-MSspectrum data that showed $[M+H]^+$ ion at *m/z* 271and 293 $[M + Na]^+$. The molecular formula of [**3**]was further supported by its ^{13}C-NMR spectral data. The UV spectrum of [**3**]also showed absorption maxima at 340 and 270nm in MeOH, together with the chemical shift at $\square_H$ 6.83 (1H, s, H-3) in ^{1}H-NMR spectrum, indicating its nature as a flavone (**Mabry et al., 1970).** The ^{1}H NMR spectrum of [**3**]showed thepresence of two meta coupled aromatic doublets at

δ_H6.62 and 6.72 corresponds to H-6 and H-8 protons, two doubletof doublets at δ 7.09 and 7.84 for H-3`/H-5` and H-2`/H-6` protons of ring B, and a singlet at δ_H6.83 corresponding toH-3 proton; characteristic for a 5,7,4'-tri-substituted flavone. The ^{13}C NMR spectrum showed the presence of twelvearomatic carbons; seven quaternary carbons, five methine carbons, and an unsaturated carbonyl carbon. The ^{1}H- and^{13}C-NMR values for all the carbons were assignedon the basis of HMBC correlations. A search in literature suggested the spectral data of [**3**]was consistent to 5,7,4`-trihydroxyflavone, also known as Apigenin (**Agrawal, 1989; Venkata et al., 2013).**

Compound 4

was obtained asamorphous yellow powder.Itgave intense yellow color with ammonia vapor. The colored spot appeared as dark purple under UV lamp, turned to intense yellow color with vaniline/ H_2SO_4 spraying reagents and heated. The positive-ion ESI-MS spectrometry of **[4]** showed ion peak at m/z 301 $(M + H)^+$ indicating its $(M)^+$ to be 300 which was compatible with the molecular formula $C_{16}H_{12}O_6$. The UV absorption spectra in MeOH (338, and 276 nm)and in presence of $AlCl_3$, $AlCl_3$/HCl, NaOMe, NaOAc and NaOAc/H_3BO_3 indicated the presence of free hydroxyl groups at C-5, 7- and 4`- positions, confirmed its nature as a flavone[Mabry, T. J., et al 1970 ]. $^{1}$H-and $^{13}$C-NMR spectra of [**4**], revealed the characteristic chemical shifts and couplingpatterns for a 5, 6, 7, 4'-oxygenated flavone. Signals for an AA`BB` spin-system appear as a doublet at δ_H 7.84 (2H d , *J* = 8.5 Hz, H-2', H-6', δ_C 130.10) and δ_H7.18 (2H d , *J* = 8.8 Hz, H-3', H-5', δ_C 119.74) indicated that the ring- B was substituted at C-4', the singlet signal at (δ_H 6.79,δ_C 108.12) was assigned to H/C-3. The other singlet aromatic proton at (δ_H 6.85,δ_C 94.30) was assigned to H-8 of A-ring. In addition, the ^{1}H- and ^{13}C-NMR spectra of [**4**] showed a methoxyl signal at δ_H3.79 (3H, s, δ_C 60.05). This methoxyl carbon was correlated to the H-8 proton, which was coupled with the quaternary carbon signals at δ_C 157.76 and 152.80 attributed to C-7 and C-5; respectively, by HMBC confirmed that the methoxyl group was placed on C-6 position. Comparison of these NMR data with compound [**3**] as well as by literature comparison (**Agrawal, 1989; Megumi et al., 2003**), compound [**4**] as was established as: 5,7,4`-trihydroxy-6-methoxy-flavone (Hispidulin or (6-methoxy-apigenin).

Compound [5]

was obtained asamorphous yellow powder.Itgave intense yellow color with ammonia vapor and yellowish brown color with vaniline/ H_2SO_4 spraying reagents and heated. The positive-ion ESI-MS showed a psudo-molecular ion peak at m/z 433 $[M + H]^+$ and 271 $[M + H - glc]^+$which in conjunction with the ^{13}C-NMR spectral data, indicating its $[M]^+$ to be 432, suggested that the molecule of **[5]** had $C_{21}H_{20}O_{10}$ molecular formula. The UV absorption maxima recorded in MeOH showed two absorptions at 342 and 268 nm, characterestic for a flavone skeleton as with compounds [**3** and **4**][Mabry, T. J.,et al .1970]. The ^{1}H- and ^{13}C-NMR spectra of **[5]**were showed signals of apigenin skeleton: [a singlet signal at (δ_H 6.86, δ_C 103.40) was assigned to H/C-3; two *meta*-coupled protons (AB-spin system) at δ_H 6.48 and 6.81 (each, 1H, d, *J* = 1.6 Hz, δ_C 99.54 (C-6), 94.79 (C-8) of ring A; characterized the 6- and 8-protons of a flavonoid with 5, 7 dihydroxy A-ring]. It also showed, four aromatic protons at [δ_H 7.94 (2H, d, *J* = 8.6 Hz, H-2`, 6`, δ_C 128.47), 6.94 (2H, d, *J* = 8.6 Hz, H-3`, 5`δ_C 117.20)] represented AA`BB` spin-pattern of 4'-oxygenated B-ring confirmed an aglycone with A-ring functionality at C-5 (δ 161.65) and C-7 (δ 166.12), and B-ring at C-4` (δ

161.10). In addition, the ^{1}H- and ^{13}C-NMR spectra of [**5**] showed the presence of resonance of an anomeric proton and carbon signals at (δ_H 4.85, 1H, d, *J*= 7.8 Hz, H1``, δ_C 99.95), consistent with the presence of an β-D-glucopyranosyl unit. A loss of 162 mass units from the molecular-ion in the ESI-MS at m/z 271 $[M + H - glc]^+$, $(M + H-glucose)^+$ and m/z 253 $(M + H- glucose - H_2O)^+$ suggested the presence of a glucose moiety in **[5]**.The sugar unit was placed on the C-4` position based on the lack of bathochromic shift with NaOMe and the characteristic correlations observed between the glucosyl anomeric proton ($\square_H$4.85) and methine carbons at (C-3 and C-5). Consequently, the structure of compound **[5]** was established as 5, 7, 4`-trihydroxy-flavone-4`-*O*-β-D-glucopyranoside and was good agreement with Apigenin-4`-*O*-β-D-glucopyranoside, previously isolated from *Ixorafinlaysoniana* (**Faten& Zedan, 2003**).

Compound 6

was isolated as a white powder. It gave positive color reaction with Liebermann-Burchard test indicating its steroidal or triterpenoidal nature. The molecular formula was determined as $C_{30}H_{50}O$on basis of the quasi-molecular ion peaks observed at 427 $(M + H)^+$, 411 $[M - CH_3]^+$, 409 $[M + H – H_2O]$,207 $[M – rings A and B]^+$ and other fragmemt ion peaks at 365, 207, 189, 161, 135, 107, 79 and 41, indicated [**6**] was a pentacylic triterpenoidal skeleton[29-32]. The ^{1}H- and ^{13}C-NMR spectra of [**6**] revealed the presence of seven tertiary methyl singlets protons and carbon signals integrated for 3H-each at $\square_H$1.03 (Me-23, $\square_C$ 28.34), 0.81 (Me-24,$\square_C$ 16.20), 0.88 (Me-25, $\square_C$ 16.58), 0.96 (Me-26, $\square_C$ 16.24), 1.12 (Me-27,$\square_C$ 14.75), 0.76 (Me-28,$\square_C$ 18.20) and 1.65 (Me-30,$\square_C$ 19.72). The deshielded singnal at H-3□ proton showed at $\square_H$3.46 (1H, dd, *J*= 11.2, 5.5 Hz, $\square_C$ 79.15) was due to a secondary carbinol group C-3.These spectral data together with signals for isopropenyl group [olefinic quaternary carbon at δc 152.10 (C-20), a pair of signals at $\square_H$ 4.58 appeared as one proton doublet with coupling constant 0.5 Hz and signals at $\square_H$ 4.72 as one proton double quartet with coupling constants 0.5 Hz and 0.8 Hz for an exomethylene group at (H-29a and b,$\square_C$ 109.60, C -29) and methyl singlet at δ_H1.65 (Me-30,$\square\square_C$ 19.72)], suggesting [**6**] was a lupane type triterpene[Kadriya, S. D., et al.2003; Reynolds, W., et al .1976; Tijjani1, A., et al.2012; Tinto,W. F., et al.1992].Themethine proton signal at $\square_H$ 2.68 (1H, td, *J* = 10.2, 10.5, 6.5 Hz, $\square_C$ 48.12) ascribable to 19H/C, further revealed a typical H-19□lupane structure[29-32]. The confirmation of the structure of [**6**] was accomplished through the 2D-NMR ^{1}H-^{13}C-HMBC experiment, the methine proton signal at $\square_H$3.46 (H-3) showed cross peaks with a methyl carbon signal (δc 28.34, C-23) by J_2correlation and a methylene carbon signal (δc 18.55, C-6) by J_3 correlation. The methine proton signal at $\square_H$ 2.68 (H-19) showed cross peaks with two methylene carbon signals δc 30.18 (C-21) and δc 109.60 (C-29)], a methine carbon signal δc 48.25 (C- 18), a methyl carbon signal δc 19.72 (C-30)] and a quaternary carbon signal δc 152.10 (C-20). The pair olefinic proton at $\square_H$ 4.58 and 4.72 showed cross peaks with a methine carbon signal δc 48.12 (C-19) and a methyl carbon signal δc 19.72 (C-30) by J_3correlations. The forgoing spectral analysis and comparison with reported data, afforded the structure of compound [**6**]as ***Lup-20(29)-en-3β-ol*** (**lupeol)**, a pentacylic tri-terpenoid.

Compound 7

was isolated as white amorphous powder.It gave positive color reactionwith Liebermann-Burchard test indicating its steroidal or triterpenoidal nature.Their ESI-MSappeared to be a lupane-type triterpenoid, as suggested by the very intense

fragment peaks at *m/z* 235, 217, 207, 193, and 189 characteristic fragmentation of lupane skeleton with an angular hydroxy methylene group[29-32]. The ESI-MS displayed the molecular ion peaks at (m/z 443 $[M + H]^+$,427 $[M –CH_3]^+$,425 $[M + H – H_2O]$,411 $[M –CH_2OH]^+$,401 $[M – C_3H_5]$,and 207 [M – rings A and B],corresponded to the molecular formula of$C_{30}H_{50}O_2$with 16 mass units higher than [**6**], suggesting an additional hydroxyl group in [**7**].The ^{1}H-and ^{13}CNMR spectra of [**7**] displayedcharacteristic proton and carbon signals of the isopropenyl group; a downfield singlet of vinylic methyl at □□2.05, □C19.60 (Me-30) and a pair of doublets for geminal protons due to exomethylene proton and carbon signalsat □□4.65and 4.81,with coupling constants 0.9 Hz,□C 109.38(H_2/C-29a and b). The double doublet at □□3.68 (1H, dd, *J*= 10.8, 5.6 Hz,□C 78.90) in that of [**7**] was typical for a triterpenoid with□-oriented hydrogen at C-3 of a 3*β*-hydroxysubstituent. The NMR data of [**7]**were similar to those of [**6]**except for the absence of one methyl singlet at C-28 and the addition ofa pair of doublets (*J* = 10.8 Hz) at □□ 3.72 and 3.90 in the ^{1}H-NMR spectrum, as well as the absence of one methyl signal in [**6**] and the addition of hydroxymethylene signal at □C64.72in the ^{13}C-NMRspectrum of [**7**]. This information suggested that **[7]**was a derivative of **[6]**, of which one methyl (Me-28) was replaced by the primary alcoholic group. In all, ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H-^{13}H-HMBC of [7] revealed a compound with six methyl groups, thirty carbon atoms (which is equivalent to the total number of carbon atoms in triterpenoid), a lupene-type triterpenoidal nucleus with two hydroxyl groups at C-3 and C-28. Consequently, the compound was determined to be the known structure, 20(29)-lupene-3, 28-diol, more commonly known as botulin (**Reynolds et al., 1976; Tinto et al.,1992; Kadriya et al., 2003; Tijjani1 et al., 2012).**

Compound [8]

was isolated as a white amorphous powder. It gave positive color reaction with Liebermann-Burchard test indicating its steroidal or triterpenoidal nature. The molecular formula was determined as $C_{36}H_{58}O_8$on basis of the quasi-molecular ion peaks observed at 619 $[M + H]^+$, 601 $[M + H – H_2O]$,457 [M + H – glc], 439 $[M + H – glc – H_2O]^+$, 395 $[M + H – glc – COOH]^+$by ESI-MS, along withother peaks at *m/z* 248, 207, 203 and 189.The ^{1}H- and ^{13}C-NMR spectra with the aid of ^{1}H-^{13}H-HMBC for **[8]** exhibited amethineproton at C-3 appearing at □H3.80 (1H, dd, *J*= 11.5, 5.4 Hz, □C 81.90) was due to a secondary carbinol groupC-3, signals for seven methyl signals, five appeared as singlet at [□H 1.20, □C28.96(Me-23), □H 0.98, □C 16.50 (Me-24), □H 0.92, □C15.70(Me-25), □H 1.08, □C 17.82 (Me-26),□H 1.27, □C 24.18(Me-27) and two doublets at □H0.84 (*J* = 6.5 Hz, Me-29, □C 17.55) and □H 0.98 (*J*= 6.5 Hz, Me-30, □C 21.95). In addition the ^{1}H- and ^{13}C-NMR spectra showed a triplet signal of a trisubstituted double bondat □H5.28(*J*= 3.8 Hz CH-12,δ_C126.60). These information along with the quaternary carbon chimical shift of C-13 at □C 145.12,suggested that compound [**8**] was a triterpene carrying a $(\Delta^{12})^{13}$ double bond. The signals appearing at □H 11.92 and □C 182.30, indicates the presence of a COOH group at C-28 position in the structure. These data are in agreement with a pentacyclic acid triterpene of the ursane seires.

Other extra signals in the ^{1}H and ^{13}C-NMR spectra characteristics of glucose substitution; an anomeric proton and carbon signals at δ_H4.55, (1H, d, *J*= 7.8 Hz,δ_C 99.30) and one oxymethylene protons atδ_H3.69 (1H, dd, *J* = 11.5, 5.8 Hz, H-6`A-glcand 3.92 (1H, dd, *J* = 11.5, 3.5 Hz, H-6`B`-glc,δ_C 62.75), indicated the presence of

a β-D-glucopyranoside moiety. Furthermore, a loss of 162 mass units from the molecular ion in the ESI-MS at m/z 457 [M + H - glc], clearly suggested the presence of *O*-glucose moiety in [**8**]. The attachment of glucose to the aglyconewas unambiguously determined to be atC-3 by the long-range correlation from (δ_H 4.55, H-1'-glc) to δ_C 81.90 (C-3) of the aglycon unit in the ^{1}H-^{13}C-HMBC spectrum. Therefore, the structureof [**8**] was determined as:urs-3□-hydroxy-12-en-28-oic acid-*3-glucopyranoside*(***ursolic acid-3-O-□-D-glucopyranoside***) (**Ahmad & Rahman, 1994; Babalola & Shoda, 2013**).

Results of Antioxidant Activity Using (DPPH) Radical Scavenging Method:

Free radicals are a major cause of oxidative stress that may lead to DNA strand breakage, gene mutation and DNA-protein cross links. Free radicals are known to be a product of normal metabolism. When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species (ROS) such as hydroxyle (OH^-), superoxide (O_2-), nitric oxide (NO), lipid peroxyle (Loo^-), radical and non-free radical species such as lipid peroxide (LOOH-) and different forms of activated oxygen (**Gordon, 2001; Lee et al., 2003**). ROS are involved in an organism's vital activities including phagocytosis, regulation of cell proliferation, intracellular signaling and synthesis of biologically active compounds. ROS have been implicated in several diseases including carcinogenesis, heart diseases, arteriosclerosis, diabetes and many other health problems related to ageing (**Gordon, 2001; Lee et al., 2003**). The role of ROS in the etiology and progression of several clinical manifestations has led to the suggestion that the antioxidants can be beneficial as prophylactic agents. Nevertheless, all aerobic organisms, including humans, have antioxidant defenses that protect against oxidative harm and repair damaged molecules. However, the natural antioxidant mechanisms can be insufficient, the supply of antioxidants through herbal ingredients, is of great interest for a healthy life. The DPPH free radical does not require any special preparation and is considered a simple and very fast method for determining antioxidant activity. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, which is an important limitation when interpreting the role of hydrophilic antioxidants (**Gordon, 2001; Lee et al., 2003**). The radical scavenging capacity of the EtOAc soluble fraction of the air-dried leaves and stems of Ixora finlaysoniana and its individual components [**1-8**] by testing its ability to bleach the stable DPPH radical was demonstrated in (**Table 1**).

The concentration of Ascorbic acid, BHT and isolated compounds [1-8]are 44-45□g/ml. The concentration of the EtOAc extract is 200 □g/ml, which is four to five times than the pure compounds. The SC(%) of any extract that are over 50% considered to have antioxidant activity. The results in (**Table 1**) showed that the EtOAc extract and its individual components [1-8], all have antioxidant activity with the ethyl acetate and isolated compounds [**3-7**], showing the highest activities than those of such typical antioxidants (Ascorbic Acid72.55%and BHT 58.82%), and that of [**1**, **2** and **8**], the least correlative of DPPH radical scavenging activity assay.The primarily results can be indicated that *Ixora finlaysoniana*WALL. EX. G. DON.,having antioxidant activities. It is reasonable to expect that high antioxidant compounds have great potential to reduce free radicals in *Ixora finlaysoniana*can be discovered.

Table (1) Effects of EtOAc soluble fraction of the air-dried leaves and stems of *Ixora finlaysoniana*and its individual components [1-8] and positive controls in DPPH

radical scavenging activity assay

Samples	SC%(Scavenging capacity)
(–) Control (5% DMSO/EtOH)	0
(+) Control (Ascorbic Acid)	72.55± 1.58
(+) Control (BHT [butylatedhydroxytoluene])	58.82± 1.25
EtOAc soluble fraction	81.63 ± 1.62
Compound-1	56.32 ± 1.18
Compound-2	59.64 ± 1.25
Compound-3	76.44 ± 1.55
Compound-4	73.82± 1.50
Compound-5	75.85± 1.52
Compound-6	79.80 ± 1.65
Compound-7	70.55 ± 1.53
Compound-8	55.68 ± 1.30

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دراسه فيتوكيميائية و بيولوجية لنبات الايسكورا

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مـــــــــــــــن

كلية الصيدلة جامعة النهضة قسم العقاقير بني سويف مصر ومستشفي الشرطة مدينة نصر القاهرة مصر

الايسكورا البيضاء هي شجرة متعددة الاغصان تنتمي للفصيلة الفوية وتزرع كنبات زينة في مصر وتختص هذه الدراسة بالفحص الكيميائي والبيولوجي لخلاصه خلات الاثيل والمركبات المفصولة منه لهذا النبات٠ثم استخلاص الاجزاء الهوائيه (الاوراق و السيقان) بالكحول الاثيلي(٧٠%)ثم ركزت الخلاصه الي الجفاف ثم اضافةماء مقطر لعمل معلق ثم بعد الترشيح تم اخذالجزء الذائب في الماءو تجزئته بين خلاصة خلات الايثيل والكحول البيوتانولي وقد تم تجزئية خلاصةخلات الا يثيل بواسطة كروماتوجرافيا العمود مستخدما جل السيليكا والسيفادكس LH20 وتم الحصول علي ثمانية مركبات في صورة نقيه،وقد تم التعرف علي التركيب الكيميائي لكل مركب باستخدام الطرق الطبيعية المختلفة مستخدما الاشعة فوق البنفسجية،طيف الكتلة،وطيف الرنين النووي المغناطيسى احادي وثنائي البعد لذرات الهيدروجين والكربون وهي كالاتي ٥ مركبات من مجموعة الفلافونيدات (١-٥) وثلاث مركبات من مجموعة التربينات الثلاثية(٦-٨)

١-٥ّ,٥-ثنائي هيدوركسي-٣,٦,٧,٣ّ,٤ّ خماسي ميثوكسي فلافون

٢-٥-هيدركسي ،٣,٦,٣ّ,٤ّ,٥ّسداسي ميثوكسي-فلافون

٣-٥,٧,٤ّ-ثلاثي-هيدروكسي فلافون(ابيجينين)

٤-٥,٧,٤ّ-تلاثي هيدروكسي-٦-ميثوكسي-فلافون(٦-ميثوكسي ابيجينين)

٥-ابيجينين-٤ّ -٥-ب-د-جلوكوسيد

٦-ليبيول

٧-بيتيولين

٨-حامض اودسليك-٣-جلوكوسيد

وقد تم دراسة النشاط المضاد للاكسدة لخلاصة خلات الايثيل وكذلك المركبات المفصولة منها باستخدام طريقة DPPH free radical scavenging

وبالمقارنة بين فيتامين سي وهيدروكسي بيوتيل التولويني كمواد قياسية وقد اثبتت النتائج ان خلاصة خلات الايثيل والمركبات المفصولة (١-٨) لها تاثير قوي كمضاد للاكسدة بنسب مختلفة تفوق احيانا فاعلية المواد القياسية المستخدمة مما يكسب اهمية خاصة لهذا النبات لما يحتوية من مواد طبيعية مضادة لاكسدة مما يمكن استخدامها كبدائل للمواد المختلفة التي تستخدم كمضادات للاكسدة.