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Phytochemical and Biological Studies of Natural Egyptian Recipe with Anticancer Effect

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Abstract: *Biologically guided screening study was done to investigate the cytotoxic activity of four plants either in separate manner or collected in the recipe from Egyptian market. The results revealed that the most promising cytotoxic activity was attributed to ethyl acetate extract of Holoptelea integrifolia Planch against cervical carcinoma HeLa cell line, larynx carcinoma HEp-2 cell line, and intestinal carcinoma CACO cell line compared to the reference drug vinblastine sulfate. So Holoptelea integrifolia Planch, family Ulmaceae was chosen for intensive photochemical investigation that led to isolation of nine compounds from the ethyl acetate extract; four terpenoids; Lupeol acetate (**1**), 3β, 22β-Dihydroxy olean-13(18)-ene (Abrisapogenol G)(**2**), 3,4-seco-Friedelane-3-oic acid (**3**), 2α,3β- dihydroxy-friedoolean-28-oic acid (**4**) ,three steroids; β- Sitosterol-β-D-glucoside (**5**), β-Sitosterol(**6**), Stigmasterol (**7**)in addition to isoflavone; Genistein (**8**) and phenolic; Ellagic acid (**9**). Their structures were determined by spectroscopic methods (UV, IR, MS, ^{1}H-NMR, ^{13}C-NMR and 2D NMR). In addition, the antimicrobial activity and antifungal activity were evaluated which exhibited significant results.*

Keywords: *Holoptelea integrifolia, Harraz Recipe, Cytotoxic activity, Abrisapogenol G, Friedelane*

I. Introduction

Neoplastic diseases are major public health concern worldwide. Treatment primarily involves surgery, radiation and chemotherapy or a combination of these. So, there is a continuous need of isolating more potent novel anticancer agents with least toxicity. Natural products presents a huge reservoir of bioactive compounds in variety of plant species, however, only a fractional percentage of these have been examined and are currently used as chemo-adjuvants or as anticancer agents. There is a global interest in identifying new anticancer compounds from plants and traditional sources [1]. Recently the WHO (World Health Organization) estimated that 80% of people worldwide rely on herbal medicines for some aspects [2]. In Egypt, the herbal market pronounced an excessive increase in selling many herbal recipes to cure some diseases. One of the most widely used anticancer recipes by the Egyptian patients in the market is Harraz Recipe which consists of four plants; *Holoptelea integrifolia* Planch.,*Ulmus parvifolia* Jacq.Family Ulmaceae, *Ficus glumosa* Del. family Moraceae and *Rumex dentatus* L. family Polygonaceae. *Holoptelea integrifolia* Planch was the plant selected for further phytochemical study as the results of cytotoxic screening revealed that the most promising anticancer activity was due to ethyl acetate extract of *Holoptelea integrifolia* Planch against cervical carcinoma HeLa cell line, larynx carcinoma HEp-2 cell line, and intestinal carcinoma CACO cell line compared to the reference drug vinblastine sulfate. *Holoptelea integrifolia* Planch. (family Ulmaceae) is known to possess medicinal value in the traditional system of medicine in which the bark and leaves of the plant are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, urinary astringent and in rheumatism. *Holoptelea integrifolia* Planch contains wide range of phytochemical such as; terpenoids, sterols, saponins, tannins, proteins, carbohydrates, and alkaloids [3, 4], in addition to, flavonoids, phenols, cardiac glycosides, coumarins, and quinines [5]. Many compounds were isolated from the plant and proven to be biologically active [6].

II. Materials

2.1. *Plant material*

Fresh leaves of *Holoptelea integrifolia* Planch, *Ulmus parvifolia* Jacq., *Ficus glumosa* Del., *Rumex dantatus* L. were collected from plants cultivated in the Orman Botanic Garden, Giza, during October (2014) and Harraz recipe purchased from Harraz market, Cairo, Egypt. The identities of the plants were established by Prof. Dr. Wafaa M. Amer, Department of Botany, Faculty of Science, Cairo University. Voucher specimens (H-5, U-2, F-10 and R-12) are kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University. The leaves were air-dried and reduced to No.36 powder and kept in tightly closed container until extraction process.

2.2. *General equipments and chemicals*

NMR analysis was carried out using BrukerAscend^{TM}850 spectrometer operating at (850, 400 MHz for ^{1}H &213,100 MHz for^{13}C). All samples have been prepared in $CDCl_3$ or DMSO-d_6 or or CD_3OD with TMS as

internal reference, with the chemical shifts expressed in ppm, and coupling constants *(J)* in Hertz. (The analysis was done in the NMR unit at the Faculty of Science –King Abdul-Aziz University in Jeddah-Saudi Arabia). 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 [7]. For column chromatography (CC), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), silica gel (Sigma, 28-200 mesh) was used for terpenoids CC, and F254 for TLC (Merck, Germany).

2.3. ***Cell lines***

Human intestinal carcinoma (CACO), human Larynx carcinoma (HEp-2), and human cervical carcinoma (HeLa) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD).

III. Methods

3.1. ***Preparation of plants extracts:***

A weighed quantity (250 g) of the powder recipe and leaves of each plant was separately subjected to successive continuous hot extraction using petroleum ether for defatting then ethyl acetate and finally pure methanol. All extracts were evaporated under reduced pressure using rotary evaporator and used in all the following experiments.

3.2. ***Evaluation of cytotoxic activity:***

The tumor cell lines were suspended in medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, and then incubated for 24 hr. The tested extracts were then added into 96-well plates (six replicates) to achieve eight concentrations for each extract. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test[8, 9] using vinblastine sulfate as reference standard antitumor compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA)**.**The cytotoxicity of the tested extracts was estimated in terms of percent growth inhibition compared to untreated control cells and their IC_{50} in µg/ml were detected. (Results complied in Tables 1-3 and Fig.1-6).

3.3. ***Evaluation of antimicrobial activity:***

In vitro screening of the potential antimicrobial activity of the methanol extracts of *Holoptelea integrifolia* Planch, *Ulmus parvifolia* Jacq., *Ficus glumosa* Del., *Rumex dantatus* L. and recipe [mixture of four extracts with equal quantities) against several Gram positive (*Streptococcus pneumonia* (RCMB 010010)and *Bacillus subtilis* (RCMB 010067)], Gram negative bacterial strains [*Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052)**]** and fungal strains[*Aspergillus fumigatus* (RCMB 02568) *Candida albicans* (RCMB 050386)]. All organisms were obtained from Regional Centre for Mycology and Biotechnology (RCMB).

Determination of antibacterial activity:

Antibacterial activities were investigated using agar well diffusion method [10]. The activity of tested samples was studied against Gram positive bacteria and Gram negative bacteria. Solution of 5 mg / ml of each extract in dimethyl sulfoxide (DMSO) was prepared for testing against bacteria. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 µl of the tested samples (10 mg/ml) were loaded into the wells of the plates. All samples were prepared in DMSO, which was loaded also as control. The plates were kept for incubation at 37^{o}C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Ampicillin and Gentamicin were used as antibacterial standard drugs.

Determination of antifungal activity:

Tested extracts were screened separately *in vitro* for their antifungal activity. The culture of fungi was purified by single spore isolation technique. The antifungal activity was investigated by agar well diffusion method [10]**.** The plates were kept for incubation at 30^{o}C for 3 – 4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin B was used as standard antifungal drug.

Determination of minimum inhibitory concentration (MIC)

The agar plate method was used to determine the MIC of tested samples reported by Damyanova *et al*. 2000 [11]. The MIC was considered to be the lowest concentration that completely inhibits against inoculums comparing with the control, disregarding a single colony or a faint haze caused by the inoculums. (Results complied in Table 4 and Fig.7).

3.4. ***Phytochemical investigation of ethyl acetate extract of Holoptelea integrifolia Planch leaves.***

The air dried powdered leaves of *Holoptelea integrifolia* Planch (1.75 kg) was extracted with 70% aqueous MeOH (5 X 7L) under reflux. Evaporation of the solvent yielded a crude extract (250 g). 70% MeOH After evaporation of the solvent, the obtained dry residue was defatted with petroleum ether under reflux (6 × 1 L). The resulting residue (180 g) was suspended in water (900 ml) and extracted with ethyl acetate (6 × 500 ml). The ethyl acetate extract gave pink color with sulphuric acid spray reagent on TLC when heated at 120ºC for three min. Ethyl acetate extract (80 g) was subjected to column chromatography over silica gel column (Ø 5.0 x 110 cm,) using a gradient of pet. ether / ethyl acetate (9:1, 8:2, 7:3, 6:4 1:1, 3:7) and 0:1,then gradient of EtOAc: MeOH up to100% MeOH (each 50 ml) to give nine fractions (A-L) according to the differences in composition indicated by TLC analyses, the collective promising fractions were subjected to further chromatography on silica gel several times using different solvent systems. For purification workup of collective fraction B by repeated CC over silica gel, using pet.ether: pet.ether/ EtOAC (100% till 95:5) led to the isolation mixture of compounds **6** and **7** then by repeated CC over silica gel, using pet.ether: pet.ether/ EtOAC (90:10) led to the isolation of compound **6** apart from compound **7**. Fraction **C** was further fractionated on silica gel using pet.ether: EtOAC (90:10 up to 80:20) to yield the compound **1**. Fraction D on chromatography over silica gel using pet.ether: EtOAC (70:30) as eluent afforded compound **2.** Fraction E on CC over silica gel, using pet.ether: EtOAC (60:40) led to the isolation of compound **3.** Fraction F subjected to successive CC silica gel using pet.ether: EtOAc (60:40 till 50:50) yield the compound **4**. Fraction J was applied on CC over silica gel pet.ether: EtOAC (10:90) led to the isolation of compound **5**. Fraction K on chromatography over silica gel using EtOAC: MeOH (90:10) as eluent afforded compound **8.** Finally, Fraction L on silica gel CC using EtOAC: MeOH (80:20) led to the isolation of compound **9,** and then crystallized from MeOH. Fractionation of the ethyl acetate extract of the leaves of *Holoptelea integrifolia* Planch leads to the isolation of 9 compounds **(1-9).** 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.

IV. Experimental Data Of Isolated Compounds

Compound 1 (Lupeol acetate): white crystalline needles (30mg). It showed a melting point of 216-217°C. IR spectrum of compound **1** showed the presence of ester carbonyl at 1721 cm^{-1} (C=O) and exomethylene group at 3019 cm^{-1}, 2950 cm^{-1}(C-H), 2416 cm^{-1}(C=C), 1217 cm^{-1}(C-O)and bending at 792 cm^{-1}.

Compound 2 (3 □, 22 □dihydroxy olean-13(18)-ene (Abrisapogenol G)) was obtained as colorless needles (25 mg), mp 223-224°C. Negative ESI-MS showed a molecular ion peak at *m/z* 440 $[M- 2H]^{-}$, corresponding to molecular formula $C_{30}H_{50}O_2$.

Compound 3 (3,4-*seco*-Friedelan-3-oic acid); white solid (20 mg) m.p 216-218 °C.ESI-MS, *m/z*445 $[M^{+\bullet}+H, C_{30}H_{52}O_2]$; IR (KBr) cm^{-1}: 3200, 2929, 2868, 1705, 11465, 1386, 1284, 1216, 919, 756.

Compound 4 (2 □,3 □ dihydroxy-friedoolean-28-oic acid); was isolated as a white amorphous solid (28 mg). ESI-MS showed $[M-1]^{+}$ at *m/z* 473.2 corresponding to the molecular formula of $C_{30}H_{50}O_4$, typical for a triterpenoid.IR ($CHCl_3$) υ 3410, 2933, 2871, 1688, 1431, 1388, 1247 cm^{-1}.

Compounds **1-4** gave positive Liebermann Burchard and negative Molisch's reactions indicating its triterpenoid non - glycosidic nature. The ^{l}H, **^{13}C**-NMR ($CDCl_3$, 850, 213 MHz) data of compounds **1-4** are shown in (Table 4).

Compound 5 (*β*- Sitosterol- □-D-glucoside); white crystals (25 mg), m.p: 272-274ºC, It gave positive Liebermann-Burchard and positive Molisch's reactions indicating its steroidal glycosidic nature; soluble in a mixture of chloroform and methanol, R_f value 0.48 (pet.ether/EtOAc, 1:9 v/v), IR υKBr max cm^{-1}**:** 3400, 2920 & 2850, 1705 and 1620, 1445, 1360, 1257, 1160, 1105 & 1020.

Compound 6 (□-Sitosterol): white powder (70 mg); mp: 134-135°C; MS (*m/z*): 414(M+), 396, 339, 325, 310, 298, 257, 227, 140, 139, 125, 97, 71, 57.

Compound 7(Stigmasterol): white powder (65 mg); mp: 174-176 °C; MS (*m/z*): 412 [M+], 394, 351, 314, 300, 271, 229, 213, 55. ^{1}H-^{13}C- NMR (DMSO-d_6, 400,100 MHz) of compounds **5-7** are shown in (Table 5).

Compound 8 (Genistein)

It was isolated as yellow crystals (16 mg) with melting point 250^{o}C, purple after examination by UV light. UV spectral data: MeOH [266, 322 (sh)], NaOMe [266, 286, 335 (sh)], NaOAC[274, 325, 348 (sh)], NaOAc/H_3BO_3 [270, 335 (sh)], $AlCl_3$ [266, 325 (sh)], $AlCl_3$/HCl [273, 349 (sh)]. ^{1}H-NMR (CD_3OD,

400mHZ) (Fig. 33): 8.1 (1H, s, H-2), 7.3 (2H, dd, *J*= 8.8, H-2', H-6'), 7.0 (2H, dd, *J*= 8.8, H-3', H-5'), 6.42 (1H, d, *J*= 2.4, H-8), 6.27 (1H, d, *J*= 2.2, H-6).

Compound 9 (Ellagic acid)

It was obtained as a yellowish brown powder (25mg); give buff fluorescence under UV light, dull yellow with NH_3 and the melting point was found to be 317°C. The compound gave a faint bluish green color with $FeCl_3$ suggesting the compound to be a phenolic compound. Analysis by mass spectroscopy gave molecular mass at 301 *m/z*. IR (Kbr cm^{-1}): 3556 cm^{-1} (OH stretch) cm^{-1}, 1699 cm^{-1} (C=O), 1618 cm^{-1} (OH), 1508 cm^{-1} (C=C), 1192 (C-O). ^{1}H- NMR:(400MHz, DMSO-d_6):7.5(s, 2H, aromatic H-5, 5´), ^{13}C- NMR:(100MHz, DMSO-d_6): 136.30(C-2), 140.07(C-3 and C-10) 148.03 (C-4 and C-11), 112.21(C-5 and C-12), 110.07(C-6 and C-13), 159.00(C-7 and C-14).

V. Result And Discussion

Cytotoxic activity:

The six dose growth inhibition percent and the IC_{50} values of the tested extracts against cervical carcinoma HeLa cell line, larynx carcinoma HEp-2 cell line and intestinal carcinoma CACO cell line are represented in tables 1, 2, and 3 and Fig. 1, 2, 3 respectively. The preliminary screening for anticancer activity of methanol extracts of *Holoptelea integrifolia* Planch, *Ulmus parvifolia* Jacq., *Ficus glumosa* Del., *Rumex dantatus* L., ethyl acetate extract of *Holoptelea integrifolia* Planch. and the recipe (mixture of the four extracts with equal quantities) on three cell lines, cervical carcinoma HeLa cell line, larynx carcinoma HEp-2 cell line, and intestinal carcinoma CACO cell line showed that the methanol extract of *Holoptelea integrifolia* Planch.was nearly equipotent to the reference drug vinblastine sulfate against cervical carcinoma HeLa cell line with IC_{50} value 53.2 µg/ml , while it showed half potency of the reference drug against larynx carcinoma HEp-2 cell line exerting IC_{50} value 58.7 µg/ml. However, it showed moderate anticancer activity against intestinal carcinoma CACO cell line with IC_{50} value 93.1 µg/ml.

In addition, the ethyl acetate extract of *Holoptelea integrifolia* Planch. showed more potent cytotoxic activity against both cervical carcinoma HeLa cell line and intestinal carcinoma CACO cell line than the reference drug vinblastine sulfate exerting IC_{50} values 26.9 and 11.6 µg/ml respectively, while it also showed nearly equipotent anticancer activity against larynx carcinoma HEp-2 cell line with IC_{50} value 28.4 µg/ml. Moreover,*Ulmus parvifolia* Jacq. methanol extract exerted more potent anticancer activity against cervical carcinoma HeLa cell line than the reference drug vinblastine sulfate with IC_{50} value 14.8 µg/ml. Finally, concerning the cytotoxic activity against intestinal carcinoma CACO cell line, we found that the methanol extract of *Ficus glumosa* Del. showed nearly double potency of the reference drug vinblastine sulfate with IC_{50} value 30.2 µg/ml .While, the methanol extract of recipe showed high potency compared to the reference drug vinblastine sulfate with IC_{50} value 70.8 µg/ml. These result in agreement with the previously reported by Sousa *et al.* 2014[12, 13]; that triterpenes show cytotoxic properties against tumor cells, at low activity toward normal cells**.** Triterpenes are compounds of natural origin, which have numerous biological activities: anticancer properties, antiinflammatory, antioxidant, antiviral, antibacterial and anti-fungal. Recently, when neoplasms are main cause of death, triterpenes can become an alternative method for treating cancer because of their cytotoxic properties and chemopreventive activities [12, 13].

Antimicrobial activity:

The tested extracts were active against Gram positive bacteria, Gram negative bacteria and fungi. The methanol extract of recipe showed antimicrobial activities the same as the standards used mainly against *Aspergillus fumigatus* (fungi) and *streptococcus pneumonia* (Gram positive bacteria) and lower than the standards used mainly against *E. coli* (Gram negative bacteria).The methanol extract of *Holoptelea integrifolia* Planch. exhibited antimicrobial activities equal to the standards used mainly against *Streptococcus pneumonia* (Gram positive bacteria) and very close to the standards used mainly against *Aspergillus fumigatus* (fungi) and *Bacillis subtilis* (Gram positive bacteria) but lower than the standards used mainly against *E. coli* (Gram negative bacteria)

Phytochemical investigation:

The promising results of the cytotoxic activity of ethyl acetate extract of *Holoptelea integrifolia* Planch leaves on specific cancer cells should be only considered a preliminary study as this gives us a new direction to isolate the active phytochemicals present in this extract. Investigation of ethyl acetate extract of *Holoptelea integrifolia* Planch. leaves led to isolation of nine compounds **1-9** for the first time except **6** and **7**. Based on chemical , physicochemical analyses and spectral data[HMBC and HSQS confirm the structure in certain compounds] that in agreement with the previously published data [14-32]**;** compounds **1-9** were identified as: Lupeol acetate (**1**) [14-16], Abrisapogenol G(**2**) [17, 18], 3,4-*seco*-Friedelane-3-oic acid (**3**) [19,20], 2α,3β- dihydroxy-

friedoolean-28-oic acid (**4**) [21], three steroids; *β*- Sitosterol-β-D-glucoside. (**5**) [22, 23], β-Sitosterol (**6**) [24, 25], Stigmasterol (**7**) [24-26] in addition to isoflavone; Genistein (**8**) [27-29] and phenolic; Ellagic acid (**9**) [30]. These compounds were isolated for the first time from *Holoptelea integrifolia* Planch leaves.

VI. Conclusion

Nine compounds among them four triterpenoids, three steroids, one isoflavone and one phenolic acid were isolated from the dried leaves of *Holoptelea integrifolia* Planch. leaves ethyl acetate extract. The ethyl acetate extract has potent cytotoxic activity against human intestinal carcinoma (CACO), human Larynx carcinoma (HEp-2) and human cervical carcinoma (HeLa) cell lines. This proves the uses of the plant in folk medicine as anticancer; in addition to promising antimicrobial activities give the plant great potential and supporting the use of the plant as a source of natural raw material for phyto-pharmaceutical preparations.

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References

[1] H. Wang, T. O. Khor, L. Shu, Z. Y. Su, F. Fuentes, J. H. Lee and A. N. Kong. Plants vs. cancer: A review on natural phytochemicals in preventing and treating cancers and their drug ability. *Anti-cancer Agents Med. Chem., 12(10)*2012, *1281-1305.*

[2] R. P. Borris Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology, 51, (1–3), 1996, 29–38.*

[3] S. Mahmud, H. Shareef, M. Ahmad, S. Gouhar, and G. H. Rizwani Pharmacognostic studies on fresh mature leaves of *Holoptelea integrifolia* (ROXB) Planch. *Pakistan Journal of Botany, 42, (6), 2010, 3705–3708.*

[4] S. Ahmad, R. Sharma, S. Mahajan, R. Agnihotri, and Gupta A. Antibacterial evaluation and preliminary phytochemical analysis of the leaf extract of *Holoptelea integrifolia*. *Journal of Pharmacy Research, (5) 2012, 3823–3825.*

[5] S. R. Kumar, C. Venkateshwar, G. Samuel, and R. S. Gangadhar. Phytochemical screening of some compounds from leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at A dilabad district, Andhra Pradesh, India. *International Journal of Engineering Science Invention, (2), 2013, 65–70.*

[6] J. Srivastava, K. N. Dwivedi, and H. P. Pandey. Assessment of antioxidant activity and total phenolic content of aqueous stem bark extract of *Holoptelea integrifolia* Planch. *International Journal of Pharmacy and Pharmaceutical Sciences, (5), 2013,. 357–361.*

[7] T.J. Mabry, K.R. Markham, and M.B. Thomas. *The systematic identification of flavonoids*. Springer Verlag, Berlin, *1970, 41–164.*

[8] T. Mosmann Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods; 65: 1983, 55-63.*

[9] M. M. Elaasser, M. M. Abdel-Aziz and R. A. El-Kassas. Antioxidant, antimicrobial, antiviral and antitumor activities of pyranone derivative obtained from *Aspergillus candidus.J. Microbiol Biotech Res. 1: 2011, 5-17.*

[10] A. W. Bauer, W. M. M. Kirby, J. C. Sherris and M. Turck. Antibiotic susceptibility testingby a standardized single disk method. *American Journal of Clinical Pathology 45, 1996, 493-496.*

[11] S. Damyanova, L. M. Gomez, M. A. Banares and J. L. G. Fierro. *Chem. Master 12, 2000, 501-510.*

[12] G.F. Sousa, D.C.F. Soares, W. Nova Mussel, *et al.*, Pentacyclic Triterpenes from Branches of Maytenus robusta and in vitro Cytotoxic Property Against 4T1 Cancer Cells. *J. Braz. Chem. Soc. 2014, 25, 1338–1345.*

[13] C. Malwina, K-S. Ilona, and K. Wojciech, Review Triterpenes as Potentially Cytotoxic Compounds. *Molecules .2015, 20, 1610-1625.*

[14] A. K. Jamal, W. A. Yaacob and B. D. Laily. A Chemical Study on *Phyllanthus reticulates. Journal of Physical Science 19(2): 2008, 45-50.*

[15] K. P. C. Lutta , A.T. Akenga Bii and W.W. Cornelius, Antimicrobial Marine Natural Products from the Sponge, *Axinella infundibuliformis. Records of natural products, 8: (2008). 116-127.*

[16] S. Imam, I. Azhar, M. M. Hasan, M. S. Ali and S.W. Ahmed. Two Triterpenes Lupanone and Lupeol Isolated and Identified from *Tamarindus indica. Pakistan Journal of Pharmaceutical Sciences, 20(2): 2007, 125- 127.*

[17] J. Kinjo, K. Matsumoto, M. Inouh, T. Takeshita and T. Nohara *Chem. Pharm. Bull, 39, 1991, 116-119.*

[18] T. Takaeshita, S. Hamada and T. Nohara.New triterpenoid sapogenols from *Abrus cantoniensis Chem. Pharm. Bull., 37, 1989, 846*

[19] C. L. Queiroga, G. F. Silva, P. C. Dias, A. Possenti and J. E. Carvalho. Evaluation of the friedelin and βfriedelan-3 -ol isolated from *Maytenus ilicifolia* antiulcerogenic activity of (Celastraceae). *J. Ethnopharmacol. 72, 2000, 465–468.*

[20] **J**. M. David, F. A. Santos, M. L. S. Guedes and J. P. David Flavonóides e triterpenos de Stigmaphyllom paralias. *Quim. 26, 2003, 484–487.*

[21] N. S. Kumar, P. M. Muthukuda and J. K. MacLeod New D: A-friedooleananes from *Euonymus revolutus* (Celastraceae). *J. Chem. Soc. Perkin Trans. I. 1985, 685-689.*

[22] T. Matsumoto, T. Shigemoto and T. Itoh. *Phytochemistry, 22, 1983, 2622-2624.*

[23] U. Kolak, G. Topcu, S. Birteksoz, G. Otuk and A. Ulubelen. Terpenoids and steroids from the roots of *Salvia blepharochlaena, Turk. J. Chem., 29, 2005, 177-186.*

[24] M. R. Habib, F. Nikkon, M. Rahman, M. E. Haque and M. R. Karim Isolation of Stigmasterol and β-Sitosterol from Methanolic Extract of Root Bark of *Calotropis gigantean* (Linn). *Pakistan Journal of Biological Sciences. 10, 2007, 4174-4176.*

[25] A. K. Jamal, W. A. Yaacob and L. B. Din A Chemical Study on *Phyllanthus columnaris,European. Journal of Scientific Research, 28, 2009, 76-81.*

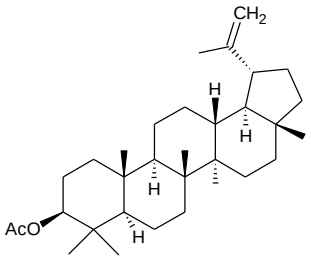
[26] F.M. Moghaddam, M.M. Farimani S. Salahvarzi and G. Amin. Chemical Constituents of Dichloromethan Extract of Cultivated *Satureja khuzistanica, Evidence-Based Complementary and Alternative Medicine, 4, 2006, 95-98.*

[27] T. J. Mabry, K. R. Markham and M. B. Thomas. *The Systematic Identification of flavonoids. Second Edition, Springer Verlag, New York*. 1996.

[28] M. Swati, G. N. Singh, K. Kohli, M. Ali, Y. Kumar, R. M. Singh and O. Prakash. Isoflavonoids from *Flemingia strobilifera* (L) R. BR. Roots. *Acta Poloniae Pharmaceutica-Drug Research 66 (3), 2009, 297-303.*

[29] I. Phongsak, R. C. Thitima, P. P. Souwalak and S. Apichart . Fuscacarpans A–C, new pterocarpans from the stems of *Erythrina fusca*. *Fitoterapia 81(6), 2010, 518–523.*

[30] M. Anil, P. Nandini, Simultaneous isolation and identification of phytoconstituents from *Terminalia chebula* by preparative chromatography. *J. Chem. Pharm. Res., 2010, 2(5):97-103.*



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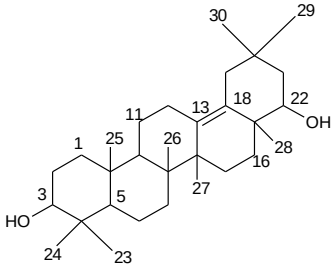

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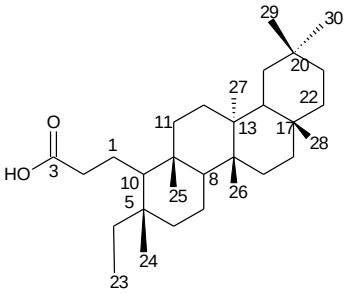

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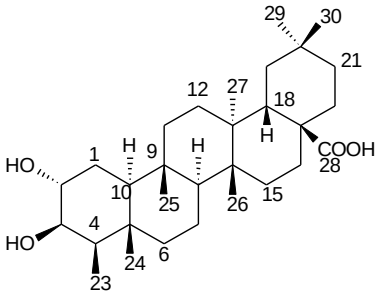

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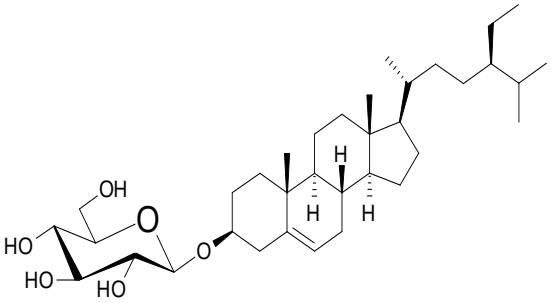

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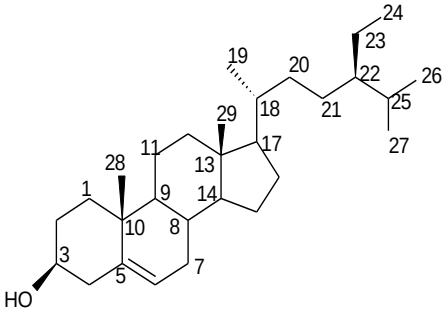

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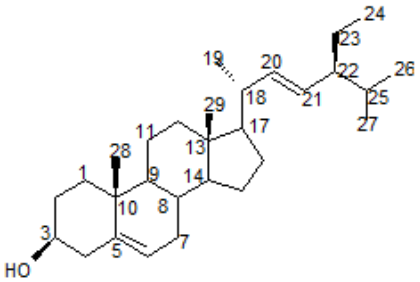

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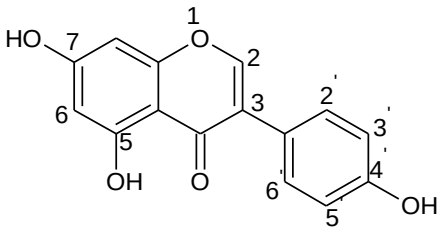

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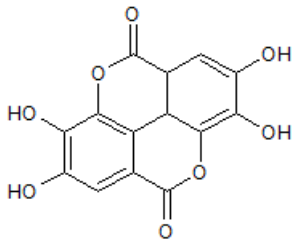

9

Table (1): Growth inhibition percent and IC_{50}values of the tested extracts in different concentrations against cervical carcinoma HeLa cell line

Sample concentration (µg/ml) Tested extrac	Growth inhibition %						IC_{50} (µg/ml)
	500	250	125	62.5	31.25	15.6	
***H. integrifolia* MeOH extract**	92.64	83.41	71.26	60.04	26.13	10.24	53.2
***H.integrifolia* EtOAC extract**	93.05	88.57	82.13	75.29	60.98	21.37	26.9
***U. parvifolia* MeOH extract**	85.47	78.22	43.51	20.38	8.53	1.22	14.8
***F. glumosa* MeOH extract**	90.73	81.29	65.32	30.18	12.84	1.92	97.8
***R. dentatus* MeOH extract**	81.24	30.15	11.38	5.84	1.28	0	347
Recipe MeOH extract	84.18	56.09	18.47	3.28	0.14	0	230
Vinblastine sulfate	90.87	77.16	65.43	52.19	28.04	17.22	59.7

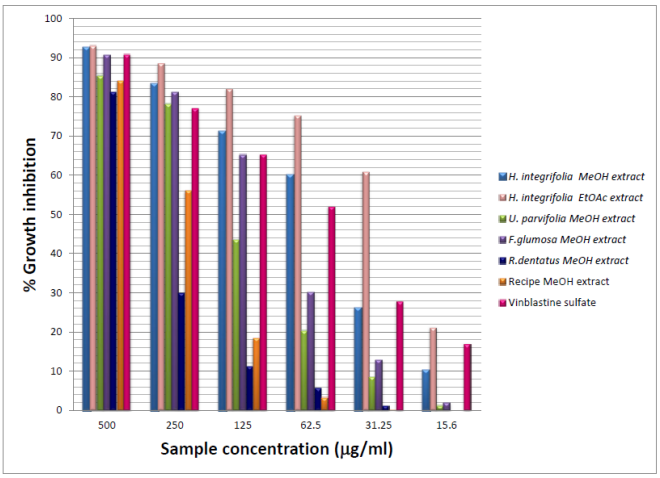

Fig. (1): Growth inhibition percent of the six tested extracts and the referencedrug (vinblastine sulfate) against cervical carcinomaHeLa cell line

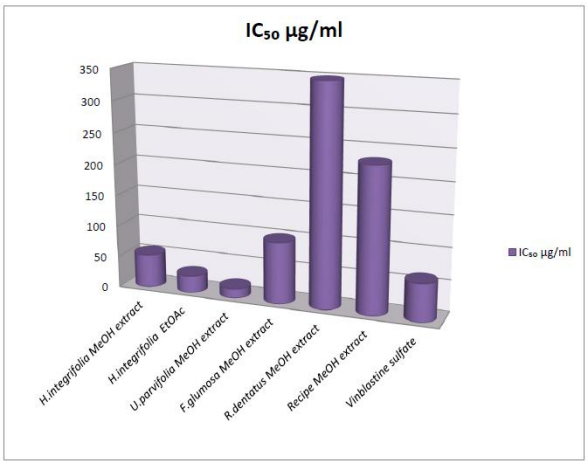

Fig.(2):IC_{50} values of the six tested extracts and reference drug (vinblastine sulfate) against cervical carcinoma HeLa cell line

Table (2): Growth inhibition percent and IC_{50}values of the tested extracts in different concentrations against larynxcarcinoma HEp-2 cell line

Sample concentration (µg/ml) Tested extract	Growth inhibition %						IC_{50} (µg/ml)
	500	250	125	62.5	31.25	15.6	
***H. integrifolia* MeOH extract**	88.03	78.18	68.06	52.72	30.13	12.41	58.7
***H. integrifolia* EtOAC extract**	92.11	86.35	79.52	65.29	55.14	26.08	28.4
***U. parvifolia* MeOH extract**	85.15	79.26	60.31	31.16	9.24	1.76	103
***F. glumosa* MeOH extract**	86.26	78.11	61.04	34.57	17.03	8.55	98.9
***R. dentatus* MeOH extract**	71.37	28.08	12.55	4.34	0.87	0	377
Recipe MeOH extract	82.56	57.13	24.79	12.48	5.11	1.37	222
Vinblastine sulfate	92.76	87.15	75.64	64.02	51.24	36.73	29.9

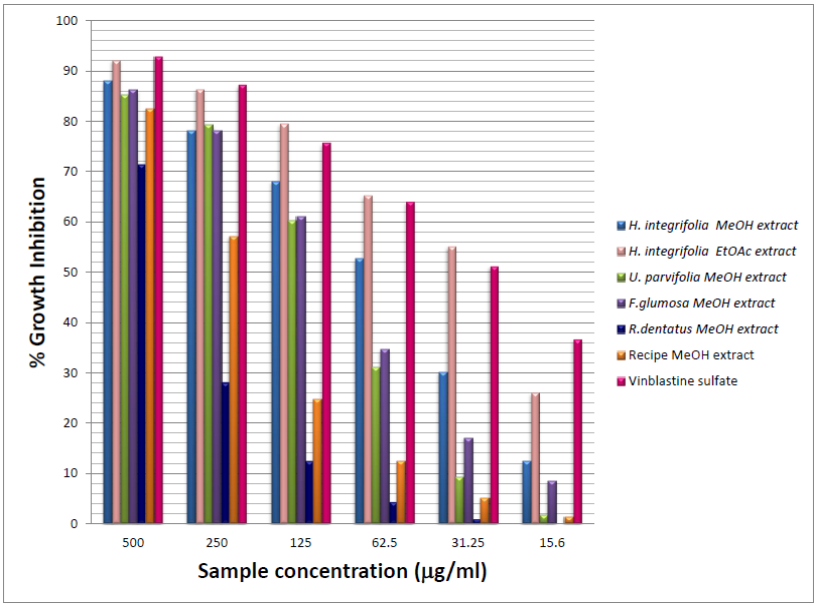

Fig. (3): Growth inhibition percent of the six tested extracts and the reference drug (vinblastine sulfate) against larynx carcinoma HEp-2 cell line

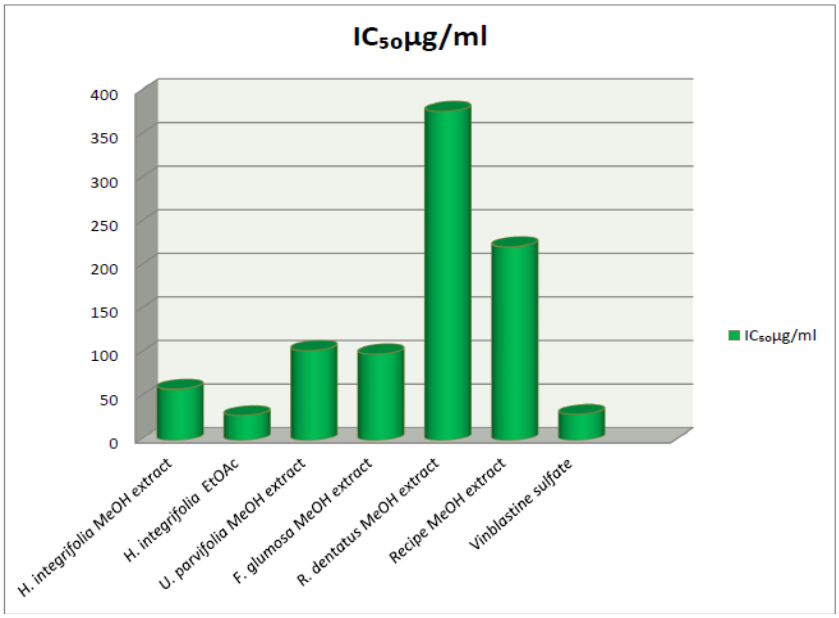

Fig. (4):IC_{50} values of the six tested extracts and the reference drug (vinblastine sulfate) against larynx carcinoma HEp-2 cell line

Table (3): Growth inhibition percent and IC_{50} values of the tested extracts in different concentrations against intestinal carcinoma CACO cell line

Sample concentration (µg/ml) / **Tested extract**	**Growth inhibition %**						**IC_{50} (µg/ml)**
	500	**250**	**125**	**62.5**	**31.25**	**15.6**	
***H. integrifolia*MeOH extract**	90.59	85.71	69.54	31.28	10.44	1.86	93.1
***H. integrifolia*EtOAC extract**	95.47	92.04	87.35	76.16	68.92	53.17	11.6
***U. parvifolia* MeOH extract**	91.63	87.16	70.22	29.71	15.07	3.11	93.8
***F. glumosa* MeOH extract**	93.69	89.72	81.64	73.22	52.37	13.59	30.2
***R. dentatus* MeOH extract**	79.41	61.73	32.81	17.58	6.15	2.36	199
Recipe MeOH extract	93.08	89.22	78.44	45.63	21.34	6.71	70.8
Vinblastine sulfate	91.03	79.64	68.58	51.07	32.42	20.59	60.7

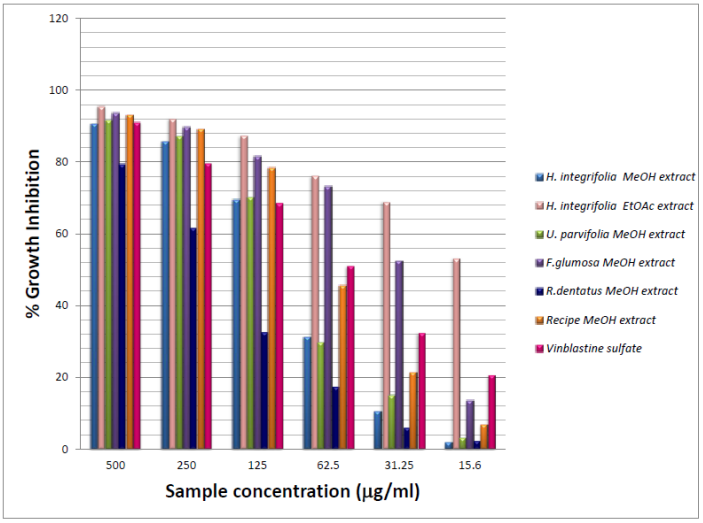

Fig. (5): Growth inhibition of the six tested extracts and the reference drug (vinblastine sulfate) against intestinal carcinoma CACO cell line

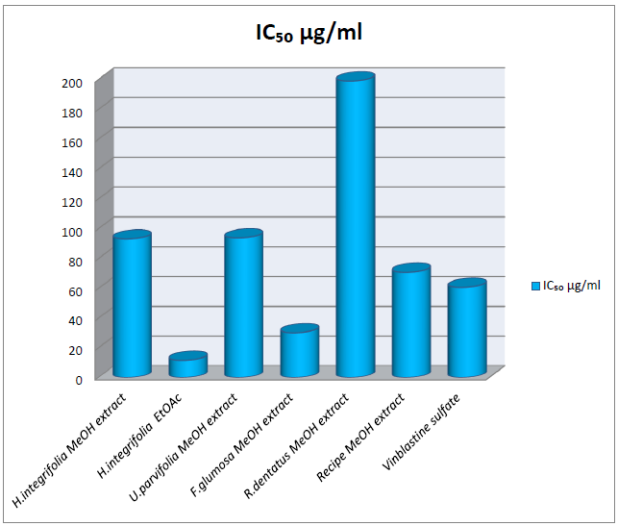

Fig.(6):IC_{50} values of the six tested extracts and the reference drug (vinblastine sulfate) against intestinal carcinoma CACO cell line.

Table (4): Antimicrobial activities of different testedmethanol extracts against tested microorganisms.

Microorganisms	Inhibition zone (mm)					
	H.integrifolia	***F.glumosa***	***R.dentatus***	***U.parvifolia***	**Recipe**	**Reference drug**
Fungi						***Amphotericin B***
Aspergillus fumigatus (RCMB 02568)	21.3± 0.58	18.3± 0.25	13.6± 0.39	20.6 ± 0.36	21.6±0.63	23.7± 0.1
Candida albicans (RCMB 05036)	20.2±.025	16.2±0.34	11.7±0.39	18.2±0.44	22.3±0.25	25.4± 0.1
Gram positive bacteria						***Ampicillin***
Streptococcus pneumoniae (RCMB 010010)	23.4± 0.15	18.3± 1.5	16.0±0.44	22.6±0.63	23.8±0.44	23.8±0.2
Bacillissubtilis (RCMB 010067)	24.2± 0.42	20.4± 0.58	18.3±0.67	23.4±0.32	26.3±0.58	32.4± 0.3
Gram negative bacteria						***Gentamicin***
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	NA	17.3± 0.1
Escherichia coli (RCMB 010052)	24.1± 0.53	20.1± 0.63	12.8±0.46	23.6±0.46	25.2±0.58	19.9± 0.3

Results are expressed in the form of mean ± SD. NA = No Activity.
RCMB = Regional Center for Mycology and Biotechnology.

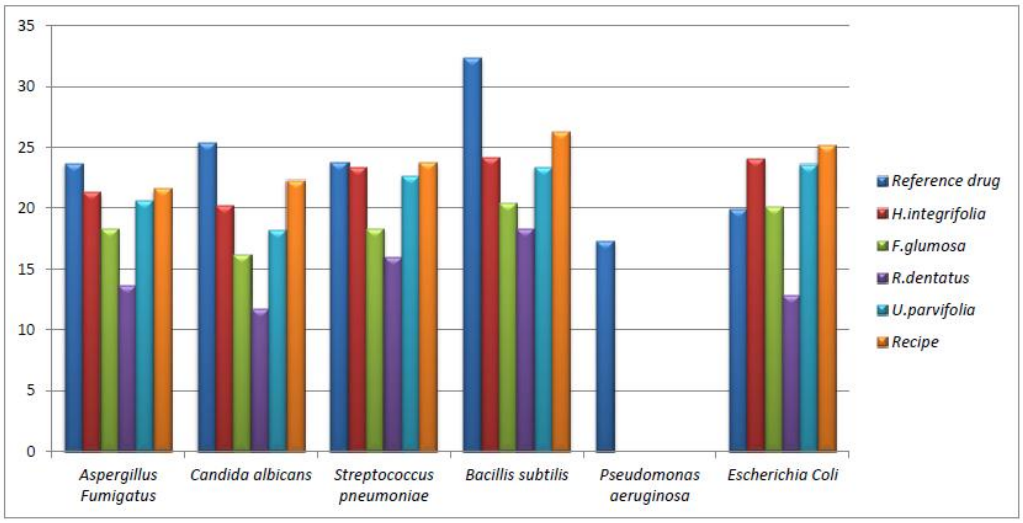

Fig. (7): Antimicrobial activities of different tested methanol extracts against tested microorganisms.

Table (5): ^{1}H, ^{13}C - NMR (850, 400 and 213, 100 MHz $CDCl_3$) spectral data of triterpenoids (1, 2, 3 and 4)

	1		**2**		**3**		**4**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		37.3	0.98/2.33	38.6	1.75	35.2	1.36, m	23.3
2		22.37	1.73/1.94	28.8	1.66	25.10	3.64, dd (7.8,2.4)	76.4
3	4.47(dd,5.5 ,10.5)	81.0	3.23,1Hdd, (11.0,5.1)	77.14		176.24	3.23, t (2.4)	76.84
4		37.4	-	40.6	1.60	35.07	1.4 ,m	43.0
5		58.9	0.70	59.4		38.97		37.3
6		14.1	1.43/1.58	24.36	1.42	39.19	1.59 ,m	41.3
7		32.77	1.58/1.46	34.01	1.44	18.01	1.26 ,m	19.6
8		38.0		44.3	1.62	41.9	1.12 ,m	52.8
9		50.3	2.45	64.6		37.74		36.3
10		37.8		37.4		39.01	1.37,m	51.3
11		19.9		37.6	1.37	35.95	1.08 , m	34.7
12		22.7	1.38	45.1	1.38	37.11	1.34 ,t	30.8
13		37.2		132.85		39.94		38.4
14		42.8		44.3		38.23		37.4
15		25.4	1.73/1.26	27.9	1.54	32.21	1.08 ,m	32.4
16		36.7	1.48/1.83	31.9	1.39	36.00	2.22 ,dd(7.8,4.2)	29.4
17		43.0		39.3		29.91		43.9
18		48.3		132.41	1.62	42.79	2.36,dd(9.8,2.4)	37.2
19	2.34(td,11, 10,5.5Hz	48.0	1.70/2.16	38.6	1.38	35.23	1.29 , d	34.6
20		148.65		33.7		28.05		28.2
21		29.44	1.44/1.38	44.4	1.48	32.78	1.35,t	32.5
22		39.3	3.35,1H,dd, (10.4,6.5)	77.19	1.69	39.49	1.36 ,m	35.4
23	1.04 (s)	27.96	1.08	28.8	0.78 (7.4)	17.51	0.77,d (7.3)	15.8
24	0.95 (s)	21.16	0.84	14.1	0.79	21.27	0.82 (bs)	15.9
25	1.4 (s)	19.37	1.16	22.62	1.04	14.82	0.76 , s	19.5
26	0.83 (s)	19.1	0.79	22.34	1.065	19.93	0.74 ,m	20.0
27	0.79 (s)	14.11	1.34	24.8	1.04	19.61	0.94 ,s	19.3
28	1.28 (s)	19.00	0.98	28.6	1.2	32.01		179.8
29	4.96,br s, 4.207,br s	110.53	0.98	33.7	0.94	34.86	0.89 ,s	29.8
30	1.68, s	19.37	0.76	20.9	0.99	31.74	0.99 ,s	35.5
1′		171.0	-	-	-	-	-	-
2′	2.04, s	27.9	-	-	-	-	-	-

J(coupling constant in Hz) between parentheses

Table (6): ^{1}H, ^{13}C - NMR (850, 400 and 213, 100 MHz, DMSO-d_6) spectral data of steroid compounds (5, 6, and 7)

	5		**6**		**7**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.25,m, 2H,	36.85		37.5		37.6
2	1.33,m, 2H	29.12		31.9		32.1
3	3.13,m, 1H	76.99	3.53, tdd, 1H, (4.5,4.2,3.8)	72.0	3.51(tdd, 1H, (4.5,4.2,3.8)	72.1
4	2.14,m, 2H	42.14		42.5		42.4
5		140.51		140.9		141.1
6	5.34 ,bs, 1H	121.29	5.36,t, 1H, (6.4)	121.9	5.31(t, 1H, (6.1)	121.8
7	1.73,m, 2H	31.41		32.1		31.8
8	1.22,m, 1H	31.46		32.1		31.8
9	1.22, m, 1H	49.83		50.3		50.2
10		36.27		36.7		36.6
11	1.33,m, 2H	20.21		21.3		21.5
12	1.33,m, 2H	38.20		39.9		39.9
13		41.91		42.6		42.4
14	1.22,m, 1H	56.36		56.9		56.8
15	1.73,m, 2H	23.79		26.3		24.4
16	1.73,m, 2H	27.76		28.5		29.3
17	1.73,m, 2H	55.66		56.3		56.2
18	0.62,s, 3H	11.27		36.3		40.6
19	0.94,s, 3H	19.10	0.93d, 3H, (6.5)	19.2	0.9, d, 3H,(6.2)	21.7
20	1.32,m, 1H	35.70		34.2	4.98,m, 1H)	138.7
21	0.84,d,3H, (6.3)	18.69		26.3	5.14,m, 1H)	129.6
22	1.73,m, 2H	33.51		46.1		46.1
23	1.73,m, 2H	25.64		23.3		25.4
34	1.12,m, 1H	45.49	0.84t, 3H, (7.2)	12.2	0.83,t, 3H, (7.1)	12.1
25	2.14,m, 1H	28.74		29.4		29.6
26	0.75,d, 3H , (7.7)	18.69	0.83,d, 3H, (6.4)	20.1	0.82,d, 3H, (6.6)	20.2
27	0.73,d, 3H, (1.6)	18.35	0.81,d, 3H, (6.4)	19.6	0.80,d, 3H, (6.6)	19.8
28	1.33,m, 2H	22.60	0.68,s, 3H	19.0	0.71,s, 3H,	18.9
29	0.77,t, 3H, (6.9)	12.29	1.01,s, 3H	12.0	1.03,s, 3H,	12.2
1′	4.11,d, 1H, (7.8)	100.82	-	-	-	-
2′	3.14,m, 1H	73.21	-	-	-	-
3′	3.14,m, 1H	76.18	-	-	-	-
4′	3.14,m, 1H	69.90	-	-	-	-
5′	3.06, m, 1H	75.62	-	-	-	-
6′	2.94,m, 1H	61.36	-	-	-	-

J(coupling constant in Hz) between parentheses.