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PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON THE LEAVES OF *TECOMA MOLLIS* HUMB. AND BONPL CULTIVATED IN EGYPT

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نبات التيكوما مولس همب وبونبل المنزرع فى مصر هو أحد نباتات الزينة التابعة للعائلة البجنونية وفى دراسة كيميائية لمكونات هذا النبات تم تحضير الخلاصة الكحولية لأوراق هذا النبات ثم تجزئتها باستخدام الهكسان والكلوروفورم وخلات الايثيل والبيوتانول وبعد ذلك تم فصل وفحص مكونات كل خلاصة على حده باستخدام كروماتوجرافيا العمود والطبقة الرقيقة. وقد تمت دراسة الصفات الطبيعية والكيميائية للمركبات المفصولة باستخدام الأشعة فوق البنفسجية ودون الحمراء ومطياف الكتلة والرنين النووى المغناطيسى الهيدروجينى والكربونى وقد أمكن التعرف على المركبات التالية: الفا آميرين (١) ، ٣-بيتا-هيدروكسى-أورس-١٢-ايـن-٢٨-الدهيد (٢) ، بيتا-سيتوستيرول (٣) ، لاكتون حمض الأورسوليك (٤) ، حمض الأورسوليك (٥) ، ٢-بيتا-٣-بيتا-١٩-ألفا-ثلاثى هيدروكسى اورس-١٢-إين-٢٨-أويك (٦) ، بيتا سيتوستيرول-٣-أ-جلوكوزيد (٧) ، أبيجنين-٧-أ-رامنوزيد (٨) ، أبيجنين-٧-أ-روتينوزيد (٩) ، ليتيولين-٧-أ-روتينوزيد(١٠) ، فايسينين-٢ (١١). وقد تم عمل دراسة بيولوجية للنبات وتبين منها أن خلاصات الهكسان ، الكلوروفورم ، خلات الإيثيل ، البيوتانول وبعض المواد المفصولة من أوراق النبات لها تأثير قوى كمضاد للإلتهابات ، مسكن ، خافض للحرارة وقد تبين أيضا أن بعض منها لها تأثير على الجهاز العصبى المركزى.

α-Amyrin (1), 3-β-hydroxy-urs-12-ene-28-aldehyde (2) β-sitosterol (3), ursolic acid lactone (4), ursolic acid (5), 2-β,3-β,19-α-trihydroxy-urs-12-ene-28-oic acid (2-tormentic acid) (6), β-sitosterol-3-O-β-D-glucoside (7) apigenin-7-O-α-L-rhamnoside (8), apigenin-7-O-rutinoside (9), luteolin-7-O-rutinoside (10) and apigenin-6,8-di-C-β-D-glucopyranoside (Vicenin 2) (11) were isolated for the first time from the ethanolic extract of the leaves of Tecoma mollis Humb and Bonpl. cultivated in Egypt. Identification of these compounds has been established by physical and spectral data (UV, IR, MS, ^{1}H- and ^{13}C-NMR) as well as by comparison with authentic samples. Moreover, the biological screening showed that the non-polar fraction of the alcoholic extract (n-hexane, chloroform), polar fraction (ethyl acetate, n-butanol) and aqueous extract as well as ursolic acid possess significant anti-inflammatory, analgesic and antipyretic activities. In addition, the polar fraction and aqueous extract possess also a significant anticonvulsant activity.

INTRODUCTION

Tecoma mollis Humb. and Bonpl, Family Bignoniaceae is an upright shrub to a large tree attaining 7 meters in height. It has been introduced to Egypt as an ornamental plant for its timber and showy bell-shaped flowers.[1] The extracts of many *Tecoma* species have been used in traditional medicine as hypoglycemic, astringent, to treat diarrhea, dysentery and enteritis, as well as in the treatment of throat diseases and stomatitis.[2] The roots of *Tecoma* species ground with lemon juice or with water is an effective remedy for snake, rat bites and for scorpion sting.[3] Quinone and iridoid contents of some *Tecoma* species have antifungal, antibacterial, antiviral, cytotoxic and analgesic activities.[4-7] The powdered roots of some species was administered as a cough remedy and in some cases of respiratory tract disorders[8] and the aqueous extract of the stem bark of some *Tecoma* species is used as antipyretic, hypnotic, antimalarial and for protozoal diseases.[9]

Previous studies of different *Tecoma* species revealed the isolation and identification of many constituents of different chemical

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classes as quinones (lapachol and lapachone derivatives,[10-12] tecomaquinones III[13] and lamatiol[10]), iridoids (ajugol derivatives,[14] plantarenaloside,[15,16] tecomoside derivatives,[17-20] amareloside[21] and tecoside[21]), flavonoids (quercetin,[22,23] kaempferol,[24] apigenin,[25] luteolin,[26,27] rutin,[22] scutallarein[26] and naringenin derivatives[23,28]), alkaloids (tecomine, tecostanine and tecostidine[29]), and triterpenoids (betulinic acid, α-amyrin, oleanolic acid[24] and β-sitosterol[30]).

The diverse chemical constituents of a considerable phytochemical interest of different *Tecoma* species as well as the important medicinal uses together with the results obtained from our screening encouraged us to carry out the present study. Reviewing the available literature on *Tecoma mollis* Humb. and Bonpl revealed the lack of information on its chemical constituents. Therefore, it was deemed of interest to carry a pharmacognostical study on this plant. We have previously reported the macro- and micro-morphological characters of the leaves and stems.[31]

In continuation, we present here the isolation and identification of triterpenes, sterols, and flavonoids from the leaves of the titled plant.

EXPERIMENTAL

General experimental procedures

1- Melting points are uncorrected and were measured by Electrothermal 9100 Digital Melting Point Instrument (England Ltd., England).
2- UV spectra are measured in methanol and different ionizing and complexing agents using an Uvidec-320 spectrophotometer with matched 1 cm quartz cells, (Jasco, Tokyo, Japan).
3- Schimadzu Infra red-470 Spectrometer (Japan) was used for measuring IR spectra in KBr discs.
4- ^{1}H- and ^{13}C-NMR spectra were run in CD_3OD, $CDCl_3$, C_5D_5N and d_6-DMSO at 400 MHz (by JEOL TNM-LA400, FT NMR system, Japan) and at 500 MHz (by Bruker Avance Spectrometer, Germany) using TMS as internal standard.
5- EIMS spectra were recorded by JEOL, JMS 600 H, Japan.
6- UV-Lamp (254, 366 nm, VL, 6 LC, Marine Lavalee-Cedex, France).
7- Silica gel for column (E. Merck, Germany).
8- TLC was performed on:
   - Precoated silica gel plates 60 F_{254} on aluminium sheets (E. Merck, Germany).
   - Reversed phase silica gel plates RP-18 F_{254} (E. Merck, Germany).
   - Sheets of Whatman No. 1 and No. 3 chromatographic paper.
9- Authentic samples were obtained from Department of Pharmacognosy, Faculty of Pharmacy, Assiut University.
10- Visualization:
   - Sulphuric acid 50% was used for visualizing sterols and triterpenes
   - Aniline phthalate reagent was used for detection of sugars.
   - Aluminium chloride 5% in methanol was used for detection of flavonoids.
   - Anisaldehyde-sulphuric acid (5% in 5% sulphuric acid) was used for detection of iridoids.
11- Solvent systems:

The following solvent systems were used for TLC and PC screening:

I- Pet. ether - ethyl acetate (90:10)
II- Pet. ether - ethyl acetate (80:20)
III- Benzene - ethyl acetate (90:10)
IV- n-Butanol - acetone - formic acid - water (60:17:8:15)
V- Chloroform - methanol (90:10)
VI- Chloroform - methanol - water (85:15:1)
VII- n-Butanol - acetic acid - water (4:1:2)
VIII- Acetic acid - water (15:85)

Materials used for biological study

- Normal saline 0.9% (El-Nasr Pharmaceutical and Chemical Co., Abou-Zaabal, Egypt).
- Indomethacin as a standard antipyretic anti-inflammatory (El-Nile Co., Cairo, Egypt).
- Acetyl salicylic acid as a standard analgesic (The Arab Drug Co., Cairo, Egypt).
- Yeast as a factor-inducing edema and pyrexia.
- Pentylenetetrazole (Sigma Co., St. Louis, Mo, USA) as a factor-inducing convulsions and death in mice.
- Phenyl-p-quinone (Sigma Co., St. Louis, Mo, USA) as a factor-inducing pain and writhing.
- Sodium valproate (T3A Pharma group, Assiut, Egypt) as a standard antiepileptic.

Plant material

The leaves of *Tecoma mollis* Humb. and Bonpl, were collected from the trees cultivated in the Experimental station of Faculty of Agriculture, Assiut University in the period from January to March 2000 (before and during the flowering and fruiting stages) and identified by Prof. Dr. Gamal Taha, Department of Horticulture, Faculty of Agriculture, Assiut University.

Extraction and fractionation

The air-dried leaves (2 Kg) of *Tecoma mollis* Humb. and Bonpl, were extracted with ethanol (70%) at room temperature by maceration and percolation then concentrated under reduced pressure. The residue left after evaporation of the solvent (193 g) was diluted with water and then subjected to fractionation with n-hexane, chloroform, ethyl acetate and n-butanol successively. Each fraction was concentrated under reduced pressure to solvent-free residue (77, 24, 36 and 44 g respectively).

Isolation

1- n-Hexane fraction

About 30 g of the n-hexane soluble fraction was chromatographed on silica gel column (E. Merck) (900 g, 5 (ID) x 150 (L) cm) and elution was started with n-hexane followed by n-hexane-ethyl acetate gradient. The eluted fractions (500 ml each) were collected, concentrated and monitored by TLC using solvent systems I, II, and III. The eluted fractions were grouped into five groups according to similar contents.

Group I was eluted with n-hexane-ethyl acetate (95:5) and revealed the presence of one spot using silica gel 60 F_{254} (systems II, V), recrystallized from hot methanol and labelled compound (**1**).

Group II was eluted with n-hexane-ethyl acetate (93:7) and revealed the presence of one major spot using silica gel 60 F_{254}, (systems II, V), concentrated under reduced pressure and rechromatographed on silica gel column (E. Merck), eluted with n-hexane-chloroform gradient to afford compound (**2**).

Group III was eluted with n-hexane-ethyl acetate (90:10) and revealed the presence of one spot using silica gel 60 F_{254} (systems II, V). Repeated crystallization from methanol afforded compound (**3**).

Group IV was eluted with n-hexane-ethyl acetate (85:15) and revealed the presence of two spots using silica gel 60 F_{254} (system V) that was subjected to preparative silica gel 60 F_{254} plates and solvent system V to yield compound (**4**).

Group V was eluted with n-hexane-ethyl acetate (80:20) and revealed the presence of one major spot using silica gel 60 F_{254} (systems V) and by repeated crystallization from methanol afforded compound (**5**).

2- Chloroformic fraction

About 15 g of the chloroform soluble fraction was chromatographed on silica gel column (E. Merck) (500 g, 5 (ID) x 150 (L) cm) and elution was initially started with chloroform followed by chloroform-methanol gradient. The eluted fractions (250 ml each) were collected, concentrated and monitored by TLC using solvent systems V, and VI. The fractions eluted with chloroform-methanol (95:5) were collected, concentrated under reduced pressure, and rechromatographed on silica gel column, elution was started with chloroform-methanol gradient. The fractions eluted with chloroform-methanol (98:2) afforded compound (**6**). Fractions eluted with chloroform-methanol (90:10) were collected, concentrated and purified by crystallization to yield compound (**7**).

3- Ethyl acetate fraction

About 18 g of ethyl acetate soluble fraction was chromatographed on silica gel column (E. Merck) (600 g, 5 (ID) x 150 (L) cm). The elution was started with chloroform followed by chloroform-methanol gradient. The eluted fractions (500 ml each) were collected, concentrated and monitored by TLC screening. The fractions eluted with chloroform-methanol (85:15) were subjected to paper chromatography (N0. 3) and solvent system (acetic acid-water (30:70) to afford compound (**8**). The fractions eluted with chloroform-methanol (80:20) and (70:30) each revealed the presence of mixture of compact flavonoid and iridoid spots using silica

gel 60 F_{254} and solvent system chloroform-methanol (80:20). Several chromatographic trials failed to produce any pure compound.

3- n-Butanol fraction

About 15 g of the n-butanol fraction was chromatographed on silica gel column (E. Merck) (500 g, 5 (ID) x 150 (L) cm). The elution was started with chloroform followed by chloroform-methanol gradient. The eluted fractions (500 ml each) were collected and screened by TLC. The fractions eluted with chloroform-methanol (60:40) were subjected to paper chromatography (N0. 3) and solvent system acetic acid-water (15:85) to afford compounds (**9** and **10**). The fraction eluted with 50% methanol was rechromatographed on silica gel column (E. Merck) and elution was started with ethyl acetate-methanol gradient to afford compound (**11**).

Acid hydrolysis

Five-mg portion of each of the isolated glycosides was dissolved in 5 ml methanol to which 5 ml of 5% sulphuric acid is added. The mixture was refluxed for 3 hours on a boiling water-bath, cooled, the aglycone was extracted with chloroform, purified and subjected to TLC. The produced sugars were identified by TLC using silica gel 60 F_{254} and solvent system IV.

Compound 1: Colourless needles [methanol], (350 mg), m.p 184-186°, R_f= 0.4 (system I), IR (KBr, u, cm^{-1}): 3420, 2980, 1650, 1460, 1380, 1050, 1010, 960 and 805.

Compound 2: Colourless needles [methanol], (40 mg), m.p 217-219°, R_f= 0.57 (system II). EI$^+$-MS at *m/z*: 440 (14.2%) $[M]^+$, other diagnostic peaks at *m/z*= 422 (55.1%) $[M-H_2O]^+$, 410 (11.8%) $[M-HCHO]^+$, 407 (17.4%), 379 (56.8%), 232 (64.2%), 207 (59.8%), 203 (100%), 189 (78.1%), 133 (80%), 55 (39.4%), 43 (30.4%) and 18 (32.2%). ^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ 0.76 (3H, s, CH_3), 0.77 (3H, s, CH_3), 0.86 (3H, d, J= 6.3 Hz, 29-CH_3), 0.91 (3H, s, CH_3), 0.95 (3H, br.s, 30-CH_3), 0.98 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.12-2.00 (m, CH_2 protons), 3.20 (1H, dd, J= 10.50 and 4.64 Hz, H-3α), 5.30 (1H, t-like, H-12), 9.30 (1H, s, CHO).

Compound 3: Colourless needles [methanol], (720 mg), m.p 135-137°, R_f= 0.52 (system II). EI$^+$-MS at *m/z*: 414 (100%) $[M]^+$, other diagnostic peaks at *m/z*= 399 (25.6%) $[M-CH_3]^+$, 396 (26.8%) $[M-H_2O]^+$, 381 (11.2%) $[399-H_2O]^+$, 371 (1.3%) $[M-C_3H_7]^+$, 273 (16%) [M-side chain]$^+$, 255 (18.4%), 231 (19.2%).

Compound 4: Colourless needles [ethanol], (80 mg), m.p 262-265°, R_f= 0.69 (system V). IR (KBr, u, cm^{-1}): 3500, 2970-2855, 1768, 1465, and 1025. EI$^+$-MS at *m/z*: 454 (65%) $[M]^+$, other diagnostic peaks at *m/z*= 426 (23.4%) $[M-CO]^+$, 410 (100%) $[M-CO_2]^+$, 409 (15.9%), 408 (20.4% $[426-H_2O]^+$, 300 (15.6%), 257 (13%), 215 20.7%), 201 (25.9%), 189 (23%), 187 (15.7%), 175 (16.7%), 145 (18.4), 133 (19.1%). ^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ 0.77 (3H, s, 24-CH_3), 0.90 (3H, s, 23-CH_3), 0.93 (3H, d, J= 6 Hz, 30-CH_3), 0.98 (3H, s, 25-CH_3), 0.99 (3H, d, J= 6.3 Hz, 29-CH_3), 1.04 (3H, s, 26-CH_3), 1.15 (3H, s, 27-CH_3), 1.20-2.20 (m, CH_2 protons), 3.20 (1H, dd, J= 11.20 and 4.60 Hz, H-3α), 5.53 (1H, dd, J= 2.90 and 10.30 Hz, H-11), 5.96 (1H, d, J= 10.3 Hz, H-12).

Compound 5: Colourless needles [ethanol], (5 g), m.p 289-292°, R_f= 0.49 (system V). IR (KBr, u, cm^{-1}): 3435, 2970, 1689, 1453, 1388, 1029 and 996. EI$^+$-MS at *m/z*: 456 (14.7%) $[M]^+$, other diagnostic peaks at *m/z*= 438 (2.8%) $[M-H_2O]^+$, 410 (4.8%) $[M-HCOOH]^+$, 383 (21.4%), 381 (21.2%), 302 (100%), 300 (15.7%), 257 (24.6%), 248 (87.2%), 220 (10.7%), 207 (61.6%), 189 (33.6%), 133 (57.9%), 119 (34.8%), 95 (29.8%), 81 (25.9%) and 18 (24.5%). ^{1}H-NMR spectrum (400 MHz, CD_3OD and d_6-DMSO): δ 0.66 (3H, s, CH_3), 0.74 (3H, s, CH_3), 0.78 (3H, d, J= 6.10 Hz, 29-CH_3), 0.85 (3H, s, CH_3), 0.87 (3H, s, CH_3), 0.88 (3H, d, J= 6.10 Hz, 30-CH_3), 1.02 (3H, s, CH_3), 1.12-2.00 (m, CH_2 protons), 2.09 (1H, d, J= 11.50 Hz, H-18β), 3.00 (1H, dd, J= 5.40, 10.24 Hz, H-3α), 5.11 (1H, br.s, H-12).

Compound 6: White amorphous powder (110 mg), m.p 303 - 305°, R_f= 0.40 (system V). IR (KBr, u, cm^{-1}): 3420, 2970, 1686, 1458, and 1043. EI$^+$-MS at *m/z*: 488 (9.7%) $[M]^+$, other diagnostic peaks at *m/z*= 470 (8.4%) $[M-H_2O]^+$, 442 (37.6%) $[M-HCOOH]^+$, 424 (10%) [442-

H_2O], 370 (16.4%), 264 (31%), 246 (48%), 218 (34%), 201 (47.9%), 187 (24.1%), 171 (12.9%), 146 (100%), 55 (34.4%), 28 (10.6%) and 18 (52.7%). ^{1}H-NMR spectrum (400 MHz, CD_3OD): δ 0.71 (3H, s, 26-CH_3), 0.83 (3H, d, J= 6.6 Hz, 30-CH_3), 0.89 (3H, s, 24-CH_3), 0.90 (3H, s, 23-CH_3), 1.15 (3H, s, 25-CH_3), 1.19 (3H, s, 29-CH_3), 1.22 (3H, s, 27-CH_3), 1.12-2.00 (m, CH_2 protons), 3.03 (1H, d, J= 3.92 Hz, H-3α), 3.90 (1H, m, H-2α), 5.18 (1H, t, J= 3.5 Hz, H-12).

Compound 7: White amorphous powder (500 mg), m.p 288-289°, R_f= 0.24 (system V). IR (KBr, u, cm^{-1}): 3455, 2935, and 1636. ^{1}H-NMR spectrum (400 MHz, C_5D_5N): δ 0.63 (3H, s, 18-CH_3), 0.83, 0.84, 0.90 (9H, m, 26-, 27-, 29-CH_3), 0.87 (3H, s, 19-CH_3), 0.97 (3H, d, J= 5.90 Hz, 21-CH_3), 1.10 – 2.80 (m, CH and CH_2 protons), 3.91 (1H, m, H-3α), 3.94-4.60 (m, sugar protons), 5.10 (1H, d, J= 7.50 Hz, H-1′), 5.32 (1H, m, H-6).

Compound 8: Pale yellow needles [methanol] (20 mg), m.p 198-200°, R_f= 0.17 (system VIII). UV (λ_{max}, nm, MeOH): 268, 335; NaOMe: 269, 384; $AlCl_3$: 277, 347, 384; $AlCl_3$/HCl: 277, 343, 382; NaOAc: 268, 354; NaOAc/H_3BO_3: 268, 340. ^{1}H-NMR spectrum (CD_3OD, 400 MHz): δ 1.30 (3H, d, J= 6.85 Hz, CH_3-rhamnose), 3.00-4.00 (m, sugar protons), 5.30 (1H, d, J= 2.00 Hz, H-1``), 6.50 (1H, d, J= 2.00 Hz, H-6), 6.64 (1H, s, H-3), 6.85 (1H, d, J= 2.00 Hz, H-8), 6.91 (2H, d, J= 8.80 Hz, H-3`,5`), 7.88 (2H, d, J= 8.80 Hz, H-2`,6`).

Compound 9: Yellow amorphous powder (45 mg), R_f= 0.52 (system VIII). UV (λ_{max}, nm, MeOH): 268, 335; NaOMe: 267, 387; $AlCl_3$: 275, 347, 384; $AlCl_3$/HCl: 276, 341, 382; NaOAc: 267, 341, 388; NaOAc/H_3BO_3: 267, 339. ^{1}H-NMR spectrum (d_6-DMSO, 500 MHz): δ 1.10 (3H, d, J= 6.60 Hz, CH_3-rhamnose), 3.00-4.00 (m, sugar protons), 4.54 (1H, br.s, H-1```), 5.05 (1H, d, J= 7.60 Hz, H-1``), 6.43 (1H, d, J= 2.20 Hz, H-6), 6.76 (1H, d, J= 2.20 Hz, H-8), 6.82 (1H, s, H-3), 6.97 (1H, d, J= 8.80 Hz, H-3`,5`), 7.92 (2H, d, J= 8.80 Hz, H-2`,6`).

Compound 10: Yellow amorphous powder (25 mg), R_f= 0.39 (system VIII). UV (λ_{max}, nm, MeOH): 254, 349; NaOMe: 264, 396; $AlCl_3$: 273, 431; $AlCl_3$/HCl: 272, 388; NaOAc: 257, 402; NaOAc/H_3BO_3: 257, 371. ^{1}H-NMR spectrum (d_6-DMSO, 500 MHz): δ 1.20 (3H, d, J= 6.60 Hz, CH_3-rhamnose), 3.00-4.00 (m, sugar protons), 4.53 (1H, br.s, H-1```), 5.05 (1H, d, J= 7.55 Hz, H-1``), 6.43 (1H, br.s, H-6), 6.69 (1H, s, H-3), 6.72 (1H, br.s, H-8), 6.92 (1H, d, J= 8.80, H-5`), 7.39 (1H, d, J= 2.00 Hz, H-2`), 7.42 (1H, dd, J= 2.00, 8.80 Hz, H-,6`).

Compound 11: Yellow amorphous powder (55 mg), R_f= 0.70 (system VIII). UV (λ_{max}, nm, MeOH): 273, 335; NaOMe: 281, 397; $AlCl_3$: 280, 353, 387; $AlCl_3$/HCl: 281, 347, 383; NaOAc: 282, 388; NaOAc/H_3BO_3: 275, 335. ^{1}H-NMR spectrum (d_6-DMSO, 500 MHz): δ 3.00-4.00 (m, sugar protons), 4.65 (1H, d, J= 9.80 Hz, H-1``), 4.82 (1H, d, J= 9.80 Hz, H-1```), 6.53 (1H, s, H-3), 6.88 (2H, d, J= 8.50, H-3`,5`), 7.92 (2H, d, J= 8.50, H-2`,6`), 13.71 (1H, s, 5-OH).

Biological Screening

Preparation of the extracts

About 500 g portion of the air-dried powdered leaves of *Tecoma mollis* was extracted with 70% ethanol by maceration and percolation till complete exhaustion. The combined alcoholic extract was filtered, the filtrate was concentrated till dryness under reduced pressure. The solvent free residue was weighed accurately to give a yield (52 g) and percent yield (10.4% w/w). The dried total ethanolic extract (52 g) was diluted with distilled water, transferred to a separating funnel and fractionated successively with n-hexane, chloroform, ethyl acetate and finally n-butanol.

Preparation of the aqueous extract

About 100 g portion of the air-dried leaves of *Tecoma mollis* was percolated with (900 ml) of boiling water; this procedure was repeated four times, then the combined solutions were collected and the solvent was removed under reduced pressure. The solvent free residue was weighed accurately to give yield (7.3 g) and percent yield (7.3% w/w).

Preparation of extracts for administration

A weighed amount of the previously obtained fractions (1, 2 and 4 g) was prepared

as an emulsion in saline containing 2% Tween 80 to obtain concentrations of 10, 20 and 40 mg/ml. Each fraction was subjected to different preliminary pharmacological screening methods for anti-inflammatory, analgesic and antipyretic activities.

Animals

Male albino rats (100-120 g) and mice (30-35 g) were used. The animals were bred and housed under standardized environmental conditions in the pre-clinical animal house, Pharmacology Department, Faculty of Medicine, Assiut University. They were fed with standard diet and allowed free access to drinking water.

Statistical analysis

Data were analysed using the student's t-test and the values were expressed as mean ± S.E.

A) Anti-inflammatory activity

The anti-inflammatory study was carried out according to the method described by Winter *et al.*,[32] where a pedal inflammation in rat paws was induced by subplantar injection of 20% yeast suspension into the right hind paw of the rats in a dose 1 ml/100 g body weight.

Male rats of (100-120 g) body weight were divided into groups of six animals. At the beginning of test. The paws thicknesses were measured in mm using Varinier Caliber. Tested fractions of *Tecoma mollis* at doses of 200 and 400 mg/kg were given 30 minutes orally before injection of the yeast. Control group received 2% Tween 80 in normal saline (solvent mixture), while reference group received indomethacin (8 mg/kg). After 30 minutes from drug administration, the inflammation was induced by injection of the yeast in the right paw while the left one was injected by an equal volume of saline solution. The difference between the thicknesses of the two paws was taken as a measure of edema. The anti-inflammatory efficacy of the tested fractions was estimated by comparing the magnitude of paw swelling in the pretreated animals with those induced in control animals receiving saline. The measurement was carried out at ½, 1, 2, 3, 5 and 12 hours after injection of the inflammatory agent. The percentage of edema[33] and percentage of inhibition[34] were calculated as follows:

$$\% \text{ edema} = \frac{(\text{Right paw thickness} - \text{Left paw thickness}) \times 100}{\text{Right paw thickness}}$$

and

$$\% \text{ inhibition} = \frac{(V_o - V_t) \times 100}{V_o}$$

where:

V_o : the average paw thickness of control group

V_t : the average paw thickness of the treated group.

The tested fractions were administered at 200 and 400 mg/kg orally with reference indomethacin at dose 8 mg/kg. The anti-inflammatory and inhibitory effects of the different fractions were listed in Tables 3 and 4.

B) Analgesic activity

1- Writhing test[33]

Groups of six male albino mice (30-35 g) were used in this test. Plant extracts, reference substance acetyl salicylic acid (ASA) at a dose of 100 mg/kg and control vehicle (2% Tween 80 in saline solution) were administered orally 60 minutes before the intraperitoneal (i.p.) administration of a solution of phenyl-p-quinone (4 mg/kg).[33]

Five minutes after algic compound injection, each animal was isolated in an individual box and the numbers of times that the animal stretched or writhed during 30 minutes were counted. The number of writhings and stretchings was calculated and permitted to express the percentage of protection[33] using the following ratio.

$$\% \text{ production} = \frac{(\text{Control mean} - \text{Treated mean}) \times 100}{\text{Control mean}}$$

2- Hot-plate test

Hot-plate method described by Jacob *et al.*[35,36] was used. The animals were placed on a metal plate surface maintained at 55±1° on top of which a transparent restraining cylinder open at both ends was placed. The reaction time in seconds was taken as the time period from the instant the animal reached the hot plate until the moment the animal licked its feet or jumped out.

Each subject was its own control; thus before treatment, its reaction time was determined twice at 10 minutes intervals, the mean of these two values constituting the reaction time before treatment (T_b). Thirty minutes after i.p. administration of fractions, the reaction time was again evaluated only once, this value representing the reaction time after treatment (T_a) and the cut-off time was 20 second for each group, the average reaction times (T) were then calculated.

The percentage of variation can be expressed by the following ratio:[37]

$$T = \frac{(T_a - T_b) \times 100}{T_b}$$

C) Antipyretic activity[33,38]

Groups of 6 male rats (100-120 g) body weight were used and rectal temperature was recorded with thermometer. Hyperthermia was induced by subcutaneous injection of a 20% (w/v) aqueous suspension of yeast in a volume of 10 ml/kg following Adams *et al.* method.[39] At four hours after pyrogen agent injection, the test fractions of *Tecoma mollis* in doses of 200 and 400 mg/kg were orally administered to rats. Animals in reference group received 8 mg/kg indomethacin, while equal volume of Tween 80 in saline was used as a control treatment. Rectal temperatures were taken after 1, 2, 3 and 5 hours from administration of tested fractions. The results were listed in Table 7.

2- Activity on the central nervous system

The activity of the alcohol fractions of *Tecoma mollis* on the CNS were evaluated by performing assays of their effects on spontaneous motor activity,[40] rectal temperature and pentylenetetrazol-induced convulsions.

Drugs and dosage

The following drugs and dosages were used: pentylenetetrazole (125 mg/kg), sodium valproate (200 mg/kg), alcohol fractions and aqueous extract of *Tecoma mollis* at doses 100, 200 and 400 mg/kg. All drugs and extracts were dissolved in distilled water immedaitely before use. Control animals received distilled water under the same conditions.

i) Effects on spontaneous motor activity[40]

The locomotor activity of mice (30-35 g) were registered with an activity cage. This cage contains an electromagnetic field that is sensitive to any motion within it. Movement of the animal causes an alteration in the energy of the field that is recorded as a locomotion count. Each animal was placed on the cage five minutes after receiving the extract as an i.p. injection and the total number of steps in 10 minutes periods over one hour were recorded. The results were recorded in Table 8.

ii) Effects on rectal temperature[41]

Rectal temperature was measured with thermometer, the temperature was recorded just before (T_o) administration and at 30, 60, 90 and 120 minutes after i.p. injection of the extracts. The effect of extracts at different dosages on rectal temperature of rats was listed in Table 9.

iii) Anticonvulsant activity[40,41]

The effect of alcohol fractions and total aqueous extract of *Tecoma mollis* on pentylenetetrazole-induced convulsions were evaluated as Marcucci method.[42] Pentylenetetrazole 125 mg/kg was administered i.p. to the mice 60 minutes after injection of test sample or control and the elapsed times before onset of clonic convulsion, tonic convulsion and death were recorded. Mice were considered to be survived if they lived for longer than 20 minutes after pentylenetetrazole administration. The effects of different fractions of *Tecoma mollis* on pentylenetetrazole-induced convulsions and death were listed in Table 10.

RESULTS AND DISCUSSION

I- Identification of the isolated compounds

Compounds **1** and **3** were identified as α-amyrin, and β-sitosterol respectively by comparison of their physico-chemical data such as m.p, IR, EI^+-MS and co-chromatography with reference samples. Compound **7** was identified as β-sitosterol-3-O-β-D-glucoside by comparing its IR, m.p, ^{1}H-NMR, and ^{13}C-NMR with reported data.[43] After its acid hydrolysis, the sugar was identified as glucose and the aglycone as β-sitosterol.

Compounds (**2, 4, 5** and **6**) gave positive colour reaction with Liebermann-Burchard and

Salkowski tests indicating their triterpenoidal nature.[44] Compound (**5**) was identified as ursolic acid by comparing its m.p, EI$^+$-MS, IR, ^{1}H-NMR, ^{13}C-NMR with those of the reported data.[45,46]

EI$^+$-MS of compound (**2**) showed molecular ion peak at m/z 440 for $C_{30}H_{48}O_2$. ^{1}H-NMR spectrum showed seven signals for seven methyl groups. The spectrum also showed a signal at δ 3.2 (1H, dd, J= 4.64 and 10.50 Hz), which was assigned for H-3α and an olefinic proton at δ 5.3 was attributable for H-12. The signal at δ 9.3 (1H, s) was assigned for aldehydic proton,[47] which was confirmed by a signal at 207.4 in ^{13}C-NMR, Table 1. The signals at δ 126.2 and 137.8 in ^{13}C-NMR indicated that compound (**2**) was Δ^{12}-ursane triterpenoid.[48] The presence of hydroxyl group at C-3 was confirmed by the appearance of a signal at δ 79.0 in ^{13}C-NMR. The downfield shift of directly attached α–carbon C-17 to δ 50.1 and the upfield shifts of β–carbons C-16, C-18 and C-22 to δ 23.3, 52.6 and 31.9 compared to those reported for α-amyrin[45] confirmed the location of CHO group at C-28. this was also confirmed by diagnostic peaks at m/z 203 (232-CHO) and 201 (203-2H). The structure was also confirmed by inspection of other quaternary carbons at position-4, 8, 10 and 14 which have similar chemical shifts as ursane series.[45] From the above-mentioned spectral data, it could be concluded that the suggested structure of compound (**2**) is 3-β-hydroxy-urs-12-ene-28-aldehyde.

IR spectrum of compound (**4**) showed a band at 3500 cm^{-1} for OH stretching and a band at 1768 cm^{-1} for carbonyl group stretching. EI$^+$-MS showed molecular ion peak at m/z 454 for $C_{30}H_{46}O_3$. ^{1}H-NMR spectrum showed seven signals for seven methyl groups, five singlets and two doublets. The spectrum also showed a signal at δ 3.2 (1H, dd) which was assigned for H-3α and two olefinic protons at δ 5.53, 5.96 for H-11 and H-12 respectively which were confirmed by two signals at δ 128.8 and 133.4 in ^{13}C-NMR, Table 1. The olefinic system should be located between C-11 and C-12 due to the downfield shift of C-9 to δ 53.0 compared with that reported for α-amyrin at δ 47.7 which contain the double bond between C-12 and C-13.[45] ^{13}C-NMR also showed a signal at δ 78.9 which was assigned for 3-β-OH. The signal at δ 179.9 was assigned for carboxyl group which was located at C-28 due to the downfield shift of C-17 to δ 45.1. This carboxyl group undergoes lactone formation with C-13 and leads to shift of double bond between C-11 and C-12, upfield shift of C-13 to δ 89.7, C-22 to δ 31.3 and the downfield shift of C-18 to δ 60.6 compared with ursolic acid. From the above-mentioned spectral data and compared to the reported data,[49] it could be concluded that compound (**4**) was identified as ursolic acid lactone.

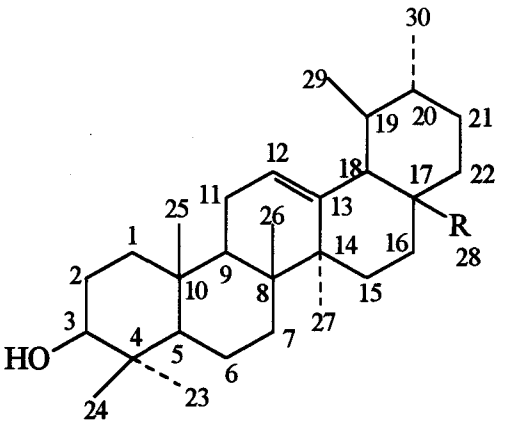
The IR spectrum of compound (**6**) showed a band at 3420 cm^{-1} for OH stretching and a band at 1686 cm^{-1} for carbonyl group stretching. ^{13}C-NMR spectrum showed seven signals for seven methyl groups, occur at the same chemical shifts as ursolic acid except 29- and 30-CH_3. 29-CH_3 is downfield shifted to δ 27.1 due to the presence of OH group at C-19 which was confirmed by its downfield shift to δ 73.6. The presence of OH at C-19 leads to downfield shift of C-20 to δ 43.0 and upfield shift of C-30 to δ 18.5 compared to ursolic acid. The presence of OH group at C-19 can be also confirmed by the downfield shift of 29-CH_3 in ^{1}H-NMR to δ 1.19 as a singlet instead of doublet in ursolic acid. The presence of OH group at C-2 and C-3 was confirmed by two signals at δ 72.1 and 79.6 respectively. ^{1}H-NMR spectrum showed one doublet at δ 3.03 (J= 3.92 Hz) and multiplet at δ 3.90 which were assigned for H-3α, and H-2α respectively, the small coupling constant value of H-3 with H-2 indicated that protons 2 and 3 have α-orientation with β-orientation of hydroxyl groups[44] and by reviewing the literature of triterpenes,[50] it was found that, the shift in C-2 and C-3 was acceptable for β-orientation of hydroxyl groups attached to these carbons. EI$^+$-MS showed molecular ion peak at m/z= 488 for $C_{30}H_{48}O_5$ and other diagnostic fragments characteristic for ursane series.[46] From the afore-mentioned studies, it could be concluded that compound (**6**) was identified as 2-β,3-β,19-α-trihydroxy-urs-12-ene-28-oic acid (2-tormentic acid).

The UV spectral data for compounds (**8-11**) in methanol indicated their flavone nature. The acid hydrolysis of compounds (**8** and **9**) yielded rhamnose, glucose and rhamnose

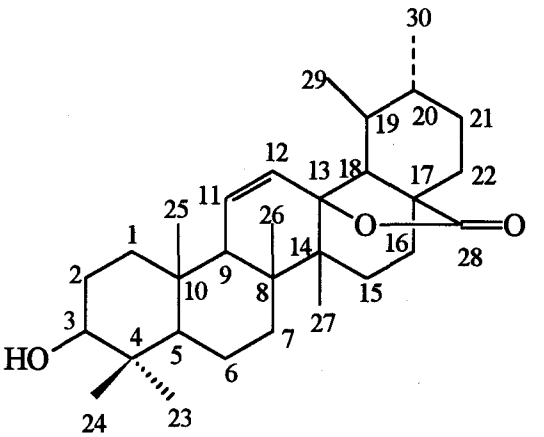
Table 1: ^{13}C-NMR data of compounds (2, 4 -7) (100 MHz, relative to TMS).

C-Atoms	2	4	5	6	7
1	38.7 t	38.2 t	38.7 t	45.5 t	37.5
2	27.0 t	27.0 t	27.3 t	72.1 d	29.4
3	79.0 d	78.9 d	77.4 d	79.6 d	78.6
4	38.7 s	38.9 s	38.9 s	39.4 s	39.3
5	55.2 d	54.7 d	55.3 d	55.6 d	140.8
6	18.3 t	17.7 t	18.4 t	19.3 t	121.9
7	33.1 t	31.2 t	33.2 t	34.2 t	32.2
8	39.8 s	41.9 s	39.6 s	39.6 s	32.0
9	47.6 d	53.0 d	47.5 d	48.3 d	50.3
10	36.9 s	36.3 s	36.9 s	39.0 s	36.9
11	23.2 t	128.8 d	23.3 t	24.7 t	21.3
12	126.2 d	133.4 d	125.2 d	130.1 d	39.9
13	137.8 s	89.7 s	138.6 s	139.9 s	42.5
14	42.2 s	41.7 s	42.1 s	42.7 s	56.8
15	27.2 t	25.5 t	28.0 t	29.5 t	24.5
16	23.3 t	22.8 t	24.2 t	26.6 t	28.5
17	50.1 s	45.1 s	47.7 s	49.6 s	56.2
18	52.6 d	60.6 d	52.9 d	56.8 d	11.9
19	39.0 d	40.3 d	39.1 d	73.6 s	19.4
20	38.8 d	38.0 d	38.9 d	43.0 d	36.4
21	30.2 t	30.8 t	30.6 t	27.3 t	19.0
22	31.9 t	31.3 t	36.8 t	37.9 t	34.2
23	28.1 q	27.7 q	28.4 q	30.2 q	26.3
24	15.5 q	14.9 q	15.4 q	17.4 q	46.0
25	15.6 q	19.1 q	16.2 q	17.1 q	30.2
26	16.6 q	18.9 q	17.2 q	18.1 q	19.2
27	23.2 q	16.1 q	23.5 q	24.9 q	19.9
28	207.4 d	179.9 s	178.8 s	182.3 s	23.4
29	17.2 q	17.8 q	17.2 q	27.1 q	12.1
30	21.0 q	17.9 q	21.2 q	18.5 q	-----
1`					102.6
2`					75.3
3`					78.1
4`					71.6
5`					78.4
6`					62.8

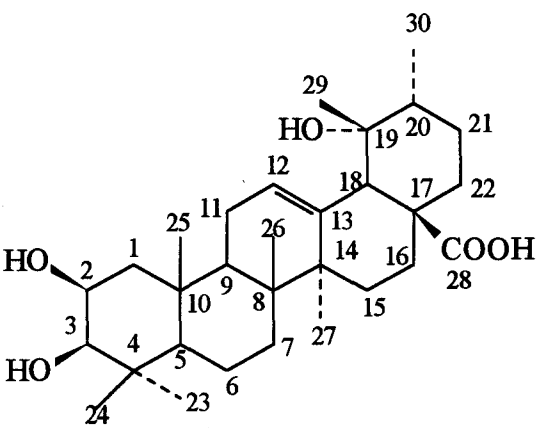
Spectra were measured in $CDCl_3$ (2, 4), CD_3OD + d_6-DMSO (5), CD_3OD (6), C_5D_5N (7).



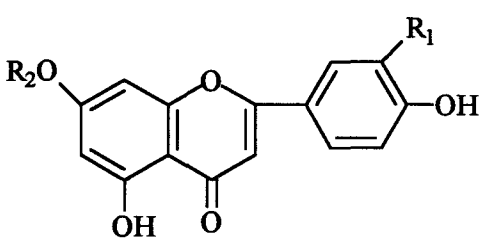
Compound **2**: R = CHO
Compound **5**: R = COOH



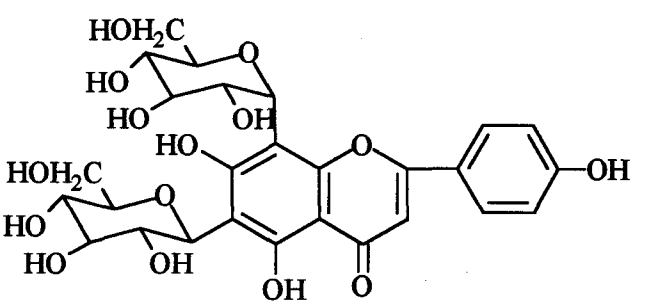
Compound **4**



Compound **6**



	R_1	R_2
8	H	Rhamnose
9	H	Rutinoside
10	OH	Rutinoside



Compound **11** (Vicenin 2)

respectively and the aglycone was identified as apigenin from the appearance of a signal for H-3 in ^{1}H-NMR, their UV data with different ionising and complexing agents[51] as well as co-chromatography with authentic apigenin. The sugar attachment was assigned to be at C-7 by comparing the UV data before and after hydrolysis. The ^{1}H-NMR was also identical to those reported for apigenin-7-O-α-L-rhamnoside[52] and apigenin-7-O-rutinoside for compounds (**8** and **9**) respectively. The acid hydrolysis of compound (**10**) yielded glucose and rhamnose, the aglycone was identified as luteolin from the appearance of a signal for H-3 in ^{1}H-NMR, its UV data with different ionising and complexing agents[51] as well as co-chromatography with authentic luteolin. The sugar attachment was assigned to be at C-7 by comparing the UV data before and after hydrolysis. The ^{1}H- and ^{13}C-NMR data, Table 2, were also identical to those reported[25,53] for luteolin -7-O-rutinoside (Scolymoside).

The UV spectral data for compound **11** showed a bathochromic shift in band I (+52 nm) upon addition of $AlCl_3$ which was not significantly changed on addition of HCl indicating the presence of free hydroxyl group at C-5 and the absence of ortho dihydroxy groups in ring B which was confirmed by the absence of shift upon addition of sodium acetate/boric acid. The ^{1}H-NMR spectrum showed two doublet signals at δ 7.92 and 6.88 (each 2 H, d, J= 8.50 Hz) which were assigned for H-2`,6` and H-3`,5` respectively. The spectrum also showed one signal at δ 6.53 which was assigned for H-3 indicating a flavone nature, two anomeric protos for two glucose units at δ 4.82 and 4.65 (each 1H, d, J= 9.80 Hz) with high coupling constant values indicating their C-type glycosidic linkage[54] which was confirmed by two signals in ^{13}C-NMR, Table 2, at δ 73.6 and 74.5 for C-6 and C-8 glucose. The position of C-linkage is also confirmed by the downfield shift of C-6 (δ 98.8 in apigenin aglycone) to δ 108.0 with upfield shift of C-5 (δ 161.5 in apigenin aglycone) to δ 159.6. Also the downfield shift of C-8 (δ 94.0 in apigenin aglycone) to δ 105.4 with upfield shift of C-7 (δ 163.8 in apigenin aglycone) to δ 160.9 and C-9 (δ 157.3 in apigenin aglycone) to δ 155.7. The ^{13}C-NMR spectral data of compound **11** showed good agreement with the reported data for apigenin-6,8-di-C-glucoside.[53]

Partial and complete acid hydrolysis of compound **11** failed to give its aglycone and this confirm its C-type glycosidic nature. From the above-mentioned data, it could be concluded that compound **11** was identified as apigenin-6,8-di-C-β-D-glucpyranoside (vicenin 2).

Conclusion

Compounds **1, 3, 7** and **10** were isolated for the first time from *Tecoma mollis;* **5, 8** and **9** for the first time from the genus *Tecoma;* **2, 4, 6** and **11** for the first time from the family Bignoniaceae.

II- Biological Studies

Anti-inflammatory activity

Average thickness of back paws and percentage of inhibition are respectively reported in Tables 3 and 4 for alcoholic fractions, ursolic acid and aqueous extract at doses 200 and 400 mg/kg with reference indomethacin (8 mg/kg).

In control group, the subplanter injection of yeast produced a local edema in the following 30 minutes, that increased to reach its maximum intensity after 3 hours after injection of the phlogistic agent, the percentage of edema being then between 63 to 67%. Afterwards, this edema was progressively decreased, but it is still remained obvious 12 hours after injection.

A pretreatment with alcohol fractions and aqueous extract significantly reduced the yeast-induced edema, in a dose-dependent way. In the case of pretreatment with n-hexane fraction (200 and 400 mg/kg), the percentage of edema was decreased gradually with time and a maximum inhibition (33.3, 43.5%) for 200 and 400 mg/kg respectively being obtained after 3 hours of yeast injection. The effect was maintained during the following 2 hours and remained significant after 12 hours with inhibition of 29.5, 40.2% for 200 and 400 mg/kg respectively. Also in the case of pretreatment with chloroform fraction, the percentage of edema was decreased gradually with time and a maximum inhibition of 34.7, 42.6% for 200 and 400 mg/kg being obtained after 2 hours of injection of phlogistic agent and still remained significant after 12 hours with inhibition of 26.4, 39.6% for 200 and 400 mg/kg, respectively.

Table 2: ^{13}C-NMR data of compounds (9 - 11) (125 MHz, d_6-DMSO, relative to TMS).

C-Atoms	9	10	11
2	164.6 s	164.5 s	163.4 s
3	103.2 d	103.3 d	102.1 d
4	182.1 s	182.2 s	180.9 s
5	161.6 s	161.5 s	159.6 s
6	99.6 d	99.8 d	108.0 s
7	163.1 s	163.1 s	160.9 s
8	94.9 d	95.0 d	105.4 s
9	157.1 s	157.1 s	155.6 s
10	105.5 s	105.4 s	103.7 s
1`	121.1 s	121.2 s	122.1 s
2`	128.8 d	113.4 d	128.6 d
3`	116.2 d	145.5 s	115.9 d
4`	161.3 s	150.0 s	160.8 s
5`	116.2 d	116.4 d	116.1 d
6`	128.8 d	119.0 d	128.6 d
	Glucose	Glucose	6-C-Glucose
1``	100.1 d	100.1 d	73.6 d
2``	73.2 d	73.3 d	71.1 d
3``	76.5 d	76.5 d	77.9 d
4``	69.8 d	69.7 d	68.8 d
5``	75.8 d	75.5 d	80.9 d
6``	66.2 t	66.2 t	59.9 t
	Rhamnose	Rhamnose	8-C-Glucose
1```	100.7 d	100.7 d	74.5 d
2```	70.9 d	70.9 d	71.7 d
3```	70.5 d	70.4 d	79.0 d
4```	72.2 d	72.2 d	70.9 d
5```	68.4 d	68.4 d	81.9 d
6```	17.9 q	18.0 q	61.3 t

Spectra were measured in d_6-DMSO.

Table 3: Results of the anti-inflammatory activity of the different fractions of *Tecoma mollis* leaves on yeast-induced edema in rats.

Group	Dose mg/kg	VL (mm) (mean±S.E.)	Thickness of the right paw, mm (mean ±S.E.), n = 6					
			½ hr	1 hr	2 hr	3 hr	5 hr	12 hr
Control	-	3.1±0.02	6.98±0.07	8.46±0.16	9.0±0.11	9.5±0.09	9.23±0.12	7.55±0.15
Indomethacin	8	3.02±0.03	4.38±0.08**	4.98±0.14**	4.96±0.09**	3.85±0.16**	4.26±0.13**	3.37±0.07**
n-Hexane	200	3.1±0.03	5.52±0.28	6.15±0.18*	6.26±0.10*	6.33±0.24*	6.86±0.16*	5.32±0.09*
	400	3.06±0.02	4.70±0.12*	5.22±0.25**	5.45±0.15**	5.37±0.21**	5.92±0.07*	4.51±0.08**
Chloroform	200	3.09±0.01	5.50±0.14	5.99±0.09*	5.87±0.22*	6.44±0.13*	6.39±0.05*	5.55±0.24*
	400	3.05±0.01	4.82±0.07*	5.21±0.13**	5.16±0.21**	5.63±0.15**	5.95±0.10*	4.56±0.06**
Ethyl acetate	200	2.99±0.02	5.08±0.09*	5.11±0.07**	4.89±0.12**	4.91±0.18**	5.28±0.16**	4.37±0.13**
	400	3.00±0.04	4.46±0.13**	4.16±0.09**	3.90±0.07**	4.30±0.24**	4.70±0.12**	3.64±0.10**
n-Butanol	200	3.07±0.06	4.94±0.11*	4.95±0.17**	4.56±0.15**	4.69±0.10**	5.12±0.08**	4.6±0.09**
	400	3.05±0.06	4.30±0.16**	3.90±0.08**	3.41±0.12**	3.78±0.13**	4.10±0.04**	3.54±0.07**
Aqueous extract	200	3.03±0.04	5.05±0.07*	5.26±0.15**	4.79±0.09**	4.62±0.16**	5.21±0.21**	4.43±0.13**
	400	2.98±0.10	4.22±0.08**	3.88±0.14**	3.48±0.09**	3.65±0.08**	4.00±0.11**	3.54±0.16**
Ursolic acid	200	3.00±0.02	6.04±0.16	6.40±0.21*	6.17±0.13*	6.12±0.18**	6.53±0.06*	5.59±0.15*
	400	3.01±0.03	5.18±0.17*	5.60±0.04*	5.74±0.09*	5.45±0.03**	5.96±0.06*	5.08±0.07*

VL: left paw thickness (mm)

Differences with respect to the control group were evaluated using Student's t-test (*P< 0.05, **P< 0.01).

Pure ursolic acid (Table 4) that was isolated from n-hexane fraction also exhibited an inhibitory activity for yeast-induced edema, the maximum inhibition of 35.7, 42.6% for 200 and 400 mg/kg being obtained after 3 hours of injection of yeast and remained significant for 12 hours. The polar fractions (ethyl acetate or n-butanol) showed strong dose-dependent anti-inflammatory activity, from the dose 200 mg/kg, 3 hours after yeast injection, a maximum inhibition of 48.3 of ethyl acetate fraction and 50.6% n-butanol fraction - being obtained at this time. This effect was maintained during the following 2 hours and remained significant after 12 hours. With the higher dose 400 mg/kg, this effect appeared 2 hours after yeast injection and reached a maximum intensity at this time with percentage of inhibition being 56.6 and 62.1% of ethyl acetate and n-butanol respectively. The total aqueous extract showed the same activity against yeast-induced edema with closed potency with that of ethyl acetate and n-butanol fractions.

The observed anti-inflammatory activity could be attributed to the presence of triterpenes (ursane derivatives[55]), flavonoids, flavonoidal glycosides (flavone type[56,57]) and iridoid glycosides.[58]

Analgesic activity

The effects on analgesic activity were tested using two different assay procedures, writhing test was used to evaluate peripheral analgesic activity and hot plate test to evaluate central analgesic activity.

The results of the phenyl-p-quinone writhing test given in Table 5 showed that the different alcohol fractions significantly ($P< 0.05$, $P< 0.01$) reduced the number of writhings and stretchings induced by the algogenic agent at the two doses assayed, the analgesic effect in this model being dose-related and the most active product was n-butanol fraction (400 mg/kg) with a protective effect (72.3%) that is more potent than that produced by ASA at a dose of 100 mg/kg (63.1%) used as reference drug. The results of hot-plate test presented in Table 6 showed that the polar fractions (ethyl acetate and n-butanol) as well as total aqueous extract exhibited central analgesic properties since they exerted a significant protective effect on thermal painful stimuli. Such stimuli are characteristic of central analgesics as morphine[37] while acetyl salicylic acid is known to be inactive on thermal stimuli. The non polar fractions (n-hexane, chloroform) as well as ursolic acid showed peripheral analgesic activity towards chemical painful stimuli caused by phenyl-p-quinone whereas they did not exhibit any central analgesic activity on heat-induced pain in mice, Table 6.

Hypothermia

From the results presented in Table 7, four hours after yeast injection, a significant hypothermia was recorded and remained stable throughout the test. Two hours after administration of the n-hexane fraction at doses of 200 and 400 mg/kg, a significant reduction of hyperthermia by 1.8 and 2.1° for 200 and 400 mg/kg as compared to hyperthermic rats.

The chloroform fraction and ursolic acid also showed an activity profile similar to the one obtained with n-hexane fraction with significant reduction of hyperthermia by 2.0, 2.4° for 200, 400 mg/kg respectively, 1.6 and 1.9° for pure ursolic acid (200 and 400 mg/kg).

The polar fractions of *Tecoma mollis* as well as the total aqueous extract displayed their maximum inhibitory effect 3 hours after oral administration with well marked hypothermic effect up to 0.9, 1.4° for ethyl acetate fraction (200, 400 mg/kg), 1.4, 1.8° for n-butanol fraction (200, 400 mg/kg) and 1.2, 1.7° for total aqueous extract (200 and 400 mg/kg) respectively, compared to non hyperthermic rats.

Activity on the central nervous system

i- Effects on spontaneous motor activity

Total aqueous extract as well as ethyl acetate and n-butanol fractions of *Tecoma mollis* caused significant reductions ($P< 0.05$, $P< 0.025$ and $P< 0.01$) on spontaneous motor activity especially at the dosages of 200 and 400 mg/kg with the dosage-effect relation. Increasing the dose leads to increasing in the CNS depression and decreasing in the movement count up to 47.3, 61.1 and 63.1% for 400 mg/kg of ethyl acetate, n-butanol and total aqueous

Table 4: Inhibitory effects of the different fractions of *Tecoma mollis* on yeast-induced edema in rats.

Groups	Dose mg/kg	Percentage of inhibition					
		½ hr	1 hr	2 hr	3 hr	5 hr	12 hr
Control	-	-	-	-	-	-	-
Indomethacin	8	37.3	41.1	44.8	59.4	53.8	55.4
n-Hexane	200	20.9	27.3	30.4	33.3	25.6	29.5
	400	32.6	38.3	39.4	43.5	35.8	40.2
Chloroform	200	21.2	29.1	34.7	32.2	30.76	26.4
	400	30.9	38.4	42.6	40.7	35.5	39.6
Ethyl acetate	200	27.2	39.6	45.7	48.3	43.0	42.1
	400	36.1	50.8	56.6	54.7	49.1	51.3
n-Butanol	200	29.2	41.5	49.3	50.6	44.5	39.0
	400	38.4	53.9	62.1	58.3	55.6	53.1
Aqueous	200	27.7	37.8	46.8	51.3	43.5	41.3
Extract	400	39.5	54.1	61.3	69.4	56.6	53.1
Ursolic acid	200	15.6	24.3	31.4	35.7	29.2	25.9
	400	25.8	33.8	36.2	42.6	35.4	32.6

Table 5: Analgesic effects of different fractions of *Tecoma mollis* and acetyl salicylic acid (ASA) on phenyl-p-quinone-induced writhing in mice.

Sample	Dose (mg/kg)	No. of writhings mean ± S.E., n= 6	% Protection
Control	-	141.3±1.04	-
ASA	100	52.2±0.96**	63.1
Ursolic acid	200	96.9±3.76*	31.4
	400	78.9±1.22**	44.1
n-Hexane	200	88.1±2.88*	37.6
	400	66.8±2.15**	52.7
Chloroform	200	91.5±3.54*	35.2
	400	68.5±0.86**	51.5
Ethyl acetate	200	72.2±1.06**	48.9
	400	43.6±2.53**	69.1
n-Butanol	200	66.1±1.70**	53.2
	400	39.1±2.26**	72.3
Aqueous extract	200	76.7±1.05**	45.7
	400	41.1±0.88**	70.9

Differences with respect to the control group were evaluated using the student's t-test (*P< 0.05, **P< 0.01).

Table 6: Antinociceptive effects of different fractions of *Tecoma mollis* and ASA on heat-induced pain in mice.

Groups	Dose mg/kg	Average reaction time (seconds) ± S.E., n = 6	P	S
Control	-	b 11.35±0.60 a 11.41±0.73	0.52	-
ASA	100	b 11.15±0.43 a 12.30±0.71	10.3	NS
n-Hexane	200	b 11.31±0.32 a 12.25±0.95	8.31	NS
	400	b 11.64±0.65 a 13.10±0.58	12.5	NS
Chloroform	200	b 11.51±0.41 a 11.94±0.53	3.74	NS
	400	b 11.25±0.23 a 12.31±0.67	9.42	NS
Ethyl acetate	200	b 11.42±0.52 a 14.95±0.35	30.96	0.05
	400	b 11.36±0.26 a 17.21±0.44	51.5	0.05
n-Butanol	200	b 11.22±0.68 a 15.55±0.36	38.59	0.05
	400	b 11.52±0.40 a 17.95±0.47	55.8	0.05
Aqueous ext.	200	b 10.96±0.83 a 15.05±0.42	37.3	0.05
	400	b 11.42±0.37 a 18.15±0.56	58.93	0.05
Ursolic acid	200	b 11.47±0.25 a 12.16±0.40	6.0	NS
	400	b 11.26±0.51 a 12.60±0.30	11.9	NS

P: Percentage of variation

b: Average reaction time before treatment; a: Average reaction time after treatment,

S: Degree of significance; NS: non-significant; control group receiving vehicle.

Table 7: Antipyretic effects of different fractions of *Tecoma mollis* on yeast-induced fever in rats.

Groups	Dose mg/kg	Average rectal temperature (°) ± S.E., n = 6				
		To	1 hr	2 hr	3 hr	5 hr
NHT	-	36.8±0.15	37.0±0.09	36.9±0.11	37.1±0.14	36.9±0.10
HT (2% Tween 80 in saline)	-	38.6±0.22**	38.8±0.14**	38.9±0.30**	38.9±0.25**	38.8±0.17**
Indomethacin	8	38.5±0.19	37.2±0.05**	36.7±0.16**	36.6±0.2**	37.0±0.07**
n-Hexane	200	38.5±0.08	37.5±0.19*	37.1±0.32**	37.3±0.17**	37.5±0.24*
	400	38.7±0.17	37.3±0.12**	36.8±0.09**	37.0±0.26**	37.2±0.08**
Chloroform	200	38.4±0.07	37.5±0.06*	36.9±0.11**	37.2±0.19**	37.4±0.15*
	400	38.5±0.2	37.2±0.09**	36.5±0.12**	36.8±0.15**	36.9±0.18**
Ethyl acetate	200	38.6±0.06	37.1±0.17**	36.6±0.09**	36.2±0.24**	36.6±0.16**
	400	38.5±0.22	36.9±0.32**	36.2±0.21**	35.7±0.18**	36.1±0.17**
n-Butanol	200	38.7±0.08	37.0±0.14**	36.3±0.19**	35.7±0.09**	36.1±0.13**
	400	38.4±0.11	36.7±0.13**	35.5±0.15**	35.3±0.17**	35.7±0.41**
Aqueous extract	200	38.5±0.15	37.1±0.23**	36.5±0.29**	35.9±0.23**	36.4±0.08**
	400	38.4±0.08	36.8±0.19**	36.0±0.13**	35.5±0.18**	35.8±0.27**
Ursolic acid	200	38.5±0.11	37.6±0.18*	37.3±0.25*	37.5±0.14*	37.5±0.09*
	400	38.5±0.13	37.3±0.16*	37.0±0.13**	37.2±0.08**	37.1±0.21**

To: Average rectal temperature just before plant extract or solvent injection.
NHT: Non-hyperthermic group receiving distilled water; HT: Hyperthermic group.
Differences with respect to the control group were evaluated using the student's t-test (*P< 0.05, **P< 0.01).

extract respectively being recorded 60 minutes after administration (Table 8). The results also showed that the administration of low dose (100 mg/kg) of ethyl acetate fraction gave a non-significant reduction on the spontaneous motor activity at least in the first fifty minutes after receiving the extract, while administration of 100 mg/kg of n-butanol and aqueous extract gave a significant reduction (P< 0.05) on spontaneous motor activity after twenty minutes with more reduction in the total movement count up to 22.8 and 26.3% for 100 mg/kg of n-butanol and total aqueous extracts. The results of experiment also showed that the total aqueous extract is more potent than that of both ethyl acetate and n-butanol fractions of alcoholic extract.

ii- Effects on rectal temperature

Total aqueous extract as well as ethyl acetate and n-butanol fractions of *Tecoma mollis* produced a significant dosage-dependent hypothermia on rectal temperature, Table 9. The maximum effect obtained 60 minutes after injection of the extracts and hypothermia was still observed up to 2 hours after administration.

Anticonvulsant activity

Blockages of pentylenetetrazole-induced convulsions in mice are characteristic effects of some CNS-depressant drugs.

Ethyl acetate fraction at a dose of 100 mg/kg displayed no significant protective effect against either clonic or tonic convulsions but delayed the lethal effects of convulsive agent. Ethyl acetate fraction at a dose of 200 mg/kg displayed significant delay of tonic, clonic convulsions and the death time, while at a dose of 400 mg/kg, it displayed significant delay of clonic convulsion with prevention of tonic and death at all (100%), Table 10.

The n-butanol fraction and total aqueous extract at doses 100 and 200 mg/kg displayed significant delay of both tonic, clonic convulsions and death time while the 400 mg/kg dose inhibited completely tonic convulsion and death by 100%.

Table 8: Effects of polar fractions of *Tecoma mollis* at different dosages on spontaneous motor activity of mice.

Group	Dose mg/kg	Steps records (mean ± S.E.), n = 6						Cumulative movement in 60 min	P
		10 min	20 min	30 min	40 min	50 min	60 min		
Control	-	96.7±4.8	101.3±5.0	103.7±8.3	97.1±5.8	98.5±7.6	97.6±4.2	590.9±17.9	-
Ethyl acetate	100	102.1±6.2	96.4±5.8	86.6±5.1	81.3±6.2	76.4±5.2	72.5±4.7*	515.3±16.1	12.8
	200	82.7±6.9	86.3±7.3	71.9±6.7*	65.4±5.8*	59.8±5.2**	52.8±4.3**	418.9±15.2	29.1
	400	73.2±6.0*	58.5±5.4**	53.4±4.9**	41.6±3.8***	46.1±4.3***	36.8±3.1***	311.6±13.8	47.3
n-Butanol	100	92.5±6.7	84.5±7.2	75.4±6.4*	68.1±5.3*	65.7±5.8*	69.4±4.6*	455.6±15.6	22.8
	200	77.6±5.7	72.1±6.5*	63.9±5.1**	47.5±4.3***	42.1±3.8***	44.9±4.1***	348.1±12.2	41.1
	400	60.1±5.2**	55.5±4.7**	33.6±3.1***	27.8±2.5***	22.3±2.1***	28.5±2.6***	229.8±10.3	61.1
Aqueous extract	100	87.3±6.9	81.6±7.1	72.5±5.7*	67.1±4.6*	66.8±5.1*	60.1±5.3**	435.4±14.4	26.3
	200	73.7±5.8*	68.9±4.4*	54.4±3.8**	50.3±4.7**	43.3±3.6***	40.8±3.3***	331.4±11.1	43.9
	400	63.4±4.2**	48.2±3.9***	40.3±3.5***	27.2±2.4***	16.5±1.3***	22.1±1.7***	217.7±9.8	63.1

Differences with respect to the control group were evaluated using Student's t-test (*P< 0.05, **P< 0.025, ***P< 0.01).

P: percentage of movement reduction.

Table 9: Hypothermic effects of polar fractions of *Tecoma mollis* on rectal temperature of rats.

Groups	Dose mg/kg	Average rectal temperature (°) ± S.E., n= 6				
		To min.	30 min.	60 min.	90 min.	120 min.
Control	-	37.5±0.14	37.7±0.08	37.5±0.19	37.6±0.21	37.6±0.13
Ethyl acetate	100	37.6±0.17	37.1±0.13*	36.7±0.28**	36.6±0.17**	37.0±0.09*
	200	37.5±0.23	36.5±0.27**	36.3±0.14**	36.16±0.16**	36.55±0.27**
	400	37.7±0.14	35.9±0.31**	35.46±0.30**	35.6±0.13**	36.26±0.19**
n-Butanol	100	37.5±0.15	36.8±0.19**	36.50±0.17**	36.56±0.32**	36.8±0.24**
	200	37.4±0.09	36.5±0.12**	35.9±0.26**	36.15±0.11**	36.2±0.21**
	400	37.5±0.16	35.9±0.14**	35.0±0.17**	35.25±0.25**	35.5±0.19**
Aqueous extract	100	37.7±0.10	36.9±0.09**	36.7±0.24**	36.5±0.19**	36.8±0.12**
	200	37.5±0.22	36.5±0.16**	36.0±0.33**	36.2±0.24**	36.2±0.26**
	400	37.6±0.13	36.0±0.24**	35.3±0.19**	35.5±0.23**	35.6±0.30**

To: Average rectal temperature just before plant extracts or solvent injection.
Significance differences with respect to the control group were evaluated by the student's t-test (*P< 0.05, **P< 0.01).

From the results listed in Table 10, it could be concluded that the polar fractions of alcohol extract and aqueous extract of *Tecoma mollis* produced a significant dosage-dependent anticonvulsant activity. At a dose of 200 mg/kg, they exhibited a significant anticonvulsant activity with closed potency to reference compound (sodium valproate) at the same dose. At higher dose (400 mg/kg), they exhibited too strong anticonvulsant activity with significant delay of clonic convulsion and complete inhibition of tonic convulsion and death by 100%.

Acute toxicity in mice

The LD_{50} of the different fractions of the leaves of *Tecoma mollis* (n-hexane, chloroform, ethyl acetate, n-butanol and the total aqueous extract) was determined according to the reported method.[59] The polar fractions as well as the total aqueous extract possessed toxicity as the LD_{50} reached 8.0 g/Kg. On the other hand, the non-polar fraction produced toxicity at 3.0 g/Kg.

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Table 10: Effects of different fractions of *Tecoma mollis* on pentylenetetrazole-induced convulsions and death.

Group	Dose mg/kg	Time of clonic convulsion (min) (mean ± S.E.), n= 6	Time of tonic convulsion (min) (mean ± S.E.), n= 6	Dead animals	Protected animals	% Protection	Time until death (min) (mean ±S.E.)
Control (Dist. Water)	-	1.28±0.11	2.99±0.10	6	0	0	3.38±0.21
Ethyl Acetate	100	2.09±0.25	4.07±0.24	4	2	33.3	9.25±0.19*
	200	3.11±0.31*	7.49±0.42*	2	4	66.6	17.38±0.5**
	400	4.1±0.36**	-	0	6	100	-
n-Butanol	100	2.8±0.27*	6.96±0.36**	3	3	50	13.82±0.5**
	200	4.0±0.22**	8.2±0.29**	2	4	66.6	18.54±0.33**
	400	5.56±0.31**	-	0	6	100	-
Aqueous Extract	100	2.32±0.11*	6.48±0.23**	3	3	50	14.19±0.40**
	200	3.36±0.28**	8.6±0.35**	2	4	66.6	19.27±0.28**
	400	5.3±0.19**	-	0	6	100	-
Sod. Valproate	200	3.4±0.26**	7.99±0.43**	2	4	66.6	19.75±0.53**

Each value represent the mean time until onset of convulsions or death, respectively.

Significanceof differences with respect to control was evaluated by the student's t-test (*P< 0.05, **P< 0.01).

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