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*RESEARCH ARTICLE*

# Chemical and Biological Assessment of *Tecoma X Smithii* Hort.

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***Chemical and Biological Assessment of Tecoma X Smithii Hort.***

***SUMMARY***

*Spectrophotometric quantitative determination of polyphenols, flavonoids, alkaloids, sterols, and carbohydrates of the leaves, flowers and fruits of Tecoma x smithii Will. Wats. was performed, as well as the lipoidal matter (unsaponifiable compounds and fatty acids methyl esters) that were investigated using gas chromatography-mass spectrometry (GC-MS). Representative aromatic acids where estimated for the three parts using HPLC. Polyphenols and flavonoids were most concentrated in the 70% ethanol extract of the leaves. The investigation of the ethyl acetate fraction of the 70% ethanol extract of the leaves resulted in isolation and identification of Chrysoeriol (E1), Luteolin (E2), and Caffeic acid (E3). Two other compounds were also isolated from the n-butanol fraction: Chlorogenic acid (B1) and Rutin (B2). The ethanolic and aqueous extracts of the leaves were evaluated for their acute toxicity, anti-inflammatory, antipyretic, analgesic, and anti-hyperglycemic activities. Both extracts were regarded safe and could significantly reduce the blood glucose level in alloxan- diabetic rats and showed a comparable antipyretic and analgesic effect to the reference standards used in the study.*

***Key Words:*** *Quercetin, sitosterol, Gallic acid, nonacosane, antidiabetic, GC-MS*

***Tecoma X Smithii Hort'un Kimyasal ve Biyolojik Değerlendirmesi***

***ÖZ***

*Tecoma x smithii Will. Wats. yaprak, çiçek ve meyvelerindeki polifenollerin, flavonoidlerin, alkaloitlerin, sterollerin ve karbonhidratların spektrofotometrik kantitatif tayini. gaz kromatografisi-kütle spektrometresi (GC-MS) kullanılarak araştırılan lipoidal maddenin yanı sıra (çözünmeyen bileşikler ve yağ asitleri metil esterleri) de yapıldı. Temsilci aromatik asitler, HPLC kullanan üç parça için tahmin edilir. Polifenoller ve flavonoidler en çok yaprakların% 70 etanol ekstraktında konsantre edildi. Yaprakların% 70 etanol ekstraktının etil asetat fraksiyonunun araştırılması, Chrysoeriol (El), Luteolin (E2) ve Kafeik asidin (E3) izolasyonu ve tanımlanmasına yol açtı. Diğer iki bileşik de n-bütanol fraksiyonundan izole edildi: Klorojenik asit (B1) ve Rutin (B2). Yaprakların etanolik ve sulu ekstreleri akut toksisite, antienflamatuar, antipiretik, analjezik ve anti-hiperglisemik aktiviteleri açısından değerlendirildi. Her iki ekstrakt da güvenli olarak kabul edildi ve alloksanabetik sıçanlarda kan glukoz seviyesini önemli ölçüde azaltabildi ve çalışmada kullanılan referans standartlarına karşılaştırılabilir bir antipiretik ve analjezik etki gösterdi.*

***Anahtar Kelimeler:*** *Kersetin, sitosterol, Gallik asit, nonacosane, antidiyabetik, GC-MS*

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## INTRODUCTION

*Tecoma* Juss. is a large genus within the family Bignoniaceae (Gentry, 1992) which has a history as a traditional folk medicine especially in South America (Kandakatla, Rao, & Banji, 2010), such as anti-inflammatory and anti-arthritic (Prajapati & Patel, 2015), antimicrobial (Adnan, Tariq, Akhtar, Ullah, & AbdElsalam, 2015; Agarwal & Chauhan, 2015) and antioxidant (Govindappa et al., 2011) activities. *Tecoma stans* L. was considered an important antidiabetic plant in Mexico (Hernandez-Galicia et al., 2002; Ramírez, Zavala, Pérez, & Zamilpa, 2012). Many chemical classes including alkaloids (Al-Azzawi, Al-Khateeb, Al-Sameraei, & Al-Juboori, 2012) flavonoids (Hashem, 2008) triterpenes (El-Emary, Kalifa, Backheet, & Abdel-Mageed, 2002) and iridoids (Abdel-Mageed, Backheet, Khalifa, Ibraheim, & Ross, 2011) were studied in *Tecoma*. *Tecoma x smithii* Will. Wats. is an upright shrub that emerged due to hybridization between *Tecoma mollis* Humb. & Bonpl. and *Tecomaria capensis* Lindl. (Bailey, Bailey, & Hortorium, 1976) Despite the availability of literature about the genus *Tecoma*, little was traced regarding *Tecoma x smithii* Will. Wats. The macro and micro-morphological characters, as well as, DNA fingerprinting were studied and illustrated (Abdrabou, Ezzat, El-Kashoury, & Taha, 2016), the volatile constituents obtained by hydrodistillation of the fresh leaves was investigated through GC-MS and were positively active as antioxidant and antimicrobial agent (Taha, El-kashoury, Ezzat, & Saleh, 2016). The target of the study was to assess some chemical properties of *Tecoma x smithii* Will. Wats. cultivated in Egypt, beside isolation and identification of characteristic constituents of the leaves and biological screening in order to explore the pharmacological properties.

## MATERIAL AND METHODS

### Plant material

Samples of the leaves, flowers, and fruits of *Tecoma x smithii* Will. Wats. were collected from the field belonging to the National Organization for Drug Control and Research (NODCAR), Egypt. The plant was kindly authenticated by Dr. Wafaa M. Amer, Professor of Flora, Botany Department, Faculty of Science, Cairo University. A voucher specimen (no. 4122012) is kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

### Preparation of the extracts

The air-dried powders of the leaves, flowers, and fruits were defatted using petroleum ether (60-80 °C) then exhaustively macerated in 70% ethanol. The concentrated alcoholic extracts were suspended in water and successively fractionated with chloroform, ethyl acetate and *n*-butanol saturated with water. All solvents were evaporated under reduced pressure to give different residues. Another amount of the air-dried powdered leaves were infused in boiling distilled water for 5 hr. The aqueous extract was then lyophilized and the residue was saved for biological investigation.

### Instrumentation

**UV spectrophotometric analysis:** UVD-2960-LABOMED Inc. USA. **GC analysis:** HP Agilent® GC-MS system were employed, comprising a 6890 gas chromatograph coupled with a 5973A Agilent® mass spectrometer. The column used for analysis of the unsaponifiable matter (Willenberg et al.); HP-5MS, (HP Agilent®, USA) (30 m x 0.25 mm, film thickness 0.25 µm). For analysis of fatty acids methyl esters (FAME), the column was 10% polyethylene glycol adipate on chromosorb W-AW (100-120 mesh) (1.5 m x 4 mm D). The oven temperature program for USM: Initial temperature, 70 °C, kept isothermal for 5 min, increased to 300 °C by the rate of 4 °C/min, then kept isothermal for 10 min, while that for FAME: Initial temperature, 70 °C increased to 190 °C by the rate of 8 °C/min, then kept isothermal for 25 min. The injection temperature was set at 250 °C, injection volume 1.0 µl, Inlet pressure: 37.1 kPa. The carrier gas: helium (1 ml/min.) Injection mode: split-less. MS interface temperature: 250°C; MS mode: EI; detector voltage: 70 eV; mass range: 50 - 550 m/z at 3.62/scan. Data handling was carried out by means of GC/MSD ChemStation software Agilent®. library searched data base Wiley7n.1 and wiley7Nist05.L. **HPLC analysis:** Hitachi LaChrom HPLC system were employed, equipped with a quaternary pump L2130 and a UV detector L2400 at 320 nm. The separation of components was achieved on a Phenomenex BDS RP-18 column, at 25 °C temperature, 20 µl injection volume, and 1ml/min flow rate. **For $H^1$NMR analysis:** Varian Mercury VX-300 NMR-spectrometer (Japan), 300 MHz spectra were recorded in DMSO using TMS as internal standard and chemical shift values expressed in δ ppm.

### Moisture and ash evaluation:

Determination of the moisture content, ash values, and crude fibres was carried out following the procedures of the Egyptian Pharmacopoeia (2005). Moisture determination was done by drying the sample at 105°C until constant weight in oven (Memert®, Germany), Ash values and Crude fibers were determined with the aid of a muffle furnace (Pyrolabo®, France) at 550 °C for 12 hr.

### Spectrophotometric determination of the total content of polyphenols, flavonoids, alkaloids, and sterols:

Powdered leaves, flowers, and fruits (2.5 g, each) were separately extracted, till exhaustion, with meth-

anol and different concentrations of ethanol (100, 95 and 70%). Stock solutions (50 mg % in ethanol) of each of these extracts were prepared. The total polyphenols content was calculated as Gallic acid equivalent (GAE) with reference to a pre-established Gallic acid standard calibration curve adopting the method of Köroglu, Hürkul, and Özbay (2012). The total content of flavonoids in the extracts was calculated as quercetin according to Eruygur, Yilmaz, and Üstün (2012). The total content of alkaloids was calculated as atropine equivalent according to Shamsa et al. (2008) procedure. The total content of unsaturated sterols in the methanol and absolute (100%) ethanol extracts was calculated as $\beta$-sitosterol equivalent (BSE) according to Daksha, Jaywant, Bhagyashree, and Subodh (2010).

**Spectrophotometric determination of carbohydrates content:**

The **total carbohydrates** were extracted; 1g powdered leaves, flowers, and fruits were defatted then refluxed with 50 ml of 6 M HCl, for 2.5 hr, adjusted to 100 ml with distilled water. While for extraction of **free sugars;** another 1g of each powder was defatted, refluxed with 80 % ethanol for 2 hr, evaporated and the residues were dissolved in distilled water to make 100 ml solutions. Carrez reagent was added to precipitate the impurities. The contents of total carbohydrates and free sugars in the extracts were calculated as fructose equivalent (FE) according to Chaplin and Kennedy (1994).

**Investigation of the lipoidal contents (Brian, Antony, Peter, & Austin, 1989; Eaton, 1989; Mendham, 2006):**

The petroleum ether extracts of the leaves, flowers, and fruits (1g, each) were separately saponified by reflux with in 10 ml 10% alcoholic potassium hydroxide and 4 ml toluene for 2 hours. Each extract was concentrated and exhaustively extracted with diethyl ether. Ether extracts were, separately, washed with distilled water, filtered over anh. Sodium sulfate, and evaporated to dryness yielding unsaponifiable matters USM. The aqueous mother liquors were acidified with 2.5% sulfuric acid to liberate the free fatty acids and then extracted with diethyl ether. The extracts were washed, dehydrated over anh. Sodium sulfate, and dried. Esterification of fatty acids was achieved by reflux for 90 min with a mixture of methanol/benzene/sulfuric acid (20:10:1 v/v). after cooling, mixtures were concentrated, diluted with excess water, and extracted with diethyl ether, which was washed with distilled water, filtered over anh. Sodium sulfate, and evaporated to dryness yielding fatty acids methyl esters FAME. The resulting USM and FAME were subjected to GC-MS analysis for components identification.

**Investigation of the free aromatic acids by HPLC:**

20 g of powdered leaves, flowers, and fruits were separately extracted with methanol till exhaustion. The extracts were filtered and evaporated and the residues were dissolved in methanol (HPLC grade) to make 10 mg/ml solutions. HPLC analysis was carried out according to the procedures of El-Hela, Luczkiewicz, and Cisowski (1999).

***Statistical analysis:*** All experiments were carried out in triplicates. The obtained data were expressed as Mean ± Standard deviation.

**Compounds isolation studies:**

Leaves extract showed fair content of polyphenols and flavonoids that encourage the attempts of polyphenol compounds isolation beside the abundance of availability of plant leaves, the 70 % alcoholic extract was fractionated to obtain the most polyphenols rich fractions; ethyl acetate and n-butanol.

**Investigation of the ethyl acetate fraction**

TLC investigation of the ethyl acetate fraction in $S_1$ (ethyl acetate-methanol-water-formic acid 100:16.5:13.5:0.2 v/v) revealed the presence of at least five constituents as detected by the number of spots. 10 g of the extract was fractionated on a vacuum liquid chromatography (VLC) (300 g, 45 cm x 5 cm D, silica stationary phase). Gradient elution was carried out with chloroform and the polarity is increased by 5 % increments of ethyl acetate till 100 %, followed by 5 % methanol. Fractions (250 ml, each) were collected, monitored by TLC ($S_1$) and pooled to give two main fractions (**Fractions I & II**).

**Fraction I**: (330 mg, eluted with 20-30 % ethyl acetate in chloroform) showed two major spots, $R_f$ values 0.89 and 0.83 in $S_1$. This fraction was subjected to repeated purification on sephadex LH-20 columns to give two **subfractions (A & B)**. **Subfraction A** showed one spot, on evaporated to dryness yielded compound $\mathbf{E_1}$ (52 mg, $R_f$ = 0.89, $S_1$) as yellow crystals. **Subfraction B**, on further purification on preparative silica gel plates using chloroform-ethyl acetate-methanol (60:30:10 v/v), it gave compound $\mathbf{E_2}$ as yellow crystals (45 mg, $R_f$ = 0.83, $S_1$).

**Fraction II**: (115 mg, eluted with 5-10 % methanol in ethyl acetate) showed one major spot, $R_f$ = 0.77 in S1.This fraction was then re-purified on sephadex LH-20 and reversed phase silica gel (RP-18) columns to yield yellowish white crystals of compound $\mathbf{E_3}$ (35 mg).

**Investigation of the n-butanol fraction**

TLC examination of the *n*-butanol fraction ($S_2$: ethyl acetate-formic acid-glacial acetic acid-water 100:11:11:26 v/v) showed five spots, four of which ap-

pearing major as shown by the intensity of the color response to the spray reagents. Ten grams of this fraction was fractionated on a vacuum liquid chromatography (VLC) (300 g, 45 cm x 5 cm D, silica stationary phase). Gradient elution was performed starting with chloroform and increasing the polarity by 10 % increments of ethyl acetate till 100 %, followed by 5 % methanol till 100 %. Fractions (250 ml, each) were collected, monitored by TLC ($S_2$, $AlCl_3$ spray reagent) and pooled to give two main fractions (**Fractions I & II**).

**Fraction I** (250 mg, eluted with 10-20% methanol in ethyl acetate) showed one major spot, $R_f$ value 0.58 in $S_2$. This fraction was further purified on sephadex LH-20 column to yield a white amorphous powder; compound $\mathbf{B_1}$ (47 mg).

**Fraction II:** (270 mg, eluted with 35-60 % methanol in ethyl acetate) revealed one major spot accompanied by other minor constituents. This fraction was further purified using sephadex LH-20 and reversed phase silica gel (RP-18) columns to yield a yellow amorphous powder of compound $\mathbf{B_2}$ (83 mg, $R_f$ = 0.51, $S_2$).

**Biological Evaluation**

The 70% ethanolic and the aqueous extracts of the leaves were dissolved in bi-distilled water by the aid of few drops of Tween 80 for the evaluation of the following biological activities. The procedures followed were in accordance with animal rights as per Guide for the Care and Use of Laboratory Animals (Council, 2010).

**Acute toxicity studies (Determination of $LD_{50}$):** The determination of $LD_{50}$ was performed by oral treatment of male albino mice (25-30 g) adopting the method described by Karber (1931)

**Evaluation of the acute anti-inflammatory activity:** The acute anti-inflammatory activity was evaluated as compared to indomethacin (20 mg/kg b.wt.) by the carrageenan-induced rat paw oedema test as described by Winter, Risley, and Nuss (1962).

**Evaluation of the antipyretic activity:** The antipyretic activity was carried out following the procedure of yeast-induced pyrexia of Tomazetti et al. (2005) and using paracetamol, a reference antipyretic, as a positive control.

**Evaluation of the analgesic activity:** The analgesic effect was carried out following the procedure of the acetic acid-induced writhing in mice method according to Koster (1959) against standard analgesic indomethacin.

**Evaluation of the anti-hyperglycemic activity:** Diabetes was induced in male albino rats by *i.p.* injection of alloxan, as described by Eliasson and Samet (1969). The tested samples and the standard anti-hyperglycemic drug, metformin, were administered orally, followed by the collection of the blood samples according to Trinder (1969) after 2 and 4 weeks intervals from the administration of the tested samples.

## RESULTS AND DISCUSSION

**Moisture and ash evaluation**

The flowers are the most humid organ (contains highest moisture percentage) of the three tested plant organs followed by the leaves (13.6 and 10.3, respectively). On the other hand, leaves powder yield more ash and shows highest crude fibers content than powders of flowers and fruits (Table 1).

**Table 1.** The pharmacopoeial constants of the leaves, flowers, and fruits of *Tecoma x smithii Will.* Wats.

| Parameter | Percentage | | |
|---|---|---|---|
| | Leaves | Flowers | Fruits |
| Moisture content | 10.3±0.13 | 13.6±0.24 | 6.4±0.21 |
| Total ash | 9.2±0.26 | 6.5±0.22 | 4.4±0.21 |
| Acid insoluble ash | 1.7±0.16 | 1.0±0.11 | 1.4±0.17 |
| Water soluble ash | 5.6±0.27 | 3.3±0.13 | 2.2±0.11 |
| Crude fibers | 4.8±0.26 | 2.6±0.08 | 3.5±0.27 |

**Spectrophotometric determination of the total content of polyphenols, flavonoids, alkaloids, and sterols:**

Both polyphenols and flavonoids were most concentrated in the 70% ethanol extract of the leaves, while the alkaloids and sterols were mainly extracted by methanol and absolute ethanol from the leaves, respectively (Table 2).

**Table 2.** The total polyphenols, flavonoids, alkaloids, steroids contents of the leaves, flowers, and fruits of *Tecoma x smithii* Will. Wats.

| | Extract | Total polyphenols (GAE) (mg/100 g dry weight) | Total flavonoids (QE) (mg/100 g dry weight) | Total alkaloids (mg/100 g dry weight) | Total steroids (BSE) (g/100 g dry weight) |
|---|---|---|---|---|---|
| Leaves | Methanol | 216±1.07 | 56.9±0.23 | 27±0.25 | 2.73±0.04 |
| | Absolute ethanol | 149.3±0.75 | 23.9±0.12 | 25.7±0.12 | 3.15±0.02 |
| | Ethanol 95% | 102±1.09 | 24.4±0.23 | 16.2±0.21 | |
| | Ethanol 70% | 250±0.91 | 93±0.33 | 8.1±0.05 | |
| Flowers | Methanol | 105.5±0.76 | 43±0.13 | 13.3±0.1 | 0.21±0.0001 |
| | Absolute ethanol | 13.1±0.21 | 6.2±0.26 | 12±0.06 | 0.71±0.0003 |
| | Ethanol 95% | 19.5±0.38 | 10.3±0.17 | 10.2±0.08 | |
| | Ethanol 70% | 61.5±0.34 | 30.6±0.33 | 5.9±0.02 | |
| Fruits | Methanol | 37±0.29 | 6.2±0.09 | 4.4±0.006 | 0.74±0.002 |
| | Absolute ethanol | 8±0.12 | 3.1±0.09 | 3.9±0.01 | 1.89±0.005 |
| | Ethanol 95% | 36±0.26 | 7.5±0.12 | 1.8±0.001 | |
| | Ethanol 70% | 42.8±0.38 | 11.4±0.17 | 1.1±0.004 | |

BSE: β-sitosterol equivalent, GAE: Gallic acid equivalent, QE:quercetin equivalent.

**Spectrophotometric determination of the total content of carbohydrates:**

The total carbohydrates (calculated as fructose equivalent FE) were 300± 4.1, 400± 3.5, and 220± 2.1 mg/100 g dry plant, while the free sugars content were 180± 2.2, 320± 1.2, and 170± 1.7 mg/ 100 g dry plant, for the leaves, flowers, and fruits, respectively. The flowers contain a relatively higher sugar content which agrees with an anatomical finding that the flower calyx frequently bears conspicuous glands that secrete sugar to attract ants and bees (Lohmann & Taylor, 2014).

**GC-MS analysis of the lipoidal matters (petroleum ether extracts)**

Nonacosane was the major hydrocarbon both in the leaves and fruits (11.7 and 36-24% respectively), while 1-octadecene (8.18%) was dominant in the flowers. *β*-Sitosterol was the major steroidal compound (13.72, 17.86 and 11.41 in the leaves, flowers and fruits, respectively) followed by stigmasterol. Squalene, *β*-amyrin, and moretenol were detected in the leaves while *α*-amyrin was detected only in the flowers (Table 3)

**Table 3.** The components identified by GC-MS in the unsaponifiable matter of the leaves (L), flowers (Fl) and fruits (Fr) of *Tecoma x smithii* Will. Wats.

| Identified compounds | $RR_t$ | RI | $M^+$ | Bp | Relative percentage | | |
|---|---|---|---|---|---|---|---|
| | | | | | L | Fl | Fr |
| • 1-Hexadecene | 0.52 | 1556 | 224 | 83 | 0.35 | 1.47 | 0.11 |
| • 5-Phenyl-undecane | 0.54 | 1631 | 232 | 91 | - | 3.35 | - |
| • 3-Phenyl-undecane | 0.55 | 1670 | 232 | 91 | - | 1.5 | - |
| • 2-Phenyl-undecane | 0.57 | 1715 | 232 | 105 | - | 2.27 | - |
| • 3-Phenyl-dodecane | 0.60 | 1767 | 246 | 91 | - | 1.8 | - |
| • 1-Octadecene | 0.61 | 1795 | 252 | 57 | 2.08 | 8.18 | 0.25 |
| • 2-Phenyl-dodecane | 0.62 | 1813 | 246 | 105 | - | 1.28 | - |
| • 6-Phenyl-tridecane | 0.62 | 1824 | 260 | 91 | - | 2.32 | - |
| • Neophytadiene | 0.63 | 1850 | 278 | 68 | 1.16 | - | - |
| • 5-Phenyl-tridecane | 0.63 | 1865 | 260 | 91 | - | 1.52 | - |
| • 6,10,14-Trimethyl-2-pentadecanone | 0.63 | 1871 | 268 | 43 | 2.02 | 2.8 | 0.16 |
| • 1-Hexadecyne | 0.64 | 1887 | 222 | 81 | 1.83 | - | - |
| • 3-Phenyl-tridecane | 0.64 | 1890 | 260 | 91 | - | 0.75 | - |
| • 6,6-Dimethyl-cyclooct-4-enone | 0.65 | 1907 | 152 | 82 | 1.23 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| • 2-Phenyl-tridecane | 0.66 | 1916 | 260 | 105 | - | 1.28 | - |
| • 4-Methyl-1,6-heptadien-4-ol | 0.68 | 2002 | 126 | 85 | 1.47 | - | - |
| • 5-Eicosene | 0.69 | 2093 | 280 | 55 | 6.15 | 5.8 | 0.31 |
| • *γ*-Undecalactone | 0.72 | 2130 | 128 | 85 | 16.6 | - | - |
| • Heptadecane | 0.73 | 2203 | 240 | 57 | 0.66 | 0.04 | 0.07 |
| • Cyclohexadecane | 0.74 | 2258 | 224 | 55 | 0.28 | - | - |
| • 1-Nonadecene | 0.75 | 2270 | 266 | 97 | - | 0.34 | 0.21 |
| • Nonadecane | 0.77 | 2297 | 268 | 57 | 3.27 | - | 7.12 |
| • Eicosane | 0.79 | 2313 | 283 | 57 | - | 4.8 | 1.04 |
| • *n*-Tricosane | 0.80 | 2341 | 324 | 57 | 0.8 | - | - |
| • 1-Eicosanol | 0.80 | 2341 | 280 | 57 | - | 0.46 | - |
| • 4,8,12,16-Tetramethylheptadecan-4-olide | 0.82 | 2397 | 324 | 99 | 0.5 | - | - |
| • Cyclotetracosane | 0.83 | 2425 | 336 | 55 | - | 4.11 | - |
| • 1-Docosene | 0.83 | 2425 | 308 | 57 | 0.43 | - | - |
| • Tetracosane | 0.84 | 2453 | 338 | 57 | 0.1 | - | 1.6 |
| • 1-Tricosene | 0.85 | 2480 | 322 | 57 | - | 0.66 | - |
| • Pentacosane | 0.86 | 2508 | 352 | 57 | - | 4.41 | 2.71 |
| • *Cis*-9-Tricosene | 0.86 | 2508 | 322 | 83 | - | 0.43 | - |
| • Hexacosane | 0.89 | 2592 | 366 | 57 | 0.64 | - | 2.97 |
| • 14-*β*-Pregnane | 0.91 | 2648 | 570 | 57 | 0.32 | - | 0.87 |
| • Heptacosane | 0.92 | 2676 | 380 | 57 | 1.52 | 3.28 | 8.77 |
| • Octacosane | 0.95 | 2760 | 394 | 57 | 1.61 | 0.46 | 7.53 |
| • Squalene | 0.97 | 2816 | 410 | 69 | 0.42 | - | - |
| • Nonacosane | 1.00 | 2940 | 408 | 57 | 11.7 | 2.06 | 36.24 |
| • Triacontane | 1.04 | 3011 | 422 | 57 | 3.84 | - | 6.04 |
| • Ergosta-4,6,22-trien-3-*β*-ol | 1.08 | 3027 | 396 | 396 | 0.3 | - | - |
| • 1-Hexacosanol | 1.11 | 3035 | 382 | 57 | 0.11 | - | - |
| • Lanol (3-*β*-cholest-5-en-3-ol) | 1.12 | 3075 | 386 | 583 | 1.37 | - | - |
| • 1-Bromo-triacontane | 1.15 | 3270 | 500 | 57 | 1.2 | - | - |
| • Cholestan-3-one, 4,4-dimethyl-cyclic-1,2-ethanediyl acetal, (5.alpha.) | 1.17 | 3304 | 458 | 99 | 2.51 | - | - |
| • 23S-Methylcholesterol | 1.20 | 3316 | 655 | 400 | 0.77 | 3.65 | 1.91 |
| • 3-*β*-4-*α*-5-*α*-4-Methyl-Cholest-7-en-3-ol | 1.21 | 3325 | 400 | 400 | 0.52 | - | - |
| • Stigmasterol | 1.22 | 3337 | 412 | 55 | 1.14 | 4.27 | 1.64 |
| • *β*- Sitosterol | 1.30 | 3376 | 414 | 414 | 13.72 | 17.86 | 11.41 |
| • *α*-Amyrin | 1.35 | 3385 | 426 | 218 | | 2.46 | - |
| • *β*-Amyrin | 1.36 | 3408 | 426 | 218 | 0.75 | - | - |
| • Moretenol (*α*-Neogammacer-22(29)-en-3-*β*-ol) | 1.47 | 3422 | 426 | 189 | 1.58 | - | - |
| **Total steroids and triterpenes** | | | | | 22.98 | 28.24 | 15.83 |
| **Total oxygenated non-steroidal compounds** | | | | | 21.93 | 3.26 | 0.16 |
| **Total hydrocarbons** | | | | | 38.04 | 52.11 | 74.97 |
| **Total identified compounds** | | | | | 82.95 | 83.61 | 90.96 |

$RR_t$: Relative retention time to Nonacosane ($R_t$: 30.51), $M^+$: molecular ion peak, Bp: base peak

Palmitic acid was the major saturated fatty acid (40.22 and 28.46 and 28.34%) in the leaves, flowers, and fruits, respectively. The major unsaturated fatty acid in the leaves was 11,14-eicosadienoic acid (19.81%, identified only in leaves) and in the fruits was linolenic acid (37.05%). While in the flowers, linoleic acid (7.14%) was the main unsaturated fatty acid (Table 4).

**Table 4.** The fatty acids identified by GC-MS analysis of the FAME of the leaves (L), flowers (Fl) and fruits (Fr) of *Tecoma x smithii* Will. Wats.

| Fatty acid corresponding to the identified FAME | $RR_t$ | RI | $M^+$ | BP | Relative percentage | | |
|---|---|---|---|---|---|---|---|
| | | | | | L | Fl | Fr |
| • Capric acid | 0.57 | 1380 | 186 | 74 | - | 0.42 | - |
| • 9-Oxo-Nonanoic acid | 0.66 | 1461 | 172 | 74 | - | 0.4 | - |
| • Octanedioic acid | 0.68 | 1529 | 171 | 138 | - | 0.23 | - |
| • Lauric acid | 0.73 | 1580 | 214 | 74 | - | 3.37 | 0.91 |
| • Nonanedioic acid | 0.75 | 1680 | 209 | 152 | - | 0.91 | - |
| • Decanedioic acid | 0.82 | 1728 | 199 | 55 | - | 0.18 | - |
| • Myristic acid | 0.88 | 1780 | 242 | 74 | 1.41 | 18.32 | 1.08 |
| • Pentadecanoic acid | 0.92 | 1878 | 256 | 74 | - | - | 0.24 |
| • Palmitic acid | 1.00 | 1973 | 270 | 74 | 40.22 | 28.46 | 28.34 |
| • Linoleic acid* | 1.10 | 1994 | 294 | 67 | 8.52 | 7.52 | 1.88 |
| • Stearic acid | 1.13 | 2032 | 298 | 74 | 6.45 | 9.85 | 8.43 |
| • Magaric acid (*n*-Heptadecanoic acid) | 1.15 | 2080 | 284 | 74 | 4.27 | - | 0.04 |
| • Nonadecanoic acid | 1.16 | 2122 | 312 | 74 | - | 0.44 | - |
| • Linolenic acid* | 1.17 | 2199 | 292 | 79 | 16.07 | 0.75 | 37.05 |
| • 11-Eicosenoic acid* | 1.20 | 2294 | 324 | 55 | - | 1.06 | 0.18 |
| • 11,14-Eicosadienoic acid* | 1.20 | 2316 | 322 | 67 | 19.81 | - | - |
| • Arachidic acid | 1.21 | 2359 | 326 | 74 | 0.55 | 4.48 | 3.14 |
| • Heneicosanoic acid | 1.27 | 2463 | 340 | 74 | 0.15 | - | 0.39 |
| • Behenic acid (Docosanoic acid) | 1.31 | 2567 | 354 | 74 | - | 3.96 | 2.44 |
| • Tricosanoic acid | 1.35 | 2668 | 368 | 74 | 0.18 | 0.78 | 0.56 |
| • 2-Hydroxy-docosanoic acid | 1.36 | 2706 | 370 | 57 | - | 0.73 | - |
| • Lignoceric acid (Tetracosanoic acid) | 1.39 | 2760 | 382 | 74 | 1.82 | 3.5 | 1.82 |
| • Pentacosanoic acid | 1.44 | 2773 | 396 | 74 | - | 0.78 | 0.51 |
| • Cerotic acid (Hexacosanoic acid) | 1.51 | 2934 | 410 | 74 | 0.09 | 1.95 | 1.03 |
| • Montanic acid (Octacosanoic acid) | 1.67 | 3161 | 438 | 74 | - | 1.3 | 0.68 |
| • Melissic acid | 1.94 | 3360 | 466 | 74 | - | 0.88 | 0.23 |
| Total saturated FA | | | | | 55.14 | 80.93 | 49.84 |
| Total unsaturated FA (*) | | | | | 44.40 | 9.34 | 39.11 |
| Total identified compounds | | | | | 99.54 | 90.27 | 88.95 |

$RR_t$: Relative retention time to palmitic acid methyl ester ($R_t$: 20.53), RI: retention index, $M^+$: molecular ion peak, Bp: base peak, FAME: fatty acid methyl ester

**Identification and quantification of the free aromatic acids by HPLC:**

Gallic, vanillic, and caffeic acids were detected in all investigated plant organs. Chlorogenic acid prevailed the detected acids in case of the leaves (49.5±0.08 mg/100 g), while cinnamic acid prevailed in the flowers and fruits (15.7±0.02 and 9.7±0.03 mg/100 g, respectively), as listed in table (5).

**Table 5.** The free aromatic acids identified in the leaves, flowers, and fruits of *Tecoma x smithii* Will. Wats. by HPLC.

| Name of standard | $R_t$ | Concentration (mg/100 g dry weight) | | | Calibration equation | LOD |
|---|---|---|---|---|---|---|
| | | leaves | flowers | fruits | $r^2$ | LOQ |
| Gallic acid | 3.617 | 5.2±0.09 | 6.1±0.04 | 6.5±0.02 | y = 3245 x + 32609 | 0.001 |
| | | | | | $r^2$ = 0.998 | 0.003 |
| Cinnamic acid | 4.6 | - | 15.7±0.02 | 9.7±0.03 | y = 939.76x - 17364 | 0.0013 |
| | | | | | $r^2$ = 0.9991 | 0.004 |
| Vanillic acid | 5.037 | 11.9±0.08 | 10.7±0.04 | 1.6±0.01 | y = 65522x - 3E+06 | 0.007 |
| | | | | | $r^2$ = 0.9921 | 0.02 |
| Chlorogenic acid | 5.953 | 49.5±0.08 | - | - | y = 62319x - 2E+06 | 0.005 |
| | | | | | $r^2$ = 0.9964 | 0.015 |
| Caffeic acid | 11.82 | 17.2±0.07 | 2.7±0.01 | 1.7±0.03 | y = 190440x - 62730 | 0.004 |
| | | | | | $r^2$ = 0.999 | 0.012 |
| Ferulic acid | 18.01 | | Traces | | y = 271250x + 38770 | 0.003 |
| | | | $r^2$ = 0.999 | | | |
| | | | | 0.009 | | |

**Identification of the isolated compounds** (**$E_1$**, **$E_2$**, **$E_3$**, **$B_1$**, and **$B_2$**)

This was achieved by analysis of their physicochemical properties and chromatographic profiles as well as their spectral data.

**Compound $E_1$:** Yellow crystals, m.p.: 320-322 $^0$C, 50 mg, soluble in methanol. $R_f$ 0.89, $S_1$. **UV $\lambda_{max}$ nm**: MeOH 239, 268, 339 (flavone), NaOMe 235, 275, 400 (free OH on ring A and B), $AlCl_3$ 232, 276, 297 (sh), 353, 384 (free OH at C-5), $AlCl_3$/HCl 234, 276, 300 (sh), 356, 382 (free OH at C-5 and no *ortho* dihydroxy at ring B), $CH_3COONa$ 247, 274, 359 (free OH at C-7 and C-4`), $CH_3COONa/H_3BO_3$ 241, 270, 340 (no *ortho* OH). **$^1$H-NMR**: δ ppm (300 MHz, DMSO) 12.94 (br. s, OH), 7.90 (1H, dd, J= 8.7, 1.8 Hz, H-6`), 7.53 (1H, s, H-2`), 6.93 (1H, d, J= 8.7 Hz, H-5`), 6.86 (1H, s, H-3), 6.50 (1H, d, J= 1.8 Hz, H-8), 6.19 (1H, d, J= 1.8 Hz, H-6), 3.88 (3H, s, -OCH3). The data complies with published data (Kang et al., 2010) for Chrysoeriol (luteolin-3`-methyl ether).

**Compound $E_2$:** Yellow crystals, m.p.: 328-329 $^0$C, 40 mg, soluble in methanol. $R_f$ 0.83, $S_1$. **UV $\lambda_{max}$ nm**: MeOH 234, 260, 300 (sh), 349 (flavone), NaOMe 233, 269, 404 (free OH on ring A and B), $AlCl_3$ 232, 271, 300 (sh), 407 (free OH on ring A and B), $AlCl_3$/HCl 234, 267, 296 (sh), 355 (free OH at C-5 and *ortho* dihydroxy at ring B), $CH_3COONa$ 234, 266, 355 (free OH at C-7 and C-4`), $CH_3COONa/H_3BO_3$ 232, 264, 360 (*ortho* dihydroxy at ring B). **$^1$H-NMR**: δ ppm (300 MHz, DMSO) 7.38 (2H, m, H-2` and H-6`), 6.86 (1H, d, J= 8.7 Hz, H-5`), 6.61 (1H, d, J= 1.2 Hz, H-8), 6.42 (1H, d, J= 1.2 Hz, H-6), 6.14 (1H, s, H-3). By comparison with published data (Hashem, 2008), compound $E_2$ could be identified as Luteolin.

**Compound $E_3$:** Yellow white crystals, m.p.: 213-216 $^0$C, 50 mg, soluble in methanol. $R_f$ 0.77, $S_1$. **UV $\lambda_{max}$ nm** (MeOH) 232, 288 (sh), 325 (a phenolic acid (Dürüst, Özden, Umur, Dürüst, & Küçükİslamoğlu, 2001)). **$^1$H-NMR**: δ ppm (300 MHz, DMSO) 7.41 (1H, d, J=15.6 Hz, H-7), 7.05 (1H, s, H-2), 6.95 (1H, d, J=8.4 Hz, H-5), 6.75 (1H, dd, J=7.8 & 1.5 Hz, H-6), 6.16 (1H, d, J=15.9 Hz, H-8). The identity of this compound as Caffeic acid was confirmed.

**Compound $B_1$:** White amorphous powder, 50 mg, soluble in methanol. $R_f$ 0.58, $S_2$. **UV $\lambda_{max}$ nm** (MeOH) 217, 240 (sh), 324. (a phenolic acid (Dürüst et al., 2001)). **$^1$H-NMR**: δ ppm (300 MHz, DMSO) 12.37 (1H, s, COO-H), 9.53 (1H, s, C3`-OH), 9.10 (1H, s, C4`-OH), 7.42 (1H, d, J=15.9 Hz, H-7`), 7.03 (1H, s, H-2`), 6.97 (1H, d, J=8.4 Hz, H-6`), 6.76 (1H, d, J=8.1 Hz, H-5`), 6.14 (1H, d, J=15.9 Hz, H-8`), 5.07 (1H, q, J=6.2, H-3), 3.92 (1H, s, H-4), 3.56 (1H, s, H-5), 3.30 (3H, s, alc-OH), 2.50 (2H, m, H-2), 1.96 (2H, m, H-6). By comparison with published data (Zhu, Dong, Wang, Ju, & Luo, 2005), this compound was identified as 3-caffeoylquinic acid (Chlorogenic acid).

**Compound $B_2$**: Yellow amorphous powder, 90 mg, soluble in methanol. $R_f$ 0.51, $S_2$. **UV $\lambda_{max}$ nm**: MeOH 257, 300 (sh), 356 (flavonol), NaOMe 281, 345 (sh), 412 (free OH on ring A & B), $AlCl_3$ 270, 306 (sh), 426 (free OH on ring A & B), $AlCl_3$/HCl 268, 298 (sh), 321, 400 (free OH at 5 & *ortho* OH at ring B), $CH_3COONa$ 271, 300 (sh), 382 (free OH at 7 & *ortho* OH at ring B), $CH_3COONa/H_3BO_3$ 268, 298 (sh), 375 (*ortho* OH at ring B). **$^1$H-NMR**: δ ppm (300 MHz, DMSO) *Aglycone: 12.59 (br.s, OH), 7.54 (1H, dd, J= 8.4, 1.2 Hz, H-6`), 7.39 (1H, br.s, H-2`), 6.83 (1H, d, J= 8.4 Hz, H-5`), 6.37 (1H, d, J= 1.8 Hz, H-8), 6.18 (1H, d, J= 1.8 Hz, H-6). *Sugar: 5.33 (1H, d, J= 7.2 Hz, H-1``), 4.38 (1H, br.s, H-1```), 0.99 (3H, d, J = 6 Hz, Me-6```). By comparison with published data (Ibrahim, Mohamadin, & Hamaad, 2004), this compound could be identified as quercetin-3-O-rhamnoglucoside (Rutin).

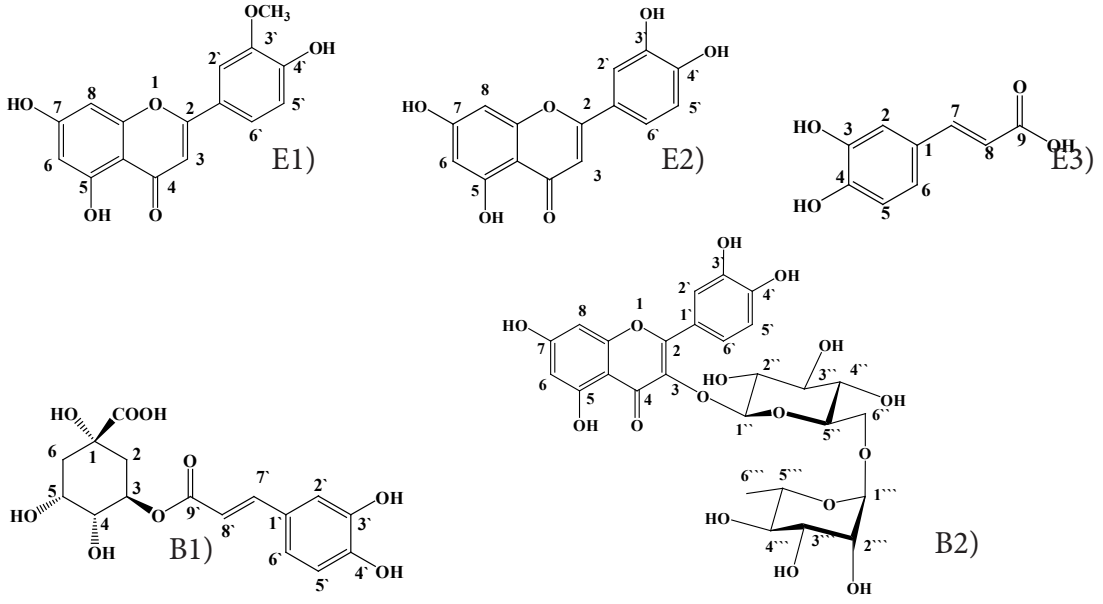


**Figure 1.** Structure formulae of the isolated compounds;
E1) Chrysoeriol, E2) Luteolin, E3) Caffeic acid, B1) Chlorogenic acid, B2) Rutin.

**Biological evaluation**

Both aqueous and ethanolic extracts are safe and non-toxic under the experimental condition with $LD_{50}$ up to 5.3 and 5.8 g/kg body weight, respectively. Extracts are thus considered to be safe in the range of the administered doses (Osweiler, Carson, Buck, & Van Gelder, 1985). The anti-inflammatory activity (table 6) estimated for both extracts exhibited a significant inhibition of the induced rat paw oedema. The ethanolic extract caused more oedema inhibition (59.77 %) than the aqueous extract (54.37 %).

**Table 6.** The acute anti-inflammatory activity of the aqueous and 70% ethanolic extracts of the leaves of *Tecoma x smithii* Will. Wats. *versus* indomethacin in male albino rats.

| Group | Dose (mg/kg b.wt) | % Oedema | | Potency *** |
|---|---|---|---|---|
| | | Mean ± S.E. | % of change ** | |
| Control | I ml saline | 62.9 ± 1.4 | - | - |
| Aq. extract | 100 | 28.7 ± 0.8* | 54.37 | 0.83 |
| 70% Eth. extract | 100 | 25.3 ± 0.6 * | 59.78 | 0.91 |
| Indomethacin | 20 | 21.6 ± 0.3 * | 65.66 | 1.00 |

* Statistically significantly different from control group at $p < 0.01$

** Percentage of change calculated as compared to the control

***Potency calculated as compared to the standard anti-inflammatory drug (indomethacin)

S.E. = standard error

The ethanol extract showed a significant antipyretic activity against yeast induced hyperthermia in rates and the effect was more pronounced after two hours, compared to paracetamol as reference drug (table 7).

**Table 7.** The antipyretic activity of the aqueous and 70% ethanolic extracts of the leaves of *Tecoma x smithii* Will. Wats. *versus* paracetamol in male albino rats.

| Group | Dose (mg/kg b.wt.) | Induced rise in temp. | Body temperature change | | | | Potency *** |
|---|---|---|---|---|---|---|---|
| | | | One hour | | Two hours | | |
| | | | Mean ± S.E. | % of change ** | Mean ± S.E. | % of change ** | |
| Control | 1 ml Saline | 38.6 ± 0.34 | 38.8 ± 0.2 | --- | 38.7 ± 0.2 | --- | --- |
| Aq. extract | 100 | 38.9 ± 0.4 | 38.6 ± 0.3* | 0.77 | 38.1 ± 0.2* | 2.06 | 0.37 |
| 70% Eth. extract | 100 | 39.3 ± 0.5 | 38.5 ± 0.2* | 2.04 | 37.1 ± 0.1* | 5.60 | 1.00 |
| Paracetamol | 20 | 39.4 ± 0.5 | 38.2 ± 0.2* | 3.05 | 37.2 ± 0.1* | 5.58 | 1.00 |

* Statistically significantly different from zero time at $p < 0.01$.
** Percentage of change calculated with reference to control (induced hyperthermia without treatment).
***Potency calculated as compared to the standard antipyretic drug (paracetamol).
S.E. = standard error

Both the aqueous and ethanolic extracts could significantly reduce the abdominal constrictions supporting the effective analgesic activity (table 8).

**Table 8.** The analgesic effect of the aqueous and 70% ethanolic extracts of the leaves of *Tecoma x smithii* Will. Wats. *versus* indomethacin in albino mice.

| Group | The dose (mg/kg b. wt). | Number of abdominal constrictions Mean ± S.E. | % of inhibition** | Potency*** |
|---|---|---|---|---|
| Control | 1 ml saline | 43.2 ± 1.3 | 0.00 | 0.00 |
| Aq. extract | 100 | 21.3 ± 0.4* | 50.69 | 0.83 |
| 70% Eth. extract | 100 | 19.2 ± 0.5* | 55.56 | 0.91 |
| Indomethacin | 20 | 16.7±0.3* | 61.34 | 1.00 |

* Statistically significantly different from control group at $p < 0.01$
**Percentage of inhibition: as compared to the control.
***Potency calculated as compared to the standard analgesic drug (indomethacin).
S.E. = standard error

Evaluation of the anti-hyperglycemic effect revealed a significant reduction (ascending with time) in blood glucose level in alloxan- diabetic rats, the ethanolic extract showed a higher activity than the aqueous extract (table 8).

**Table 9.** The Anti-hyperglycemic activity of the aqueous and 70% ethanolic extracts of the leaves of *Tecoma x smithii* Will. Wats. *versus* metformin in male albino rats.

| Group | Control | Diabetic untreated | Diabetic treated with aq. extract (100 mg/kg) | | Diabetic treated with 70% eth. extract (100 mg/kg) | | Diabetic treated with metformin (150 mg/kg) | |
|---|---|---|---|---|---|---|---|---|
| | M ± S.E. | M ± S.E. | M ± S.E. | % of change ** | M ± S.E. | % of change ** | M ± S.E. | % of change ** |
| Zero | 89.2 ± 1.7 | 248.6 ± 5.9 | 241.3 ± 6.4 | 2.94 | 256.5 ± 7.1 | 3.18 | 259.2 ± 6.9 | 4.26 |
| 2weeks | 86.9 ± 2.1 | 253.1 ± 6.5 | 193.2 ± 4.5* | 23.67 | 184.6 ± 4.8* | 27.06 | 138.7 ± 3.5* | 45.20 |
| 4weeks | 87.4 ± 1.8 | 255.4 ± 6.1 | 151.4 ± 3.9* | 40.72 | 142.8 ± 4.1* | 44.09 | 91.2 ± 2.8* | 64.29 |
| Potency *** | --- | --- | --- | 0.63 | --- | 0.69 | --- | 1 |

* Statistically significantly different from control group at $p < 0.01$.
**Percentage of change is calculated regarding the "diabetic untreated" group.
***Potency calculated as compared to the effect of metformin after 4 weeks treatment.
S.E. = standard error

## CONCLUSION

The authors aimed at the previous work to shoot different points, collecting wide range of information, draw a preliminary total picture about the chemistry of the plant not neglecting the possible therapeutic properties by rough screening of selected pharmacological actions

The flowers proved to be the most humid organ by having very high moisture content, and this made it somewhat not a good candidate for isolation studies because of the difficulty to obtain a good dry yield for further extractions. Both polyphenols and flavonoids were most concentrated in the 70% ethanol extract of the leaves, that support the selection of leaves for isolation and evaluation of biological activities guided by some available literature about other species of *Tecoma*. It was interesting to relate that flowers contain a relatively higher sugar content with an anatomical finding that they contain nectar- rich disc to attract bees.

The study of the lipoidal matter content revealed that $\beta$-Sitosterol was the major steroidal compound in all studied plant parts, followed by stigmasterol, and that palmitic acid was the major saturated fatty acid. Unsaturated fatty acids were also detected such as Linoleic, Linolenic. Chlorogenic acid prevailed the detected acids in case of the leaves, Gallic, vanillic, and caffeic acids were also detected. It was noted that aromatic acids composition of the flower and fruits extracts are so much close but different from that of leaves. both aqueous and ethanolic extracts of leaves exhibited a significant anti-inflammatory, antipyretic, analgesic, and anti-hyperglycemic effect at the selected non-toxic dose (100 mg/kg body wt), and authors strongly recommend detailed pharmacological and clinical investigations in that aspect.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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