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Short Communication

GAS CHROMATOGRAPHY/ MASS SPECTROSCOPY FOR PHYTOCHEMICAL SCREENING OF *TECOMA STANS*

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ABSTRACT

Tecoma stans (*Bignoniaceae*) is a Central and South American tree, and popularly used for the control of diabetes. The alkaoidal fraction isolated from the dried leaves of *Tecoma stans* collected from the gardens of Al-Jadria, Iraq were investigated for its phytochemical constituents through the Gas chromatography/ mass spectroscopy with preparative thin layer of chromatography. The analysis of the alkaloidal fraction confirmed the presence of previously reported alkaloids and two new indolic alkaoids.

Keywords: *Tecoma stans*, gas chromatography/ mass spectroscopy, indolic alkaloids.

INTRODUCTION

Plant *Tecoma stans* commonly growing in Latin America and is used traditionally in Mexico for the control of diabetes. The presence of alkaloids in *T. stans* was first reported in 1899 (Boorsma and Meded, 1899) and the first alkaloid was isolated by Hanrnouda and Motawi (1959). The structure of tecomine was determined Jones *et al*. (1963) and a number of pyrindane alkaloids of obvious monoterpene relationship have been isolated from the plant Dickinson and Jones (1969). Hammouda *et al.* (1971) and Youssef and Nawal (1971) indicated that the degradation of the alkaloid is dependent on the pH of its solution and that antioxidants are beneficial in delaying its deterioration. In 1983, the indolic alkaloids were isolated from the leaves of *T. stans* and a new indole oxygenase from the leaves of *T. stans* was isolated and purified (Kunapuli and Vaidyanathan, 1983; Satya and Vaidyanathan, 1984).

In 1988 GCMass of the crude base fraction indicated the presence of several related alkaloids as very minor constituents (Harris *et al*., 1988). In 1993 phytochemical investigation of an ethanolic extract from fruits of *Tecoma stans* led to the isolation of two monoterpenic alkaloids, 7-hydroxyskytanthine and 4-hydroxytecomanine (Arlete and Joana, 1993). The objective of this study was to investigate the phytochemical constituents of *Tecoma stans* using Gas chromatography–mass spectrometry (GC-MS).

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MATERIALS AND METHODS

Collection of plant material

The leaves of *Tecoma stans* (*Bignoniaceae*) grown in Iraq were collected from the area of University of Baghdad (Al-Jadria) in August and November 2004. The specimen was authenticated by Dr. Ali Al- Mousawi, Department of Biology, College of Science, University of Baghdad. The leaves were dried at room temperature in the shade and pulverized by mechanical mills.

Extraction procedure

The 50g dried leaves were extracted by a Soxhlet apparatus using diethyl ether/ammonia (15%) (80:20) as a solvent for four hours. The filtrate was in turn extracted with 4N HCl at room temperature. The aqueous phase was extracted three times with hexane, then ethyl acetate and finally with diethyl ether. The acidic aqueous phase was then treated with ammonia (30%) until pH 12 which was accompanied with a change in the color to a dark greenish brown, and introduced to a separatory funnel, where it was extracted with dichloromethane three times (1:3v/v). The organic layer was then dried with anhydrous sodium sulphate and the solvent was removed under reduced pressure at 40^{o}C, giving dark brown greenish crude alkaloidic fraction (4g) (Madhavi *et al*., 1998). A column with a length of (75cm×20mm) was packed with (50g) of silica gel (kieselgel 60) suspended in dichloromethane (100mL). The crude alkaloidic fraction was fractioned using as mobile phase dichloromethane/ methanol (from 0 to 40% v/v). Fractions were collected and each fraction was evaporated from the solvent (Peter, 2002).-All fractions were sent for GC-Mass, Fractions 5 and 12 they were further purified by preparative TLC.

Preparative Thin layer chromatograph

The analysis was performed on precoated 20×20cm (2mm thickness) TLC Kieselgel GF254 plates (Merck, Germany) and then activated by heating at 110°C for an hour before use. The fraction which contained more than one compound was dissolved in minimum quantity of dichloromethane and applied on a number of preparative TLC plates using -Dichloromethane: methanol: ammonia (89.5:10: 0.5 v/v)solvent system, major bands were observed under UV light (254nm), this was assured by spraying side of the plate with dragendroff spraying reagent to indicate the position of the bands. The major bands were scrapped off, eluted with dichloromethane then filtered; the filtrate was evaporated to dryness under vacuum (Peter, 2002).

Gas chromatography/mass spectroscopy

Shimadzu 2010 QB gas chromatography with a MSD detector equipped with HP-5 fused silica capillary Column (30m×0.25mm×25µm film thickness) is used for this purpose. The samples were injected via an all- glass injector working with split mode, with the Heleium as the carrier gas with a flow rate of 1ml/min (Hegazi and Abd El Hady, 2002). Temperature program: Injected temp 200°C, Ion source 200°C, Interphase 200°C. Column temperature was raised to 45°C (3min hold at 45°C, 4°C/min), then gradually increased to 150°C (3min hold at 150°C, 4°C/min) then raised to 250°C and a 15min hold (Antoanela *et al*., 2002).

1DNMR spectrum

^{1}H NMR data were acquired at room temperature on a Bruker AMX 300 spectrometer operating at ^{1}H (300MHZ) using ($CDCl_3$) as a solvent chemical shifts are shown in δ(ppm) value with TMS (tetramethylsilane) as an internal standard coupling constant (*J*) are given in hertz.

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry (GC-MS)

1- Fraction 5 showed major peaks, as shown in (Fig. S1).

The analysis for each peak in the chromatograms according to Library WILEY229.LIB suggested:

1- Formula $C_{10}H_{11}NO$, mol.wt. 161, retention time 36.958, mass peak 80, base peak 161. As shown in the mass spectrum (Fig. S2), this gave us an idea the presence of Boschniakine.
2- A new indolic compound: 2,3-dihydro-4, 4- dimethyl indol-4-ol-2-one, mol.wt. 177, retention time 43.292,

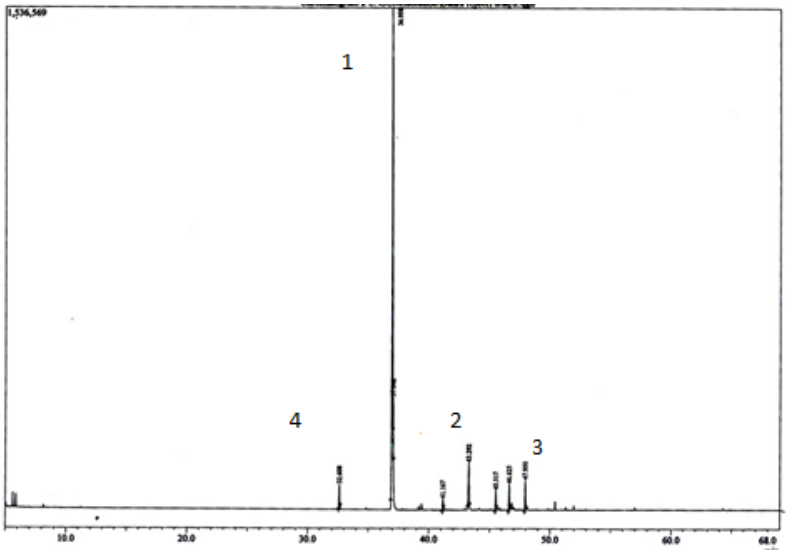


Fig. S1. Gas chromatography/ mass spectroscopy for fraction 5 showing major peak and two other new indolic compounds.

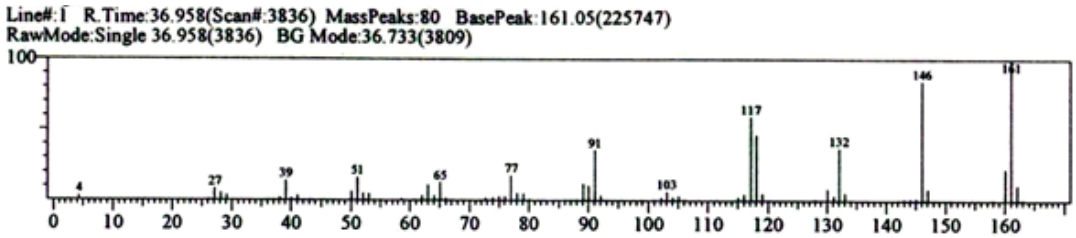


Fig. S2. Mass spectrum of Boschniakine.

mass peak 44, base peak162. Formula $C_{10}H_{11}NO_2$. As shown in the mass spectrum (Fig. S3).

3- A new indolic compound: Indole-2,3-dione, 1-methyl, 3- oxime, mol.wt 176, retention time 47.95, mass peak 35, base peak 176, fromula $C_9H_8N_2O_2$. As shown in the mass spectrum (Fig. S4).

4- Precursor for the Boschniakine, benzoic acid amide, mol.wt.121. Retention time 32.608, mass peak 18, base peak 105. Formula C_7H_7NO. As shown in the mass spectrum (Fig. S5).

2- Fraction 12 showed major peaks, as shown in (Fig. S6).

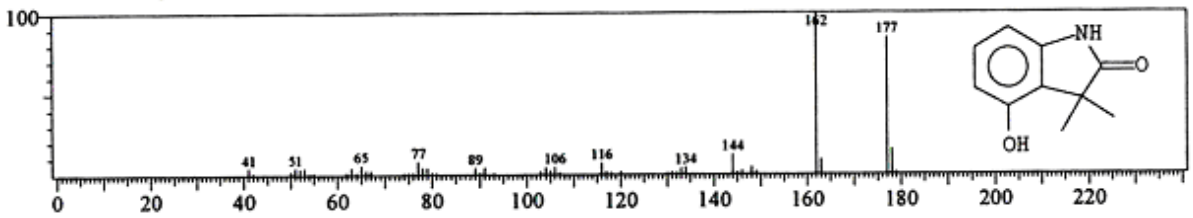


Fig. S3. Mass spectrum of a new indolic compound.

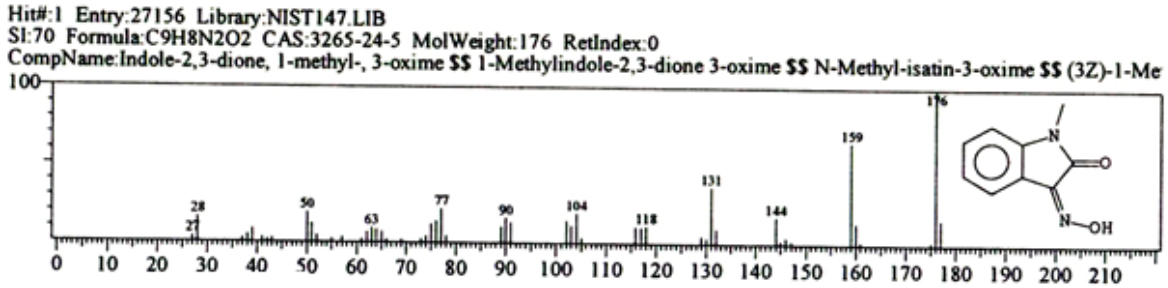


Fig. S4. Mass spectrum of a new indolic compound.

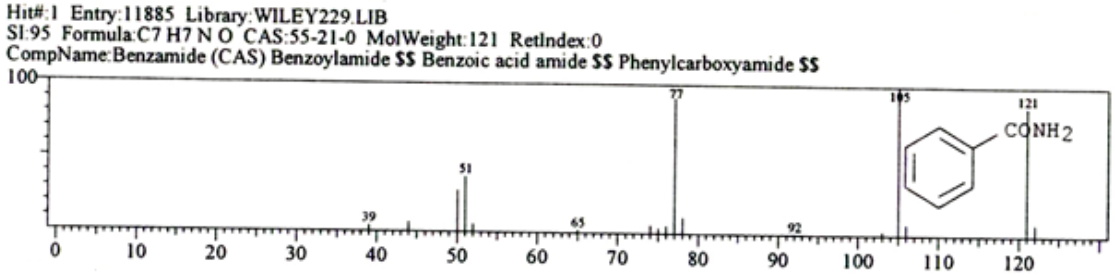


Fig. S5. Mass spectrum for a precursor for the Boschniakine.

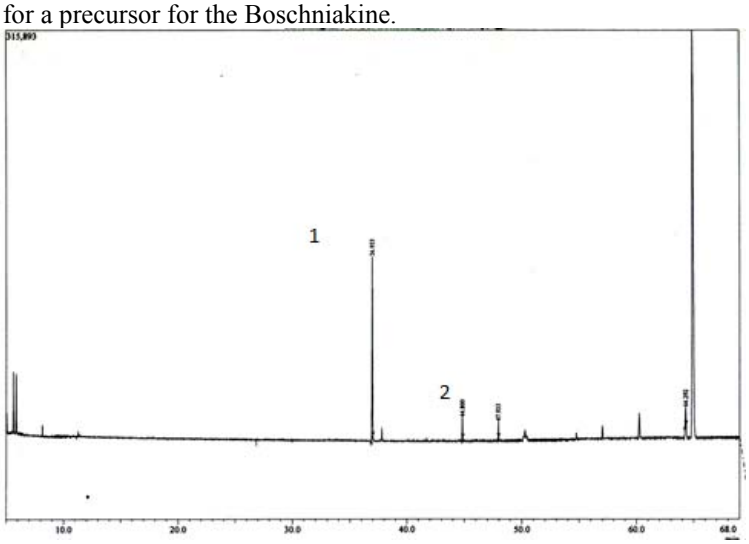


Fig. S6. Gas chromatography/ mass spectroscopy for fraction 12 showing major peak and minor peak.

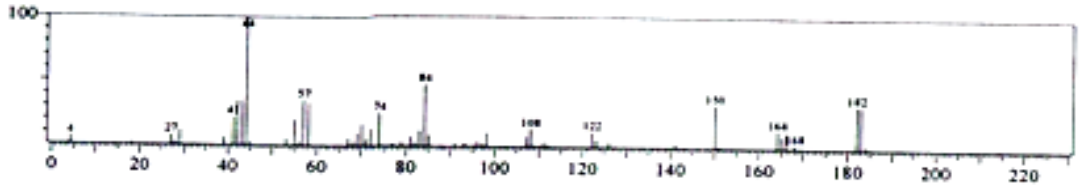


Fig. S7a.

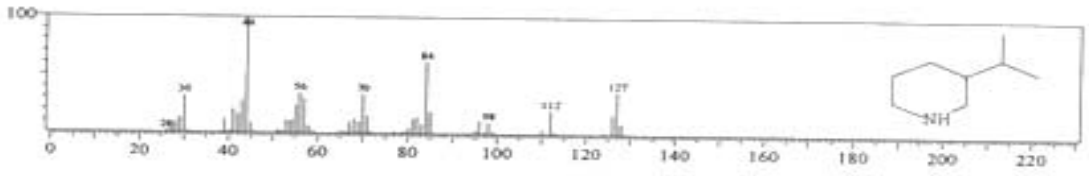


Fig. S7b.

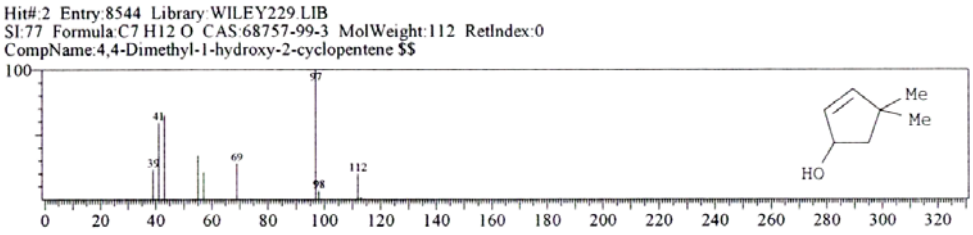


Fig. S8. A precursor of the indolic alkaloids.

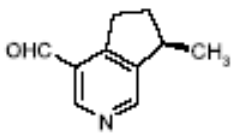
The analysis for each peak in the chromatograms according to Library WILEY229.LIB suggested:

1- A compound with a molecular weight of 182 as shown in (Fig. S7a) or a precursor for the tecomine compound as shown in (Fig. S7b).
2- A precursor of the indolic alkaloids:4, 4, dimethyl-1-Hydroxy-2-cyclo pentene. Mol.wt. 112, mass peak 11, base peak 97, retention time 44.8. Formula $C_7H_{12}O$. As shown in the mass spectrum (Fig. S8).

Spectroscopic

1- Boschniakine chemical structure as shown in (Fig. S9), an oil of a formula $C_{10}H_{11}NO$ mol.wt. 161 with pungent odor yields 0.1g. UV λmax 250 and 263 nm (ethanol). IR (KBr) cm^{-1}: 3028, 2960, 2856, 1739 and 758. ^{1}H-NMR (CDCl3) (300 MHz): 1.12 (3H , d, J 6.7) , 1.5-1.8 (1H , m) , 2.9-3.4 (3H , m) , 8.5 (1H , s) , 10.2 (1H, s) (Costantino *et al*., 2003).
2- Beta-Hydroxyskitanthine, chemical structure as shown in (Figure S10), an oil of a formula $C_{11}H_{20}NO$ mol.wt. 182; yields 0.1g. UV λmax 220nm (ethanol). IR (KBr) cm^{-1}: 3396, 2923, 2854. ^{1}H-NMR (CDCl3) (300 MHz): 0.85 (3H , d, J 6.9) , 0.95 (3H , d, J 7.0), 1.3-1.35 (1H , m) , 1.7 (2H , m) , 2.1 (3H, s), 3.3-3.6 (1H ,ddd, J 10.3, 4.2, 2.1) (Costantino *et al*., 2003).

GC-MS is a valuable tool to screen alkaloids, present investigation of the Iraqi *T. stans* revealed four alkaloids by GC-MS. The combination of two techniques such as GC-MS and preparative TLC has led to a rapid chemical screening of already two known alkaloids (Boschniakine, Beta- Hydroxyskitanthine) and two unknown indolic alkaloids. This method is simpler, fast and inexpensive method to search for secondary metabolites from natural sources in order to be further investigated for biological activity.



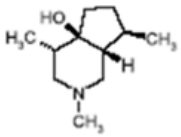


Fig. S9. Boschniakine.Fig. S10. Beta-Hydroxyskitanthine.

CONCLUSION

To the best of our knowledge, this is the first report of the Iraqi *tecoma stans* confirming the presence of known alkaloids and using GC-Mass for screening of new indolic alkaloids.

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