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# Identification of limonoids and steroids from the leaves of *Turraea heterophylla* (Smith) by HPLC-ESI-QTOF-MS/MS methods

**Kouadio Kouassi Blaise, Kablan Ahmont Landry Claude, Konan Dibi Jacques, Oussou Kouamé Raphael, Dongui Bini Kouamé and Attioua Koffi Barthélemy**

**Kouadio Kouassi Blaise**
[a)] Laboratoire des Sciences et Technologies de l'Environnement, UFR Environnement, Université Jean Lorougnon GUEDE, Daloa, BP 150 Daloa, Côte d'Ivoire
[b)] Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

**Kablan Ahmont Landry Claude**
Département de Mathématiques, Physique et chimie, UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire

**Konan Dibi Jacques**
Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

**Oussou Kouamé Raphael**
Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

**Dongui Bini Kouamé**
Laboratoire des Sciences et Technologies de l'Environnement, UFR Environnement, Université Jean Lorougnon GUEDE, Daloa, BP 150 Daloa, Côte d'Ivoire

**Attioua Koffi Barthélemy**
Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

**Corresponding Author:**
**Kouadio Kouassi Blaise**
[a)] Laboratoire des Sciences et Technologies de l'Environnement, UFR Environnement, Université Jean Lorougnon GUEDE, Daloa, BP 150 Daloa, Côte d'Ivoire
[b)] Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

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**Abstract**

*Turraea heterophylla* (Smith) is a species used in Ivoirian medicine as antimalarial, aphrodisiac and various human diseases. Using the dereplication method, the analysis of the crude methanol extract of the leaves led to the identification of six compounds including three limonoids (28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy) 14,15- deoxyhavanensin-1,7-diacetate; mzikonol; 1,2- dihydro-azadirone) and three steroids (3ß-acetoxy-5□-pregn-7,20-dien-6-one; 3β-hydroxy-5α-pregn-6-one; villosterol). These secondary metabolites, which are reported for the first time in *T. heterophylla*, were identified according to their HPLC-ESI-QTOF-MS/MS data.

**Keywords:** *Turraea heterophylla*, dereplication, identification, limonoids, steroids

## 1. Introduction

Medicinal plants are widely used in Africa traditional medicine to treat various diseases such as malarial, hemorrhoids, hypertension and Buruli ulcer [Gnaoré *et al*., 2020; Jacques *et al*., 2019] [1, 2]. Many of them are used without their chemical composition being known; among them are species of *Turraea*. The genus *Turraea* belongs to the Maliaceae family. Species of this family are widely distributed in tropical regions of the world [APG III, 2009; ATIBT, 2016] [3, 4]. *Turraea* plants grow as trees or shrubs [Mabberley *et al*., 2008] [5], and their chemical composition is varied [Wang *et al*., 2018; Yuan *et al*., 2013] [6, 7]. The species that interests us here is *Turraea heterophylla* (Smith). In Côte d'Ivoire, this species is found in both forest and savanna regions; and it is known as aphrodisiac [Neuwinger 1996] [8]. It is also used to treat asthma, emphysema, lumbago and headaches [Bouquet et Debray, 1974] [9]. Concerning the chemistry of this species, the literature reports the presence of tannins, saponins, terpenes, steroids and limonoids in the roots bark [Boua *et al*., 2013; Akrofi, 2011] [10, 11]. In addition to this, our previous study of the leaves revealed the presence of polyterpenes, steroids, alkaloids, saponins, polyphenols and flavonoids; with a very high content of flavonoids and polyphenols in this organ [Kouadio *et al*., 2021] [12]. Present study aims to identify the structure of these secondary metabolites. For this, extraction was performed using methanol and compounds were detected and identified by HPLC-ESI-QTOF-MS/MS method.

## 2. Material and Methods

### 2.1 Plant material

The fresh leaves of *T. heterophylla* were collected from the forest area of Daloa department, west of Côte d'Ivoire, and identified by the National Floristic Center of the Félix HOUPHOUËT-BOIGNY University (CNF), where a specimen is kept under the herbarium number 31235. The fresh leaves were then cleaned with distilled water and dried in an oven at 50 °C for 4 days before being reduced to powder using an electric mill (RETSCH, type SM 100).

### 2.2 General Experimental Procedures

The secondary metabolites were extracted using methanol 90% (v/v), and the solvent was removed using a rotary evaporator BÜCHI461 type. The sample analysis were carried out in positive polarity, using Analytical HPLC runs coupled to an Agilent LC-MS system (Agilent 1260 Infinity HPLC and to an Agilent 6530 ESI-QTOF-MS/MS. The HPLC device was equipped with a $C_{18}$ column (150 x 4.6 mm, i.d. 5 µm, Waters) and a binary pump

(Waters 2525); visualization was realized upon a UV-visible diode array detector (190-600 nm, Waters 2996).

## 2.3 Methods

### 2.3.1 Preparation of methanol extract from the leaves of *T. heterophylla*

The crude methanol extract of the leaves of *T. heterophylla* was prepared following the method descripts by Kouadio *et al*. 2021 [12]. The dried and ground plant material (200.0 g) was extracted by maceration in 600 mL of methanol for 24 hours. After filtration on Whatman n°2 paper, the Mac is extracted twice in a row with the same amount of methanol for 24 hours each. The filtrates were combined, concentrated and evaporated to dryness under reduced pressure (at 60 °C), using a rotary evaporator. Part (5mg) of crude methanol extract was dissolved in 1 mL of methanol (analytic, grad HPLC), and then with the help of a syringe, 1mL of this extract is taken in 1 mL of methanol. The extract is filtered again through a nylon syringe filter 0.5 µm. Finally, 300 µL is taken to be stored in a Vial before HPLC-QTOF-MS/MS analysis.

### 2.3.2 Analysis of the methanol extract by HPLC-ESI–QTOF-MS/MS method

The HPLC-ESI–QTOF-MS/MS analysis, or dereplication, is a new method that allows identifying very quickly and efficiently the known compounds in a complex mixture, such as crude plant extracts. With this method, it becomes easy to tell the difference between known and unknown compounds in a complex mixture, based on database search [Jongmin *et al*., 2017] [13]. This analytical method is based on the coupling system ESI-QTOF-MS/MS (electrospray ionization-quadrupole-time of flight-mass spectroscopy), that provides sufficient information to determine the exact structure of a known compound [McFarland *et al*., 2004] [14]. For the analysis of the crude methanol extract, using the system an Agilent 6530 Accurate-Mass QTOF coupled with a 1260 Agilent Infinity LC system, the flow rate was fixed to 0.25 mL/min, and mass spectra were acquired in positive mode in the mass range of m/z 100 to 1200 Da.

## 3. Results and Discussion

The automated processing of raw data obtained from HPLC-ESI-QTOF-MS analysis of *T. heterophylla* (Smth) leaves extract, followed by analysis by Agilent Masshunter Workstation Software, led to identify known constituents of this crude extract. From the LC-ESI-MS and MS / MS spectra obtained, a list of raw formulas was generated; then the structure corresponding to the formula closest to the mass is retained. Each protonated molecules $[M+H]^+$ were selected according to their great abundance and their greater regularity of fragmentation. To confirm the structures of the molecules, the ESI-QTOF-MS/MS data were compared to those in the public databases (DNP, Reaxys and SciFinder). The analysis of the crude methanol extract from *T. heterophylla* gave the LC-ESI-MS chromatography (Figure 1), which reveals several peaks corresponding to the major constituents. Each peak was analysis by Agilent Masshunter Workstation Software.

**Fig 1:** HPLC-ESI –QTOF-MS chromatogram of the methanol extract of the leaves of *T. heterophylla*

### 3.1 28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy) 14, 15-deoxyhavanensin-1, 7-diacétate

In the HPLC-ESI–QTOF-MS chromatogram (Figures 1), the peak 1 at retention time $R_T$= 19.125 min was identified to 28-nor-4α-carbomethoxy-11β-acetoxy-12α(2methylbutanoyloxy) 14,15- deoxyhavanensin-1,7-diacetate. To generate its ESI-MS and ESI-MS/MS spectra (Figure 2), this peak was analysis by Agilent Masshunter Workstation Software.

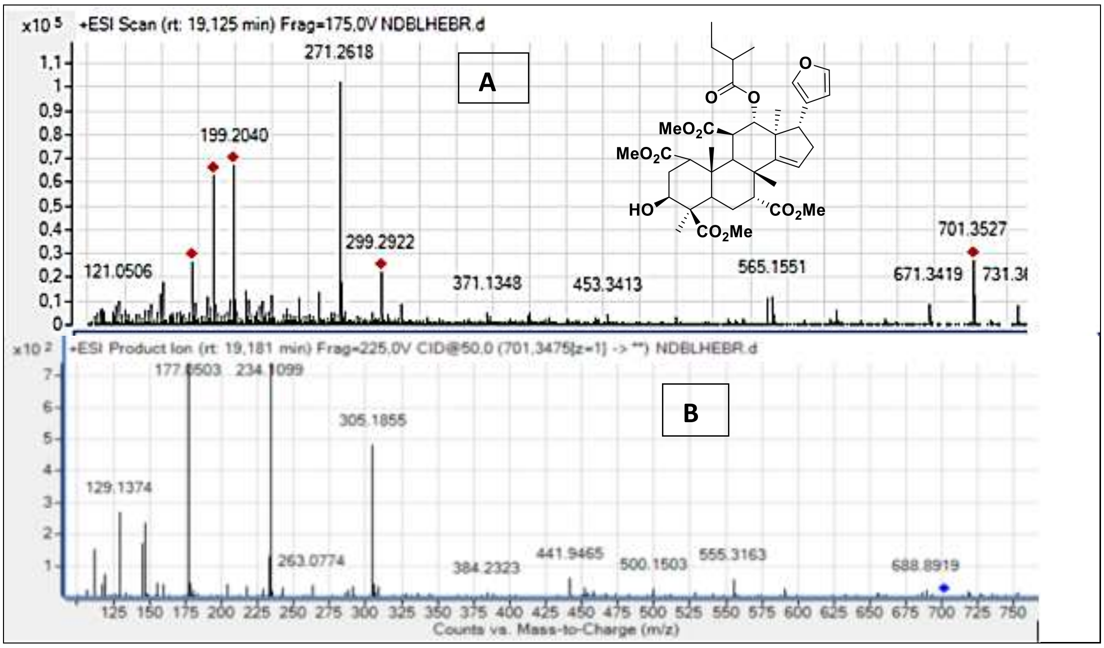

**Fig 2:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of *28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy) 14,15-deoxyhavanensin-1,7-diacetate*.

According to the Masshunter software data, the molecular ion peak at m/z 701.3527 $[M+H]^+$ on the ESI-MS spectrum (Figure 2A) matches the molecular formula $C_{38}H_{52}O_{12}$ (Cal.700.3449). In addition, several characteristic ion fragments MS/MS (Figure 2B) were observed at m/z: 555.3163 $[M+H-143]^+$; 441.9465$[M+H-143-114]^+$; 305.1855$[M+H-143-114-57-79]^+$; 234.1099$[M+H-143-114-57-79-71]^+$. According to this fragmentation pathway, the corresponding structure was found in the databases Reaxys and Dictionary of Natural Products (DNP) to be *28-nor-4α-carbomethoxy-11β-acetoxy-12α-(2-methylbutanoyloxy) 14,15-deoxyhavanensin-1,7-diacetate*. It is a known limonoid previously isolated of *Turreae floribunda* [Torto *et al*., 1995; McFarland *et al*., 2004; Mulholland, 1998, 1999; Ndung'u *et al*., 2004] [15, 14, 16, 17].

### 3.2 1, 2-dihydroazadirone

The compound corresponding to peak 2 at retention time $R_T$= 32.593 min (Figure 1) was identified to 1,2-dihydroazadirone (Figure 3). Its ESI-MS and ESI-MS/MS spectra (Figures 3A and 3B) were generated by Agilent Masshunter Workstation Software. The molecular ion peak corresponding to this compound was observed at m/z 457. 2954 $[M+H]^+$ on the ESI-MS spectrum (Figure 3A), and this matches the molecular formula $C_{27}H_{36}O_4$ (Cal. 424.2650). Several characteristic ion fragments MS/MS (Figure 3B) were observed at m/z: 425.5810 $[M+H]^+$; 380.2351$[M+H-44]^+$; 346.1933$[M+H-44-34]^+$; 298.2297$[M+H-44-82]^+$; 190.1358$[M+H-44-82-108]^+$.

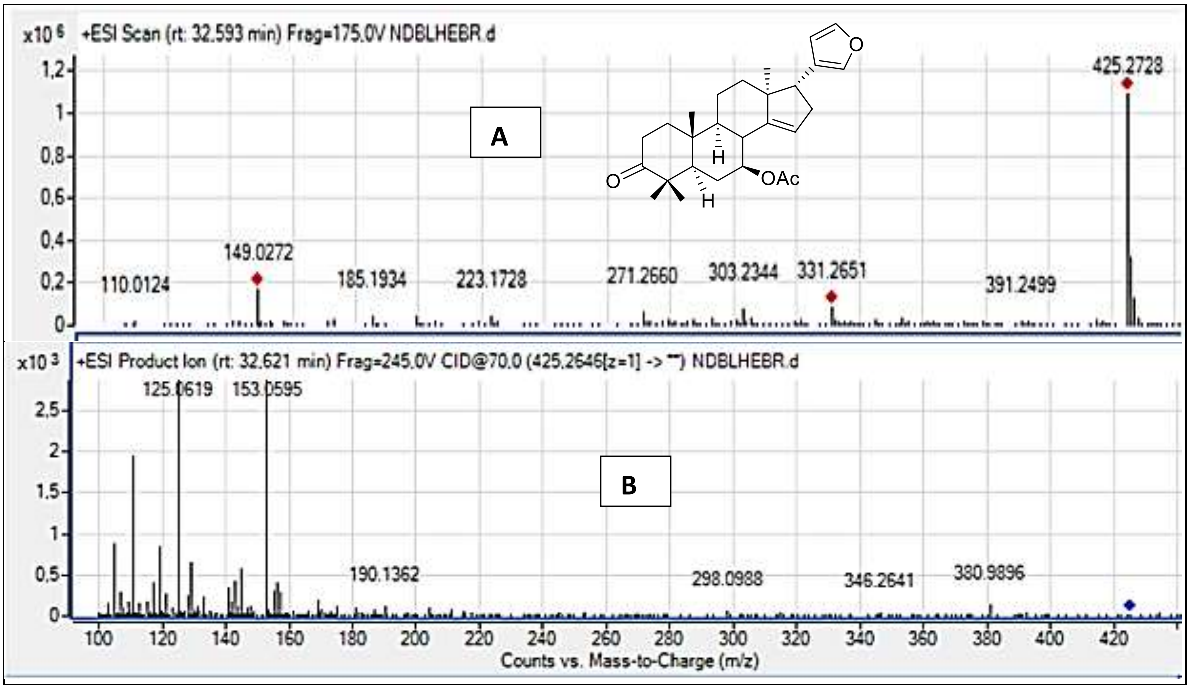

**Fig 3:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of 1,2-dihydroazadirone

This fragmentation pathway matches the chemical structure of 1,2-dihydroazadirone (Figure 3); what structure was found in the databases Reaxys and Dictionary of Natural Products (DNP). This molecule is also a known limonoid previously isolated from the roots bark and stem bark of *Turraea robusta* [Rajab *et al*., 1988; Bentley *et al*., 1992] [18, 19].

### 3.3 Mzikonol

The ESI-MS and ESI-MS/MS spectra (Figures 4A and 4B) of the compound that gave the peak 3 at retention time $R_T$= 32.944 min (Figure 1) were generated by Agilent Masshunter Workstation Software. The molecular formula corresponding to the molecular ion peak at m/z 457.2954 $[M+H]^+$ (Figure 4A) was deducted as $C_{28}H_{40}O_5$ (Cal. 456.2876).

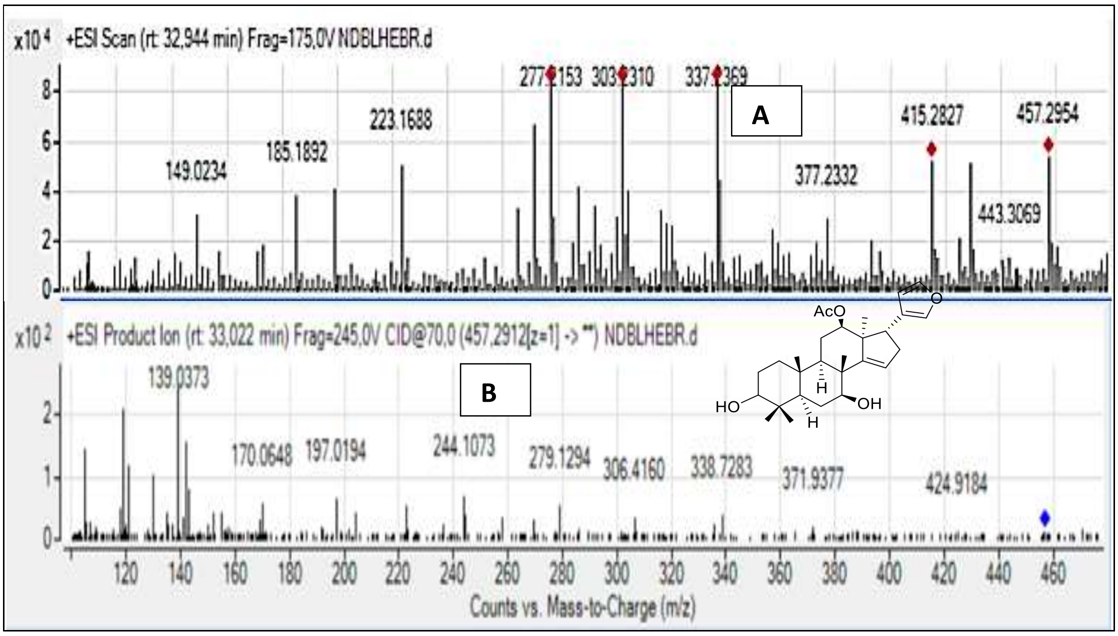

**Fig 4:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of *Mzikonol*

The corresponding structure was founded in the databases Reaxys and Dictionary of Natural Products (DNP) to be Mzikonol. This known limonoid was previously isolated from the roots bark and stem bark of *Turraea robusta* [Rajab *et al.*, 1988; Bentley *et al.*, 1992] [18, 19]. Several characteristic ion fragments MS/MS (Figure 4B), confirming this structure, were observed at m/z: 457.2954 $[M+H]^+$; 371.9377 $[M+H-86]^+$; 279.1294 $[M+H-86-92]^+$; 244.1073 $[M+H-86-92-35]^+$.

### 3.4 Villosterol

The peak 4 at retention time $R_T$= 30.836 min (Figure 1) was analysis by Agilent Masshunter Workstation Software to generate the ESI-MS and ESI-MS/MS spectra (Figures 5A and 5B).

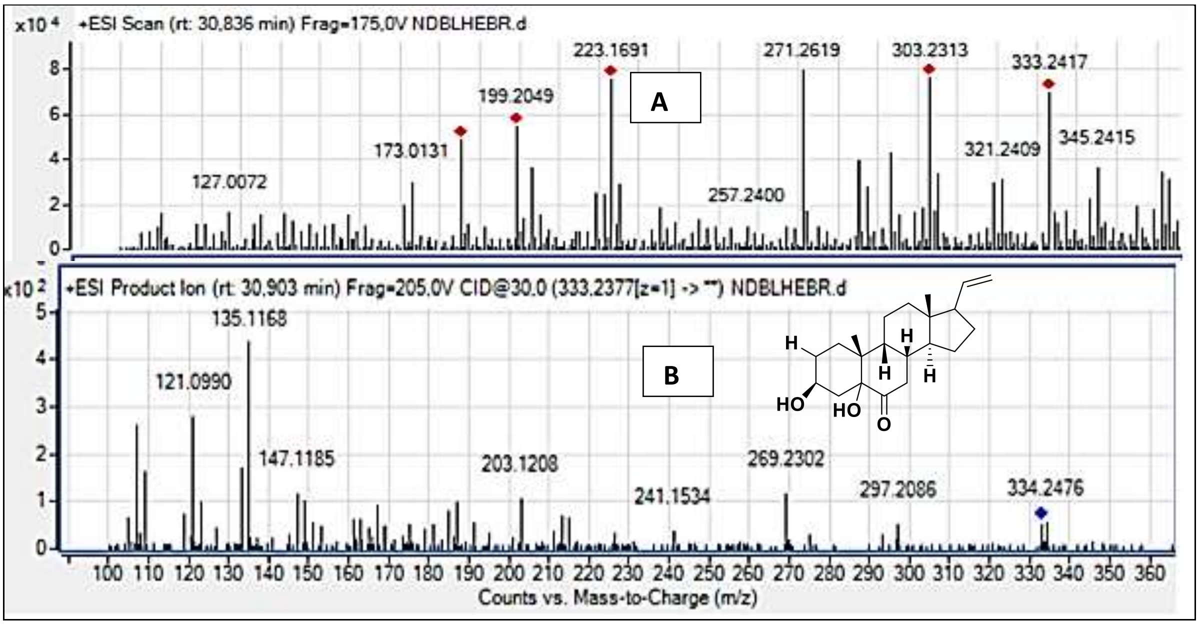

**Fig 5:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of Villosterol.

On the ESI-MS spectrum (Figure 5A), the molecular ion peak was observed at m/z 333.2417$[M+H]^+$, and the corresponding formula was deducted as $C_{21}H_{32}O_3$ (Cal. 332.2339). In addition, several characteristic ion fragments MS/MS (Figure 5B) were observed at m/z: 334.2476 $[M+2H]^+$; 297.2086 $[M+H-36]^+$; 269.2302$[M+H-36-28]^+$; 241.1534$[M+H-36-28-28]^+$; 203.1208$[M+H-36-28-28-38]^+$. According to this fragmentation pathway, the corresponding structure was founded in the data bases Reaxys and Dictionary of Natural Products (DNP) to be Villosterol. This compound is also a steroid derivative which was previously isolated from the seeds of *Turraea obtusifolia* [Fraser *et al*.,1995; Mulholland *et al*., 1998; Sarker *et al*.,1997] [20, 21, 22].

### 3.5 3β-acetoxy 5α-pregn 7,20-dien-6-one

In the Figure 1, the peak 5 at retention time $R_T$= 32.241 min was analysis by Agilent Masshunter Workstation Software to generate the ESI-MS and ESI-MS/MS spectra (Figures 6A and 6B). According to these data, the ion peak at m/z 345.2428[M+H]$^+$ was attributed to the molecular ion (Figure 5A), which value matches the molecular formula $C_{22}H_{32}O3$ (Cal. 344.2350). Several characteristic ion fragments MS/MS (Figure 6B) were observed at m/z: 345.2351 [M+H]$^+$; 328.3534 [M+H-17]$^+$; 299.3652 [M+H-17-29]$^+$; 275.1020[M+H-17-29-24]$^+$; 196.3782 [M+H-17-29-24-79]$^+$. According to this fragmentation pathway, the corresponding structure was founded in the data bases Reaxys and Dictionary of Natural Products (DNP) to be 3ß-acetoxy-5a-pregn-7,20-dien-6-one. This steroid derivative was previously isolated from the leaves and roots bark of *Turraea pubescens* [Wang *et al*., 2008; Yuan, *et al*., 2013] [6, 7].

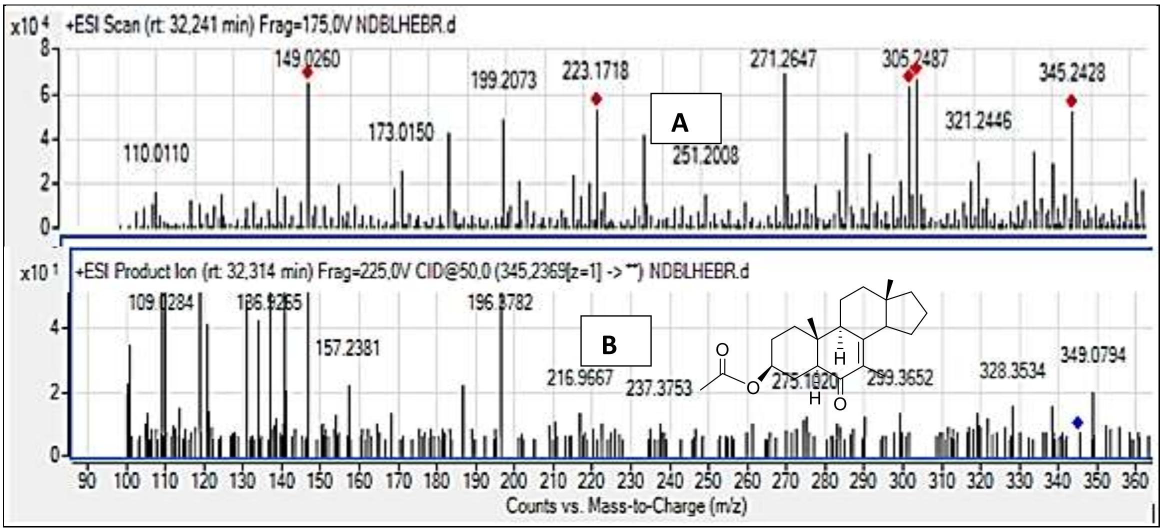

**Fig 6:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of 3ß-acetoxy-5a-pregn-7, 20-dien-6-one

### 3.6 3β-hydroxy-5α-pregn-6-one

The analysis of the peak 6 at $R_T$= 40.556 min (Figure 1), using Masshunter software, generated the ESI-MS spectrum (Figure 7A) with the molecular ion peak at m/z 305.1867[M+H]$^+$. This value matches the molecular formula $C_{20}H_{32}O2$ (Cal. 304.1789). Several characteristic ion fragments MS/MS (Figure 7B) were observed at m/z: 305.1867[M+H]$^+$; 286.2297[M+H-18]$^+$; 270.0274[M+H-18-16]$^+$; 217.1565[M+H-18-16-53]$^+$. According to this fragmentation pathway, the corresponding structure was founded in the data bases Reaxys and Dictionary of Natural Products (DNP) to 3β-hydroxy-5α-pregn-6-one. This molecule is steroid derivative previously isolated from the leaves of *Turraea pubescens* [Wang *et al*., 2007, 2008; Yuan, *et al*., 2013] [20, 7].

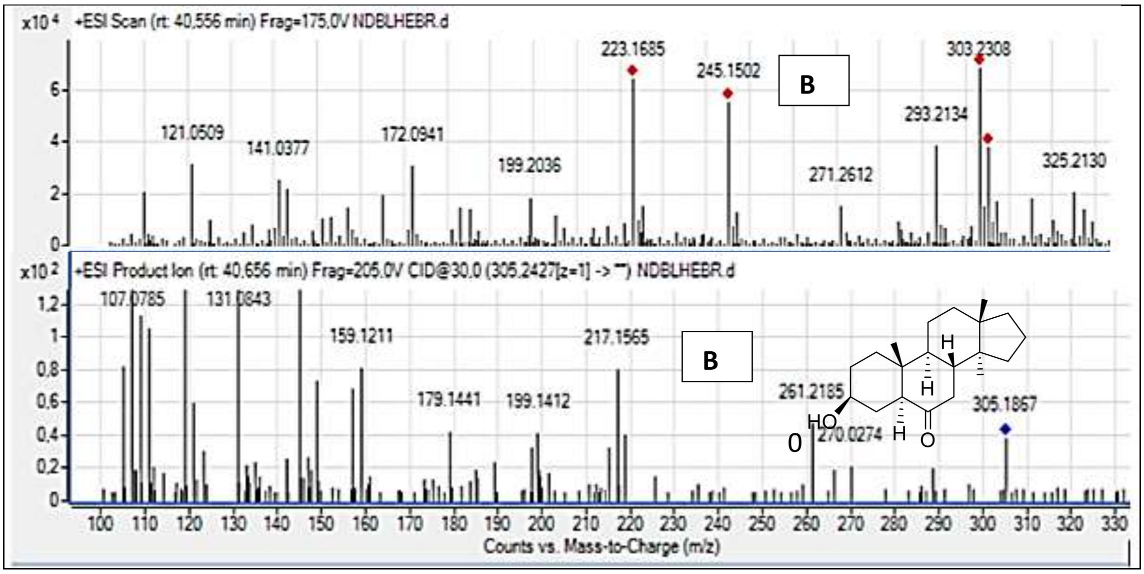

**Fig 7:** ESI-MS spectrum (A) and ESI-MS/MS spectrum (B) of 3ß-acetoxy-5□-pregn-7,20-dien-6-one

### 4. Conclusion

The analysis by the HPLC-ESI-QTOF-MS / MS method of the methanol extract of the leaves of *T. heterophylla* made it possible to identify the structures of three limonoids (1 - 3) and three steroids (4 - 6). In addition, several peaks (A - F) revealed were not identified because their structures were not found in the databases used. Regarding the identified compounds, they could justify the traditional used of *T. heterophylla* as aphrodisiac. Indeed, limonoids and steroids are known for their beneficial effects on the functioning of the heart.

## 5. Notes

The authors declare no competing financial interest.

## 6. Acknowledgments

None

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