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# Table of Contents: Volume 9  Number 30, 10 August, 2015

## ARTICLES

### Research Articles

**Effect of the speed of the drying air on the quality of essential oil from Aristolochia cymbifera Mart. and Zucc.** 806
André Luiz Montes, Daniel Emanuel Cabral de Oliveira, Osvaldo Resende, Fabiano Guimarães Silva and Juliana de Fátima Sales

**Isolation and analgesic property of lupeol from Diospyros mespiliformis stem bark** 813
Bulus Adzu, Ben Ahmed Chindo, Florence David Tarfa, Oluwakanyinsola Adeola Salawu and Ogbaji John Igoli

**Ethnobotanical study of some medicinal plants from Hoggar, Algeria** 820
Farah Ramdane, Mahfoud Hadj Mahammed, Mohamed Didi Ould Hadj, Amoura Chanai, Roukia Hammoudi, Naima Hillali, Houria Mesrouk, Imane Bouafia and Chaima Bahaz
Full Length Research Paper

Effect of the speed of the drying air on the quality of essential oil from *Aristolochia cymbifera* Mart. and Zucc.

André Luiz Montes¹, Daniel Emanuel Cabral de Oliveira¹, Osvaldo Resende², Fabiano Guimarães Silva²* and Juliana de Fátima Sales²


Received 2 January, 2015; Accepted 23 July, 2015

Dehydrating plant material ensures the conservation of active compounds in medicinal plants. Thus, the object of the present study was to evaluate the effect of three drying air speeds on the content and the chemical composition of essential oil from *Aristolochia cymbifera* Mart. and Zucc. The tests were conducted in a fixed-layer dryer with drying chambers measuring 0.60 x 0.60 x 0.60 m on a plate with 25% perforation. The treatments consisted of three drying air speeds (0.5, 1.0, and 2.0 m·s⁻¹), with four replicates and a mean temperature of 34.7±15°C. The experimental design was in randomized blocks. The essential oil was extracted by hydrodistillation. It was concluded that the drying air did not influence the essential oil content; however, there was a slight influence on the minor constituents of the essential oil extracted.

Key words: Medicinal plants, chemical composition, content of essential oil, extraction.

INTRODUCTION

Brazil is a leader in the natural product market, which includes essential oils. According to Bizzo et al. (2009), there is growing interest in natural products, and there are appeals for environmental preservation policies, which can be used as marketing tools and provide a great opportunity for the development of sustainable processes of biodiversity exploitation.

The Cerrado is one of the biomes that contains genetic resources of great medical diversity; a bibliographic survey performed only for the State of Mato Grosso found a total of 509 species described as medicinal, surpassing the estimates made by other works, and projected more than 600 species around the biome (Neto and Morais, 2003).

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Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
This species is an herbaceous perennial vine native to Brazil that is characteristically vigorous and better adapted to hot environments (Lorenzi and Matos, 2002). According to these authors, *A. cymbifera* Mart. and Zucc. contains mono/diterpenes and sesquiterpenoids in the leaves, stems and roots.

In folk medicine, *A. cymbifera* Mart. And Zucc. is used for various problems and is considered to be a diuretic, antiseptic and antispasmodic (Lorenzi and Matos, 2002). The work of Urzúa and Sotes (2008) compared several species of the *Aristolochia* genus in terms of the presence or absence of compounds in the essential oil, such as linalool monoterpenoid, sesquiterpene compounds derived from farnesane, bisabolane, elemane, germacrane, bicyclogermacrane, humulane, aristolane, caryophyllanes, eudesmane, cadinane, guainane, aromadendrane, cubebane, himachalane, santalane, copane and bourbonane. All of these compounds are found, in varying amounts, in the species of the genus *Aristolochia*. *A. cymbifera* is thought to contain few components, but one reason for this finding may be the lack of studies on this species. The components found include germacrone A, α-farnesene and α-trans-bergamotene (Urzúa and Sotes, 2008).

The collection method is an important factor in the quality of medicinal plant essential oils because features such as plant organ, stage of development, time of year, and time of day can influence the production of substances with therapeutic activity (Blank et al., 2007; Gobbo-neto and Lopes, 2007).

In addition to the above aspects, the quality of the essential oils will depend on their processing. Soares et al. (2007), have been able to obtain extractive yields of *Ocimum basilicum* L. essential oils when the drying process was accomplished with an air temperature of 40°C. However, the highest linalool yield was obtained when the drying process was accomplished with an air temperature between 50 and 60°C. However, the drying of the material will not always be suitable for the samples. According to Rocha et al. (2011a), there was a downward trend in the level of essential oil obtained from *Mikania glometa* Sprengel with increasing temperature compared with the fresh plant.

The dehydration of plant material ensures that the compounds do not deteriorate because enzyme activity is inhibited or reduced, allowing the preservation of active compounds. Additionally, dehydrated plant materials are easily stored for a long time (Chudnicka and Matysik, 2005). There are many studies on the effects of drying on the quality of bioactive compounds. These studies are necessary because each species behaves differently (Soares et al., 2007; Rocha et al., 2011b).

Therefore, the objective of the present study was to assess the effect of three drying air speeds on the content and the chemical composition of essential oil from *A. cymbifera* Mart. and Zucc.. The species *Aristolochia cymbifera* Mart. and Zucc., popularly known as “jarrinha”, “milhomem”, or “cassau”, is a species of the genus *Aristolochia* (Aristolochiaceae).

**MATERIALS AND METHODS**

**Harvest and selection of plant material**

Plants of *A. cymbifera* Mart. and Zucc. were collected in the Rio Verde region, at coordinates S 17°55′56.8″ WO 50°56′33.2″, between 7:00 and 8:00 in the morning, in October, 2011. The exsiccate material is registered with the Herbarium Jataiense under number 5,642. The plants were harvested by cutting the shoots 5 cm above the ground, packing them in a row inside plastic bags, and sending them to the Natural Products section of the Plant Tissue Culture Laboratory in the Federal Institute of Education, Science, and Technology of Goiania, Campo Verde campus. After harvesting, the plants were subjected to defoliation and selection, and plants that were diseased or had been attacked by insects were discarded.

**Determination of moisture content**

The moisture content was determined before and after drying, as described by Asae (2000) for forage species and similar plants (plants or leaves). To determine the moisture content, the leaves were placed in a convection oven at 103± 2°C for 24 h, with four replicates (Asae, 2000).

**Drying**

The initial moisture content of the leaves was approximately 75.5 (% wet basis, w.b.). During the drying process, the samples were weighed periodically until they reached water levels of 11.1 (% w.b.). The drying was conducted in a fixed-layer dryer manufactured from #16 metal sheets. The drying chamber measured 0.60 x 0.60 x 0.60 m, for a total volume of 0.216 m³, and contained a plate with 25% perforation placed at a height of 0.33 m. The fan was of the centrifugal type, driven by a three-phase motor with a power of 1.5 HP and rotation at 1,720 rpm, consisting of a rotor, palettes, a volute and support. The connection between the drying chamber and the fan was held by an expanding element that shifted from the 0.20 x 0.20 m cross-section at the fan output to 0.57 m x 0.03 m at the entrance of the drying chamber over a distance of 0.64 m (Figure 1). Each dryer was composed of six swinging temperature sensors and four electrical resistors of 1,500 ohms, for a total of 6,000 ohms. The sensors were positioned before and after the resistance and inside each tray. Four removable trays with perforated bottoms that measured 0.28 x 0.28 x 0.15 m were placed in the drying chamber (Figure 2). The system also featured an automatic controller that managed the system and stored the data generated. *A. cymbifera* Mart. and Zucc. leaves were woven into a voile fabric and spread on the tray. The system was set to 34.7±1.5°C with controlled air speeds of 0.5, 1.0, and 2.0 m·s⁻¹.

**Obtaining essential oil**

Essential oil was extracted using a clevenger appliance adapted into a 3 L flask. The sample was placed in the flask along with 2 L of distilled water. Approximately 60 g of dried leaves were used, which were ground in a Willye TE – 648 micro mill (TECNAL). The extraction time was 150 min, counted from the time of boiling. The essential oil was extracted from the aqueous phase using...
dichloromethane (3 x 6 ml/20 min each). The obtained organic fractions were combined and mixed with anhydrous sodium sulfate, and the sulfate was withdrawn by filtration after 30 min. The mass of the obtained oil was determined by weighing on an analytical balance accurate to 0.0001 mg. The obtained oil samples were transferred to amber glass bottles capped with aluminum foil, and small holes were made in the lids to allow solvent evaporation. The bottles were stored in a refrigerator at 4 to 8°C until analysis.

**Chemical analyses by gas chromatography/mass spectrometry**

The chemical analyses were performed at the Department of Chemistry of the Federal University of Lavras, Lavras-MG, on a gas chromatography apparatus coupled to a Shimadzu QP5050A quadrupole mass spectrometer (GC-MS) (Kyoto, Japan) under the following operating conditions: fused silica capillary column, DB-5 model (30 m long X 0.25 mm internal diameter X 0.25 µm film thickness) (Shimadzu, Japan), with a flow of 1 ml·min⁻¹ of helium as the carrier gas; heated to programmed temperatures (60°C with a gradient of 3°C·min⁻¹ up to 240°C, then a gradient of 10°C·min⁻¹ up to 270°C, keeping an isotherm of 7 min, with a total run time of 70 min). The ionization energy of the detector was 70 eV, and the sample injection volume of 1.0 ml was diluted in dichloromethane (ultra residue grade, Baker, EUA) and an injection ratio of 1:20. The detector and injector temperatures were maintained at 220 and 240°C, respectively. The analysis was conducted in scan mode at a speed of 2.0 scans·s⁻¹, with a mass range of 45 to 500 m/z.

**Statistical analysis**

The experimental design was in randomized blocks, with three drying air speeds. Each treatment had three replicates, for a total of ...
RESULTS AND DISCUSSION

The *A. cymbifera* Mart. And Zucc. leaves were dried to a moisture content of 11.1±0.3 (% w.b.). Figure 3 shows the temperatures on the inner side of the trays containing the leaves for the three drying speeds, as well as the room temperature and the relative humidity.

The temperature inside the trays ranged from 36.2 to 33.2°C. The mean temperatures were 34.2, 35.6, and 33.6°C, respectively, for the 0.5, 1.0, and 2.0 m·s⁻¹ air speeds. The mean room temperature and the relative humidity were 26.3°C and 61.8%, respectively. Barbosa et al. (2006), studied the influence of drying air temperature (room, 40, 50, 60, 70, and 80°C) on the content and chemical composition of *Lippia alba* (Mill) N. E. Brown essential oil, and found that there was no significant difference in the content of essential oil extracted from the product after drying at different temperatures.

Figure 4 shows the drying curves for different drying air speeds. It is evident that water was removed from *A. cymbifera* Mart. and Zucc. leaves faster at higher air speeds. Drying times of 22, 21, and 16 h were required to decrease the moisture content from 75.5 to 11.1 (% w.b.) for air speeds of 0.5, 1.0, and 2.0 m·s⁻¹, respectively. Martins (2000), evaluated drying lemongrass at temperatures of 40, 50, and 60°C with speeds of 0.5 and 1.0 m·s⁻¹ and found that the drying air speed decreased the drying time for all of the temperatures evaluated but did not influence the essential oil content or the major components.

Oliveira et al. (2013), who found that the major contents of *A. cymbifera* Mart. And Zucc. essential oil were bicyclogermacrene, spathulenol, (E)-nerolidol, δ-cadinene, α-himachalene and viridiflorol, which represent approximately 67.93, 65.52 and 69.93% of the oil obtained from plants dried in the temperatures 44.8, 36.4
and 28.4°C, respectively. One of the reasons for volatilization not occurring may be the temperature of 34.7±1.5°C used in the present study, which may have minimized the loss. According to Soares et al. (2007), the levels of O. basilicum essential oil declined at higher drying temperatures, and the greatest concentrations were obtained at 40°C with air flows of 1.9 and 0.9 m·s⁻¹.

The minor constituents germacrene B, hex-2-enal, viridiflorol and cedrol were influenced by the speed of the drying air. The contents of germacrene B and hex-2-enal

Figure 4. The drying curves for Aristolochia cymbifera Mart. and Zucc. at different drying air speeds.

Figure 5. The content of essential oil extracted from leaves of Aristolochia cymbifera Mart. and Zucc. subjected to drying at different drying air speeds. Tukey’s test at a 5% level of significance.
Table 1. The chemical composition of essential oil from the leaves of *Aristolochia cymbifera* Mart. and Zucc. subjected to three drying air speeds.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Oil compound</th>
<th>Ki²</th>
<th>0.5 m·s⁻¹</th>
<th>1.0 m·s⁻¹</th>
<th>2.0 m·s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hex-2-enal</td>
<td>839</td>
<td>0.26±0.20ᵃ</td>
<td>0.00±0.00ᵇ</td>
<td>0.00±0.00ᵇ</td>
</tr>
<tr>
<td>2</td>
<td>&lt;butyl&gt; butanoic acid ester</td>
<td>984</td>
<td>0.01±0.01ᵃ</td>
<td>0.03±0.03ᵇ</td>
<td>0.02±0.04ᵇ</td>
</tr>
<tr>
<td>3</td>
<td>Limonene</td>
<td>1024</td>
<td>0.38±0.20ᵃ</td>
<td>0.30±0.23ᵇ</td>
<td>0.43±0.29ᵇ</td>
</tr>
<tr>
<td>4</td>
<td>Linalool</td>
<td>1097</td>
<td>0.03±0.04ᵃ</td>
<td>0.07±0.05ᵇ</td>
<td>0.06±0.05ᵇ</td>
</tr>
<tr>
<td>5</td>
<td>cis-Limonene oxide</td>
<td>1137</td>
<td>0.40±0.46ᵃ</td>
<td>0.50±0.37ᵇ</td>
<td>0.34±0.35ᵇ</td>
</tr>
<tr>
<td>6</td>
<td>α-Terpineol</td>
<td>1187</td>
<td>0.01±0.02ᵃ</td>
<td>0.03±0.03ᵇ</td>
<td>0.03±0.04ᵇ</td>
</tr>
<tr>
<td>7</td>
<td>Geraniol</td>
<td>1249</td>
<td>0.07±0.06ᵃ</td>
<td>0.06±0.04ᵃ</td>
<td>0.05±0.06ᵃ</td>
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<tr>
<td>8</td>
<td>Undec-10-enal</td>
<td>1301</td>
<td>0.16±0.23ᵃ</td>
<td>0.15±0.11ᵃ</td>
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<td>9</td>
<td>Cyclosativene</td>
<td>1368</td>
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<td>2.67±0.77ᵇ</td>
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<td>α-Copaene</td>
<td>1368</td>
<td>1.41±0.86ᵃ</td>
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<td>1.20±0.71ᵇ</td>
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<tr>
<td>11</td>
<td>β-bourbonene</td>
<td>1387</td>
<td>2.70±0.73ᵃ</td>
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<tr>
<td>12</td>
<td>β-Elemene</td>
<td>1390</td>
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<td>Aromadendrene</td>
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<td>β-chemigrene</td>
<td>1476</td>
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<td>0.49±0.71ᵇ</td>
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<td>α-Curcumene</td>
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<td>0.79±0.89ᵇ</td>
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<tr>
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<td>Germacrene D</td>
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<tr>
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<td>2.23±0.21ᵃ</td>
<td>0.76±0.41ᵇ</td>
<td>1.91±1.08ᵇ</td>
</tr>
<tr>
<td>36</td>
<td>α-Murolol</td>
<td>1643</td>
<td>0.45±0.46ᵃ</td>
<td>0.56±0.40ᵇ</td>
<td>0.23±0.37ᵇ</td>
</tr>
<tr>
<td>37</td>
<td>β-Eudesmol</td>
<td>1649</td>
<td>0.38±0.75ᵃ</td>
<td>0.13±0.26ᵇ</td>
<td>0.21±0.24ᵇ</td>
</tr>
<tr>
<td>38</td>
<td>(Z)-α trans-Bergamotol</td>
<td>1690</td>
<td>0.46±0.61ᵇ</td>
<td>0.00±0.00ᵇ</td>
<td>0.26±0.44ᵇ</td>
</tr>
<tr>
<td>39</td>
<td>Farnesol (cis, cis)</td>
<td>1715</td>
<td>0.02±0.04ᵃ</td>
<td>0.22±0.32ᵇ</td>
<td>0.04±0.07ᵇ</td>
</tr>
<tr>
<td>40</td>
<td>Lanceol</td>
<td>1759</td>
<td>0.19±0.10ᵃ</td>
<td>0.14±0.28ᵃ</td>
<td>0.08±0.17ᵃ</td>
</tr>
<tr>
<td>-</td>
<td>Total identified</td>
<td>-</td>
<td>93.91±1.69</td>
<td>86.64±4.29</td>
<td>86.39±2.37</td>
</tr>
</tbody>
</table>

The mean of four independent extractions followed by the standard deviation. Kovats Index. Means followed by the same letter on the rows do not differ by Tukey's test at a 5% probability.

were lower at higher drying air speeds. In contrast, the viridiflorol content increased. The cedrol content did not differ between the 0.5 and 2.0 m·s⁻¹ speeds; however, the content decreased at the 1.0 m·s⁻¹ speed (Table 1). Certain components are present in small amounts at higher air speeds due to volatilization, as reported by
Soares et al. (2007), in which the duration of the exposure of *O. basilicum* leaves to drying air strongly influenced the magnitude of the effect that higher temperature and speed had on increasing levels of compounds.

The major component of *A. cymbifera* Mart. and Zucc. oil was spathulenol, which ranged from 26.65 to 29.56%. This compound has the smell of dry wood and can be used in flavoring compositions for food and sophisticated perfumes. It can also be applied in food, medicine, toothpaste, soaps, detergents, cleaning agents, cosmetics, skin care solutions, and other products (Naarden, 1985, cited by Mendes et al., 2008).

**Conclusion**

Increasing the speed from 0.5 m·s⁻¹ to 2 m·s⁻¹ reduced the drying time from 22 to 16 h. The speed of the drying air did not influence the content of essential oil extracted from *A. cymbifera* Mart. and Zucc. leaves. The minor constituents germacrene, hex-2-enal, viridiflorol and cedrol were influenced by the drying process. The major constituents, mainly spathulenol, were not influenced by the drying air speed.

**Conflict of Interest**

The authors have not declared any conflict of interest.

**ACKNOWLEDGMENTS**

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**REFERENCES**


Ethnobotanical study of some medicinal plants from Hoggar, Algeria

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Study was conducted from July, 2011 to March, 2012 to explore and enumerate the medicinal uses of some plants in folkloric medicine of Hoggar (Algerian Sahara). Semi-structured questionnaires were used to conduct interviews with traditional healers, herb sellers and other knowledgeable individuals on use of medicinal plants. The informants (100) consist of 63% females and 37% males of which 6% were traditional healers, 6% herb sellers, and 7% tourist guides while the others were knowledgeable individuals on medicinal plant utilization. A total of 31 plant genera belonging to 15 different families were recorded where Lamiaceae 19 (35%), Astéraceae 16 (12%), and Zygophylaceae 12 (90%) were the important families. This study provides preliminary data for further phytochemical investigation of wild plants with therapeutic potentials. Little data presented on the common usage of plants in Algeria Sahara, not only those elements of credibility to be attributed to the plants cited, also illustrated some endemic interesting plants were traditionally used for curing various health disorders in Tamanrasset (Hoggar).

Key words: Algeria, Hoggar, ethnomedicinal use, medicinal plants, Tamanrasset.

INTRODUCTION

Medicinal plants have provided modern medicine with numerous plant derived therapeutic agents. Most of these plant derived drugs were originally discovered through the study of traditional cures and folk knowledge of native population. Some of these could not be substituted despite the enormous advancement in synthetic chemistry (Hudaib et al., 2008). The World Health Organization (WHO) has reported that about 80% of the world’s population mainly depend on traditional medicine, and the use of plant extracts is mainly involved in the traditional treatment (Beverly and Sudarsanam, 2011). Plants have been used as a medicinal agent since ancient times, first only on a folkloric basis and later developed on a scientific way into a single agent drug
(Shuvasis et al., 2012). Identifying the marker compounds and the biological activities are some of the important parameters of quality assessment. Since some of the polyherbal formulations contain as much as 108 ingredients, assessing and monitoring their quality including the identification of the marker compounds and the biological activities becomes complex, difficult to achieve and is an expensive process.

An ethnobotanical identification is the first stage in the quality assurance of traditional medicine and further in discovering new drug leads from the medicinal plants (Phurpa et al., 2011). Documentation of the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. Therefore, establishment of the local names and indigenous uses of plants has significant potential societal benefits (Ugur, 2011). Plant use for medicine varies among different ethnic and cultural groups (Elufioye et al., 2012). The present work was carried out to explore the medical remedies of some medicinal plants used by the rural people of Algeria, to ascertain the detailed information on plants used by Saharan people and their usage based on ethnobotanical knowledge.

MATERIALS AND METHODS

Study area

Study area was located in the south arid zones of Algeria (2000 km from Algiers), Hoggar, with Tamanrasset town as its administrative headquarter. Ethnobotanic enquiry was performed in different areas of Hoggar region including the following: Tamanrasset, In Salah, Ablasta, Tazrouk, In Mgue, Idless, Tis, Outoul, Silta and In Gazem (Figure 1). Hoggar is divided into several natural regions, the mountainous massifs divided into two main regions: the Tefedest to the North and the Atakor in the center of the massif where it culminates the highest summits of Algeria (Tahat: 3003 m; Ilman: 2739 m; Asserkem: 2726 m) and surrounding wall tassilian to the periphery. Crossed by the tropic of cancer (22° 33’N), the Hoggar submitted to an influence of two climatic regimes: The Mediterranean regime (moderate) and the tropical regime (Sudanese). With its exceptional geographical situation; Hoggar is between a real ecological shelter of strong floristic and faunistic diversity. Several types of florae were different according to their biogeographic origins: A Mediterranean flora, a flora saharo-sindian; a flora soudano-décanian; a cosmopolitan flora; and an endemic flora (Sahki and Sahki-Boutamine, 2004; Chenoune, 2005).

Ethnobotanical survey

Ethnobotanical data were collected between July, 2011 to March, 2012. The information was mainly gathered through semi-structured interviews (Eddouks et al., 2002) that were held with selected knowledgeable individuals. Few were carried out in Arabic language and some times in Tamahq with the help of local people (Touaregs). Information regarding gathering, preparation, use, and practice of medicinal plants were also collected. In this study, 100 knowledgeable elders between the ages of 20 and 80 were randomly selected. 22% were above 61 years, 43% were aged between 41 and 60 and 36% were between the ages of 20 and 40, both rural and urban communities were visited to collect varying information on local remedies. For each medicinal plant, its use against a particular disease was assessed. Plants were collected and identified by National Institute of Forest Research (Algeria) and by the use of the flora of Ozenda (1983); as well as by the use of other publication on medicinal plants (Benchalah et al., 2004).

RESULTS

In the present study, a total of 31 plant genera belonging to 15 families were reported of which Lamiaceae 19 (35%), Astéaraceae 16 (12%), and Zygophylaceae 12 (90%) were the most important. The detailed information about the local name, parts of the plants used and medicinal uses were documented from the local people of Hoggar. The 31 species were used to treat different types of diseases such as wound and related injuries, body sickness, diarrhoea, skin problems, cephalic pains, bronchitis, cough, cold, fever, kidney problems, stomach problems, ulcer, sore throat, urinary bladder and rheumatism. The results revealed that a major proportion of medicinal knowledge comes from people living in Tamanrasset (17%), Tazrouk (14%), Idless and Ablasta (12%).

In terms of the number of important plant cited, Lamiaceae is the most predominant family of ethnomedicinal importance with six species (Salvia aegyptica, Teucrium polium, Salvia chudaei, Mentha longifolia, Marrubium deserti, Lavandula pubescens). It was followed by Astéaraceae with five medicinal plants (Atractyliis aristata, Matricaria pubescens, Asteriscus graveolens, Artemisia judaica, Artemisia campestris) and Fabaceae, Apiceae, Caparidaceae with two medicinal plants each. Other families (Chenopodiaceae Salvadoraceae, Solanaceae, Resedaceae, Polygonaceae, Myrtaceae, Axlepiaceae, Ramnaceae, Rutaceae, Poaceae) were represented with one species of ethnomedicinal importance to cure various ailments among Hoggar people. Whereas, Hammiche and Maisa (2006) have reported Astéaraceae family as the dominant family in their investigation with 12 endemic species in Tassili N’ajjer. Persons interviewed mentioned that they collected plant parts mostly in spring or summer, as Hudaib et al. (2008) have recorded that medicinal plants are collected in spring and used all year long. Mostly, plant parts were used for herbal preparations in dried form rather than in fresh form (Lone et al., 2014). Preparation of medicinal plants is varied such as: poultice, powder, and inhalation. However decoction, powder and infusion were the most form of preparation. These results were in agreement with the literature of Kola et al. (2008), Zheng and Xing (2009) and Pascal et al. (2011), wherein preparations were made with water as a solvent. The parts of the plant primarily used are the aerial parts, leaves, while roots and seeds are sometimes used. Some plants are cited in the survey as endemic and have little data or have never been studied, and are
Figure 1. Geographical situation of Tamanrasset (Hoggar) in Algeria.

taken for their phytochemical screening as has been described by Chew et al. (2011). For example: *A. graveolens, Lavandula antineae* and *Artemisia judaica* (Table 2). Women (63%) use medicinal plants more frequently than men (37%); these results are in agreement with Jouad et al. (2001) and Tahraoui et al. (2007). This could be explained by: women are more attached than men to everything traditional; the relative frequency of analphabetism of women in our society could be behind at the credulity toward information and particularly toward indication on the use of medicinal plants and the easiness of transmission of this information between women. This may explain their relative knowledge in this area (Jouad et al., 2001). Likewise the following plants are reported to have different types of uses by the various local communities and according to the literature elsewhere.

*Cymbopogon schonenthus* is reported to have sedative, digestive and aromatic properties (Katiki et al., 2012). In the South of Tunisia, this plant is also used for the treatment of rheumatism, and to diminish fever, which is in agreement with our study (Khadri et al., 2008).

*Solenstema argel* was used in folk medicine as a remedy for bronchitis, gastrointestinal cramps, stomachache, colic, cold and urinary tract infections. In Libya and Chad, a decoction of leaves is used to treat neuralgia and sciatica (Innocenti et al., 2006). It is commonly used for the treatment of cough and as a purgative (Hassana et al., 2001; Plaza et al., 2005). *Capparis spinosa* flowers, buds, fruits, seeds, shoots and bark of roots were traditionally used for pharmacological purposes, especially for rheumatism (Jiang et al., 2007; Fu et al., 2008), their floral buttons were employed as a flavouring in cooking and are also used in traditional medicine for their diuretic, antihypertensive, poultice and tonic properties (Panico et al., 2004). Dried seed of *Zizyphus jujuba* has been used as a tranquilizer, an analgesic and an anticonvulsant in oriental countries such as Korea and China for centuries (Ma et al., 2007). Some of the plants reported in the present study are interrelated with the study of Benchallah et al. (2004) and Hamich and Maisa (2006).

Similarities in the use of a Saharan species between local people may support the presence of specific active compounds in these plants, which may be useful for finding cures for specific ailments. *Salvia chaidaei* is used in the treatment of digestive diseases (diarrhoea, ulcer) rheumatisms, kidney diseases, by the studied local people. In the literature the plant is reported to dysmenorrhea, abdominal pains, spasms sun stroke, gonorrhoea (Hamiche and Maisa, 2006). Sahki and Boutamine (2004) reported that there are more than 292 plants which have been reported to have medicinal uses among the various region in the Hoggar, and these plants were frequently used to treat stomach problems, poisonous bites, nervous disorders, cough, fever, asthma
and diabetes but some of them were exotic for example: Argania spinosa, Moringa oleifera, Eucalyptus bosistanana and Populus nigra. But there is not much information available in the literature about the composition or biological activities of these plants used in this region with an exception to the study of Chentoufe at al. (2012), Roukia et al. (2013), Hammoudi et al., 2013 and Bouzabatta el al. (2013).

DISCUSSION

In Hoggar, the traditional pharmacopoeia exposes a wide arsenal of plant remedies, because it represents a key biodiversity site in the central Saharan ecosystem in Algeria; and it potentially constitutes one of the prime sites in the world for phytochemical investigation. Floristic diversity is presently estimated at about more than 292 species with high levels of endemism (Sahki and Sahki-Boutamine, 2004). This study revealed that about 31 threatened plant species are being used as medicine by local people in Hoggar especially in rural. So we can observe that phytotherapy is frequently practiced by Sahara population in this region. The reasons of the use of medicinal plants are that these natural remedies are less cheap and more efficient than modern medicines. Digestive disease is the most important ailment treated on the basis of number of citations for medicinal uses (Table 1). This is followed by fever, rheumatism and diabetes. Teucrium polium, Myrtus nivellie and Cymbopogon schonenthus are the three leading species being used as remedies against a variety of complaints in the area. The high diversity of use of these three species could be attributed to their relative abundance in the area. The high consensus of the informants on the medicinal use of these species shows the importance of these plants to the Sahara people. Some plants that were cited during the course of this study were also reported by authors elsewhere in other parts of Algeria (Hammiche and Maisa, 2006; Rebbas et al., 2012; Miara et al., 2013) and in Morocco (Jouad et al., 2011; Tahraoui et al., 2007). It is tempting to speculate that a high frequency of use of plant is related to high efficacy and safety of the plant material (Tahraoui et al., 2007). The biological activities of some of Algeria medicinal plants are known already from other studies that were carried out elsewhere (Djeridane et al., 2006; Atmani et al., 2009, 2011; Chelli-Chentouf et al., 2012; Bakchiche et al., 2013; Benariba et al., 2013; Bouzabata et al., 2013). Most preparations are made with water as a solvent and the majority of the remedies are taken orally from a single plant; mixtures are used rarely. Some people have told us that Artemisia judaica is recognised in mixture in multiple prescriptions mainly for wounds. The reason for the dominance of herbaceous medicinal plants could be because of their abundance and year round availability in the study area. The phytochemical investigations (Table 2) revealed the presence of several secondary metabolites, alkaloids, tannins, saponins, flavonoids and terpenoids, hence signifying the therapeutic effect which strongly supports the conventional use of this plant against various diseases.

A. judaica susp sahariensis Chev (Astéracea) known under the names of "Tehereglet" in Tamahaq and "Chih" in Arabic is an endemic species, mainly used as powder (43, 18%) or infusion (27, 84%) preparation by the local people in Hoggar to treat digestive diseases (42, 99%). Studies on the chemical constituents of A. judaica have been carried out by many investigators and have shown the presence of various compounds, for example flavonoids and sesquiterpene lactones. The essential oil of A. judaica contains piperitone (61, 9%) terpine-4-ol (4, 6%) and bornyl acetate (3, 0%) (Dob and Chelghoum, 2006). The water and alcoholic extracts of A. judaica from Egypt significantly reduced the blood glucose level in experimentally diabetic rats (Nofal et al., 2009).

Asteriscus graveolens Forsk (Astéracea) known under the names of "Tamayu" in Tamahaq and "Tafss" or "Noug" in Arabic was frequently (leaves and stems 60, 34%) used for diabetes (27, 91%) and rheumatism (26, 36%). According to literature Asteriscus genus was characterized by the presence of sesquiterpenes (Rauter et al., 2001), but flavonoids, bisabolone hydperoxides were also described as constituents in their extracts (Aks Sira et al., 2006). The essential oil of A. graveolens from Bechar in Algeria is characterized by the main constituents: 1, 8 cinéol 21, 5% in leaves, 16, 5% in flowers and δ cadinol 19, 1% in leaves, 13, 9% in flowers (Cheriti et al., 2007). Oxygenated sesquiterpenes with 6-oxocyclonerolidol (74.9%) and 6-hydroxycyclonerolidol (11.8%) are the major components to this oil from Morocco. The inhibition of the corrosion of mild steel in sulphuric acid solution by A. graveolens essential oil has been studied. Investigation was found to increase with increasing concentration of the essential oil to attain 82, 89% at 3 g/L (Znini et al., 2012).

Myrtus nivellii Batt and Trab (Myrtace) known under the names of "Tafaltasset" in Tamahaq and "Rainhane Essahara El Wousta" in Arabic were reported by local people for diabetes (34, 15%) and digestive diseases (25, 20%). The chemical composition of essential oil from central Sahara of Algeria is largely dominated by 1,8-cineole (33.6 to 50.4%) and limonene (17.5 to 25, 20%). The structure of two new compounds bearing the isoamylicyclopentane skeleton has been elucidated. The oil was more active against Cryptococcus neoformans with MIC of 0.16 μl/ml followed by dermatophytes, with MICs of 0.64 and 1.25 μl/ml. Furthermore, evaluation of cell viability showed no cytotoxicity in HaCaT keratinocytes at concentrations upto 0.25 ml/ml. Rached et al. (2013) have been reported the phenolic compounds and its antioxidant activity to this species.

Cymbopogon schonanthes Spreng known under the names of “Teberint” or “Teberimt” in Tamahaq and “El
### Table 1. Medicinal plants used in Hoggar, Algeria.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Tamahag name</th>
<th>Plant part used</th>
<th>Preparation</th>
<th>Therapeutic uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvia aegyptiaca L</td>
<td>Sassaf</td>
<td>Seeds, aerial parts</td>
<td>Infusion, powder, decoction</td>
<td>Fever, chills, Eye wash, digestive, diseases</td>
</tr>
<tr>
<td>Teucrium polium susp. Lam</td>
<td>Talmezout</td>
<td>Leaves, aerial parts</td>
<td>Decoction, infusion, powder, poultice</td>
<td>Fever, diabetes, digestive diseases, Menstrual disorders, arterial, Hypertension, Wounds</td>
</tr>
<tr>
<td>Salvia ch уд ai Batt and Trab</td>
<td>Aouit</td>
<td>Leaves, aerial parts</td>
<td>Decoction, powder</td>
<td>Digestive diseases (diarrhoea, ulcer) Rheumatism, kidney diseases</td>
</tr>
<tr>
<td>Mentha longifolia L</td>
<td>Taïhart</td>
<td>Leaves, aerial parts</td>
<td>Powder, decoction, infusion</td>
<td>Diabetes, Fever Arterial hypertension Jaundice</td>
</tr>
<tr>
<td>Marrubium deserti De Noë</td>
<td>Telheret</td>
<td>Leaves, aerial parts</td>
<td>Decoction, infusion</td>
<td>Fever, arterial Hypertension</td>
</tr>
<tr>
<td>Lavandula pubescens (Maire) susp antiniae Lam</td>
<td>Adjoua</td>
<td>Aerial parts, leaves, leaves+ stems</td>
<td>Infusion</td>
<td>Diabetes Cough, chills Rheumatism</td>
</tr>
<tr>
<td>Matricaria pubescens Scultz</td>
<td>Ameskik</td>
<td>Aerial parts</td>
<td>Decoction, infusion</td>
<td>Fever, digestive diseases, Allergies</td>
</tr>
<tr>
<td>Lavandula pubescens (Maire) sus</td>
<td>aya nesit</td>
<td>Aerial parts</td>
<td>Decoction, infusion powder</td>
<td>Allergies, digestive diseases, Fever, Spasms</td>
</tr>
<tr>
<td>Asteriscus graveolens Forsk</td>
<td>Tamayu</td>
<td>Leaves, aerial parts, (leaves+stems)</td>
<td>Décoction, ointment</td>
<td>Diabetes, Rheumatism Migraines, Dermatosis Respiratory diseases</td>
</tr>
<tr>
<td>Artemisia judaica susp. sahariensis (Chev) Astéracées</td>
<td>Tshenegle</td>
<td>Leaves+ flowers, Aerial parts</td>
<td>Décoction, infusion, Poultice, powder, Inhalation</td>
<td>Digestive diseases: (vomits), fever, Respiratory diseases, Wounds</td>
</tr>
<tr>
<td>Artemisia campestris L. Astéracées</td>
<td>Tedjik</td>
<td>Leaves, aerial parts</td>
<td>Décoction, infusion, powder</td>
<td>Digestive diseases, Fever, after childbirth Hair loss</td>
</tr>
<tr>
<td>Fagonia bruguieri DC zygophyllaceae</td>
<td>Afessour</td>
<td>Leaves, aerial parts</td>
<td>Décoction, infusion, Powder</td>
<td>Jaundice, Digestive diseases, anemia kidney diseases</td>
</tr>
<tr>
<td>Tribulus terrestris L. zygophyllaceae</td>
<td>Tadjaroft</td>
<td>Leaves, aerial parts</td>
<td>Décoction, infusion, powder</td>
<td>Diabetes, Digestive diseases Fever, kidney diseases</td>
</tr>
<tr>
<td>Balanites aegyptiaca Del zygophyllaceae</td>
<td>Tabournak</td>
<td>Roots, Leaves, aerial parts, cortex, fruit</td>
<td>Décoction, infusion</td>
<td>Diarrhoea, fever, Migraine, pain of the stuffs Cough</td>
</tr>
<tr>
<td>Zygophyllum album L. Zygophyllaceae</td>
<td>Abebekz</td>
<td>Leave, aerial parts</td>
<td>Décoction, powder</td>
<td>Fever, diabetes, Digestive diseases Hypertension, Wounds</td>
</tr>
<tr>
<td>Acacia nilotica L. Fabaceae</td>
<td>Taggart</td>
<td>Root, leaves, aerial parts, cortex</td>
<td>Décoction, infusion, Powder</td>
<td>Diabetes, Digestive diseases,Anemia, fever, Arterial hypertension,</td>
</tr>
<tr>
<td>Acacia tortilis (Forsk) Fabaceae</td>
<td>Abser</td>
<td>Leaves, aerial parts, cortex, latex</td>
<td>Infusion, powder Décoction</td>
<td>Ulcer stomach Fever, arterial hypertension, wounds</td>
</tr>
<tr>
<td>Deverra scoparia Cass and Dur Apiaceae</td>
<td>Tattalt</td>
<td>Leave, aerial parts</td>
<td>Décoction, infusion, Powder</td>
<td>Digestive diseases Rheumatism, Rheumatism, Diabetes</td>
</tr>
<tr>
<td>Ammodaucus C and D Meutricotrichus Apiaceae</td>
<td>Akamman</td>
<td>Seeds, aerial parts</td>
<td>Décoction, infusion, Powder</td>
<td>Digestive diseases, vomiting, fever Appetite</td>
</tr>
<tr>
<td>Cleome arabica subsp. amblyocarpa (Barrate and Murb) Caparidaceae</td>
<td>Shouya r</td>
<td>Leaves, aerial parts</td>
<td>Décoction, infusion, powder, poultice</td>
<td>Digestive diseases, Cough, Rheumatism, Respiratory diseases,</td>
</tr>
<tr>
<td>Caparidaceae</td>
<td>Tahoulout</td>
<td>Leaves, aerial parts</td>
<td>Décoction, poultice, Infusion</td>
<td>kidney diseases, Articular pains</td>
</tr>
<tr>
<td>Atriplex halimus L Chenopodiaceae</td>
<td>Arames</td>
<td>Leaves, aerial parts, roots</td>
<td>Décoction, infusion, powder</td>
<td>Cysts</td>
</tr>
</tbody>
</table>
Lamad” in Arabic was mentioned for the treatment of digestive (32, 39%) and renal (38, 06%) diseases. Essential oils of C. schoenanthus of Borkina Faso were determined. Among the identified compounds, two monoterpenes (peperitone and δ2 carene) remain the principal components in the oil (Onajah et al., 2007). The insecticidal effect of essential oil of this species from Togo has been studied (Ketoh et al., 2004). Aqueous extract, proanthocyanidin rich extract, and organic extracts of C. schoenanthus shoots from three different locations in South Tunisia were screened for their antioxidant, acetylcholinesterase and antimicrobial activities (Khadri et al., 2010).

Lavandula pubescens susp (Maire) is known under the names of “Ajoua” or “Ttehenok” in Tamahaq. The Arabic name was not found. It was widely used in infusion form for cough, chills and rheumatisms by nomads, is an endemic species from central Sahara of Algeria. No data has been published on the constituents of this plant. These are probably similar to those in other Lavender species camphor, linalool and linalyl acetate

| Table 2. Preliminary phytochemical screening of selected plant from Hoggar |
|------------------|------------------|------------------|------------------|------------------|
| Class            | Asteriscus graveolens | Artemisia judaica | Myrtus niveili | Lavandula antineae | Cymbopogon shonenthus |
| Alcaloids        | ++                | +++              | ++             | ++                | ++                |
| Tanins           | ++                | +++              | +++            | ++                | ++                |
| Flavonoids       | ++                | +++              | +++            | ++                | +                 |
| Saponins         | +                 | +++              | +++            | ++                | +                 |
| Terpenoids       | ++                | +++              | +++            | ++                | ++                |

+ = Present, ++ = Present appreciable, +++ = Present very appreciable.
CONCLUSION

Plants constitute an unlimited source of medicine for the local people living in Hoggar and phytochemicals available for improving human health. Knowledge on utilization of plant resources for health care delivery varies with cultural background globally. This study presents a useful documentation which can contribute to preserving knowledge on the use of medicinal plants in this region, yielded 31 candidate plants with important compounds that can be researched further in areas of Phytochemistry for possible leads in the development of novel drugs with little or no side effects and transferring it to future generation.

ACKNOWLEDGEMENTS

We thank all the persons of the study area for their cooperativeness. We are also very thankful to National Institute of Forest Research (Algeria) for their help in collecting and identification of plant species.

Conflict of interest

The authors declare that they have no conflict of interest.

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We thank all the persons of the study area for their cooperativeness. We are also very thankful to National Institute of Forest Research (Algeria) for their help in collecting and identification of plant species.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


Isolation and analgesic property of lupeol from *Diospyros mespiliformis* stem bark

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*Diospyros mespiliformis* Hochst (Ebenaceae) stem bark is used in traditional medicine for the management of pain related ailments. Several bioactive compounds have previously been isolated from the plant material that includes pentacyclic triterpenes. This study sequentially extracted and carried out a bioassay-guided fractionation of the plant crude material with solvents of varying polarity using analgesic efficacy in rats as bioactivity marker, aimed to isolate the active constituent. Powdered stem bark of the plant was sequentially extracted with hexane, chloroform and methanol; and preliminary tested for analgesic activity. The chloroform extract being the most active amongst the three extracts was subjected to column chromatography, and a fraction was eluted with mixture of hexane and ethyl acetate (50:50%) which yielded a compound. Three dose levels (25, 50 and 100 mg/kg) of the compound were administered orally to rats. Acetylsalicylic acid (100 mg/kg, p.o.) was used as the positive control. Nociception was induced mechanically using analgesy meter, and chemically with formalin. The compound alleviated the pain stimulus induced by the analgesy-meter and formalin in rats. The isolated compound was identified as lupeol using thermo-analysis (DSC), colorimetric, chromatographic and spectrometric techniques that included: UV-visible, IR, and ¹³C- and ¹H NMR. It was concluded that lupeol acting alone or synergistically might be responsible for the beneficial effect of the plant in treatment of pain related ailments.

**Key words:** *Diospyros mespiliformis*, lupeol, analgesic.

**INTRODUCTION**

*Diospyros mespiliformis* Hochst. ex A.DC. -- Prodr. (A. P. de Candolle) 8: 672. 1844 (mid Mar 1844) (IK) family: Ebenaceae (http://www.ipni.org), is a tree with white fragrant flowers and soft sweet pulp fruit that grows wild in tropical regions of Africa and Asia. The plant is reputed for its medicinal values, and is used in ethnomedical...
practice for treating various ailments that include sleeping sickness, malaria, headache, cough, leprosy, helminth infection (Belemotougi et al., 2006) and toothache (Etkin, 1981). Its seeds are also known to have nutraceutical value in managing high cholesterol, reducing risk of type-2 diabetes, and for weight control (Chivandi and Erlwanger, 2011). Useful biologically active compounds including naphthoquinone epoxide, α-amyrin, β-sitosterol, betulin and betulinic acid amongst others were isolated from the plant (Lajubutu et al., 1995; Mohamed et al., 2009).

Despite some advantages in the medical use of plant extracts over isolated entities, there is a need to identify the component which is responsible for the observed beneficial effects. This study sequentially extracted and fractionated the stem bark of *D. mespiliformis* in a bioassay-guided manner using analgesic activity in rats as bioactivity marker in order to identify its active component.

**MATERIALS AND METHODS**

**Plant material**

*D. mespiliformis* was collected at Chaza village, near Suleja (9°10'49 N; 7°10'45 E), Niger State, Nigeria. It was authenticated by Ibrahim Muazzam of Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (#5120) was deposited at the herbarium of the Institute. *D. mespiliformis* is not an endangered plant species, and therefore its collection for purposes of use and scientific study does not require prior authorization.

**Extraction, fractionation and compound isolation**

Stem bark of the plant was collected, cleaned, dried under shade and ground into powder. The powdered material (500 g) was sequentially extracted with hexane (DM-1), chloroform (DM-2) and methanol (DM-3) to yield 0.97, 1.23 and 7.16% of the extracts, respectively. The three extracts (DM-1, 2, 3) were preliminary tested for analgesic potency in a pilot experiment (data not shown) using the formalin test (described below); being a model in which both peripheral and centrally mediated pain relief could be measured. The chloroform extract (DM-2) was found to be the most active amongst DM-1, 2, and 3, and was subjected to column chromatography (Still et al., 1978) using silica gel 230 to 400 mesh (Sigma-Aldrich Co., St. Louis, MO, USA). The column was eluted first with hexane, followed by mixtures (100 ml) of hexane: ethyl acetate; and ethyl acetate: methanol in increasing polarity gradient. Fifty ml of the eluates were individually collected, monitored with analytical TLC on precoated silica gel adsorption plates with 250 micron layer thickness (Whatman K5 150 A, Waltham, MA, USA) and visualized under Ultraviolet (UV) light (254/365 Eagle Scientific Ltd, UK). Eluates which were found to have the same thin-layer chromatography (TLC) profile were combined together. A fraction (initially denoted DM-2B) was eluted with mixture of hexane and ethyl acetate (50:50%), and on drying yielded a compound.

**Structural elucidation**

The isolated compound was identified using calorimetric, chromometric, chromatographic and spectroscopic techniques. Differential scanning calorimeter (DSC) (NETZSCH DSC 204F1, Netzsch-Gerätebau GmbH, Selb, Germany) was used for thermochemical analysis. UV-visible spectra were recorded on UV-160A instrument (Shimadzu Corporation, Kyoto, Japan) by recording the absorption of 1 mg of the isolated compound in 10 ml ethanol (99%). IR spectra were taken in KBr pellets (FTIR-8400 S (CE), Shimadzu, Japan). The 13C- and 1H NMR spectra including 2-dimensional 1H–13C and 1H–1H correlation spectroscopy (COSY) were recorded on Bruker DRX 500 NMR (Bruker BioSpin, Rheinstetten, Germany) equipped with 5-mm QNP probe, 2H lock switch box and BVT 2000 heater. CDC13 was used as solvent and Tetramethysilane (TMS) as internal reference. The chemical shifts were recorded in δ (ppm) and coupling constant in Hz. Distortion enhancement by polarisation transfer (DEPT) analysis was performed for proton attachment, heteronuclear multiple bond correlation (HMBC) for proton-carbon (1H–13C) H–H coupling and correlation spectroscopy (COSY) coupling constants.

**Animals**

Wistar rats of both sexes obtained from Animal Facility Centre, NIPRD, Abuja, Nigeria, were used for the study. The animals were kept in propylene cages with saw-dust as bedding, and maintained on standard laboratory feeds with water ad libitum. They were used in accordance with Ethical Guidelines for Investigation of Experimental Pain in Conscious Animal (Zimmermann, 1983), in line with NIPRD’s standard procedures on laboratory animal usage (NIPRD QMS/SOP no. 05:3:06).

**Analgesy (Randall-Selitto test)**

This test was performed using the modified Randall-Selitto (1957) test with Ugo Basile Analgesy-Meter (No. 7200, Italy). In the test, a meter exerts force at a constantly increased rate on rat paw monitored by a pointer moving along a linear scale. Twenty five rats were grouped into five groups (*n* = 5) and treated p.o. with vehicle (distilled water; 10 mL/kg), lupeol (25, 50 and 100 mg/kg), or acetylsalicylic acid (ASA) (100 mg/kg). The rat paw was gently placed between the plinth and plunger of the instrument and increased pressure (exerted by 20 g) applied to the middle dorsum of the rat’s left hind paw. Stimulus was terminated and force threshold readings taken as soon as nociceptive response were elicited by the rats. Readings were taken pretreatment and at 15, 30 and 60 min after treatment.

**Formalin test**

The method described by Dubuisson and Dennis (1977) was adopted for this assay, with little modification (Adzu et al., 2014). Briefly, the animals were treated p.o. with water (10 mL/kg), lupeol (25, 50 and 100 mg/kg), or acetylsalicylic acid (ASA) (100 mg/kg). They were then injected s.c. with 50 µL solution 2.5% formalin into the sub-plantar surface of rat left hind paw, 30 min after the treatment. Severity of pain was rated in two distinct phases for 60 min: the first phase (0 to 10 min) taken every 2 min and late phase (15 to 60 min) every 5 min using 3 pain-induced behaviour in the following scoring manner: 0 - normal weight bearing on the injected paw; 1 - light resting on the paw on the floor; 2 - elevation of the injected paw and 3 - for licking, biting or grooming of the injected paw. The mean (+SEM) of the readings was recorded as the pain score, after which the left paw oedema volume of each rat was measured and compared with that of the right hind paw using a digital plethysmometer (LE 7500, LETICA, Spain) 1 h after the formalin injection.
RESULTS AND DISCUSSION

In a previous study, crude extracts of *D. mespiliformis*’s pain and fever relief activity in rodents was shown (Adzu et al., 2002). This study carried out a bioassay-guided investigation to identify the active compound using analgesic effect in rats. In the course of the evaluation, a fraction eluted by mixture of hexane/ethyl acetate (50:50%) from the CHCl₃ extract was obtained. On drying, it yielded a white powdered compound. Calorimetric, colorimetric, chromatographic and spectroscopic investigations of the compound showed: mp 197°C on DSC (Figure 1); UV MeOH 209 nm; and IR 771.55 (C–H), 1043.52 (C–O), 1215.19 (C₃–C), 2926.11 (C–H) and 3018.70 cm⁻¹ (O–H). The NMR spectra signals were obtained for: 1H (Fig. 2); 13C (Figure 3); and 1H–1H COSY (Figure 4). The highlights are: one H protons (δ 4.69 and 4.59 ppm), and carbons (δ 109.32 and 150.98 ppm); hydromethine proton (δ 3.19 ppm) and carbon (δ 79.05 ppm); singlet signals (δ 0.77, 0.80, 0.84, 0.95, 0.98, and 1.04) assigned to tertiary methyl group; and absence of aromatic proton (δ 6 to 8 ppm). Other details were shown in Figures 2 to 4. The assignment of these NMR signals, aided by the UV-visible, IR and DSC data; and comparisons with relevant literatures (Igoli and Alexander, 2008; Bagalkotkar et al., 2011) identified the compound as lup-20(29)-en-3β-ol (lupeol; Figure 5).

The analgesic potency of the isolated compound was investigated using rats. Such animal models generate reliable data that gives high predictive value in humans (Normandin, 2007); by identifying target and provide proof of efficacy (Hart et al., 2004). In some instances, these in vivo models have advantages over vitro techniques (Houghton et al., 2007). The compound was first tested on mechanical model using analgesy-meter. The test is based on the principle that inflammation increases the sensitivity to nociception and this sensitivity is susceptible to modification by analgesics. The average pre-treatment response of the rats to the model was 4 min, which was maintained throughout the 60 min duration of the experiment by the vehicle control groups. Lupeol and the standard drug (ASA) alleviated the induced pain by prolonging the rats’ responses significantly (*p* < 0.05; Table 1). The fact that lupeol increased the threshold of the intact paw suggests analgesic effect involvement of both peripheral and centrally mediated activity (Vongtau et al., 2004).

The compound was also evaluated against chemically induced pain using formalin test. The test is biphasic, and measures pain of both neurological (first phase) and inflammatory origin (second phase). The test is recommended as a basic pain research for studying the
Table 1. Inhibition of analgesy in rats by lupeol isolated from the CHCl₃ extract of *Diospyros mespiliformis* stem bark.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Threshold (mean ± SEM) a at time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre b</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>4.16 ± 1.5</td>
</tr>
<tr>
<td>Lupeol</td>
<td>25</td>
<td>4.07 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.96 ± 0.4</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>4.60 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.24 ± 0.8</td>
</tr>
</tbody>
</table>

*Weight (20 g), mean ± SEM; bPre-treatment; n = 6; one-way ANOVA, followed by Student-Newman–Keuls test for multiple comparison. *p < 0.05; **p < 0.01 vs. vehicle, ASA – acetylsalicylic acid.

mechanisms of analgesic agents because of its connection to tissue injury (Tjolsen et al., 1992). Lupeol exhibited significant analgesic activity on both phases of the formalin test; with maximal % inhibition of 60% in the first phase, and 31% at the second phase (Table 2). The formalin test model is accompanied by the development
Table 2. Inhibition formalin induced noxious stimulus test in rats by lupeol isolated from the CHCl₃ extract of *Diospyros mespiliformis* stem bark.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Pain inhibition (%)</th>
<th>Oedema volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First phase</td>
<td>Second phase</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>2.56 ± 0.2</td>
<td>2.48 ± 0.1</td>
</tr>
<tr>
<td>Lupeol</td>
<td>25</td>
<td>2.2 ± 0.2*</td>
<td>1.98 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.75 ± 0.2*</td>
<td>1.72 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.88 ± 0.2**</td>
<td>1.91 ± 0.1*</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>1.0 ± 0.3**</td>
<td>1.15 ± 0.1*</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01 vs. vehicle; ASA – acetylsalicylic acid

Figure 3. The $^{13}$C NMR spectra of lupeol isolated from the CHCl₃ extract of *Diospyros mespiliformis* stem bark.

of oedema in the injected left paw due to release of inflammatory mediators, and the oedema volume after the assay was taken to evaluate the action of lupeol against this process. Suppressing the induced pain and oedema by lupeol in this study reaffirmed its pain relief effect.

Lupeol, a safe and pharmacologically active triterpenoid is widely distributed in plant kingdom, but less applied in therapy (Gallo and Sarachine, 2009; Siddique and Saleem, 2011). It is known to elicit its activity mainly via the inhibition of tissue response to the induced nociception (Geetha and Varalakshmi, 2001; Chen et al., 2012), especially through the involvement of cytokines (De Lima et al., 2013).
Figure 4. The $^1$H – $^1$H COSY spectra of lupeol isolated from the CHCl$_3$ extract of Diospyros mespiliformis stem bark.

Figure 5. (lup-20(29)-en-3β-ol).
Conclusion

This is neither the first time lupeol is isolated from *D. mespiliformis* nor its bioactivity being demonstrated. However, this study is unique because it linked the analgesic effect of the plant material to the isolate lupeol; acting alone or synergistically with other phytochemical constituents. This might stimulate more interest in this compound since analgesics prototype like salicylic acid and morphine, and several other bioactive agents in modern pharmacopoeia were derived from products initially used in traditional medicine.

Conflict of Interest

The authors have not declared any conflict of interest.

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