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# COMPARATIVE STUDY OF MORPHOLOGICAL, ANATOMICAL, MOLECULAR AND ESSENTIAL OIL OF *LANTANA HORRIDA* AND *LANTANA CAMARA* VERBENACEAE)

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**Abstract**

*Lantana horrida*, which is frequently used as an ornamental, is not as well-known or marketed as *L. camara*. *Lantana horrida*. It distributed from West Indies and Mexico to central America. Its habitats include tropical savanna, forests, montane areas, shrublands, and grasslands. To develop effective control strategies, it is crucial to accurately distinguish this species from others within the Lantana genus. This is particularly important given the close resemblance between L. horrida and L. camara. Taxonomic analysis of viable specimens is typically used for this purpose. This study aims to investigate the use of morphological, anatomical, molecular, and chemical features of the leaves from both species to support precise classification.

Anatomical research was done on *L. horrida* and *L. camara* leaves using light and scanning electron microscopy. The main difference was detected in the petiole, which offered secretory idioblasts in *L. horrida*. Gel electrophoresis of *L. horrida* and *L. camara* total DNA digested by SaII enzyme. There was partial genetic variability between *L. horrida* and *L. camara* DNA. Gas chromatography-mass spectrometry (GC-MS) was also used to perform metabolic profiling of the investigated species. Through GC-MS summarizing, 38 compounds were known with clear fluctuations in the extracts for each species. Among them, the naturally occurring bicyclic sesquiterpene b-caryophyllene stood out and could help in the chemical classification of the extracts of the studied species. The b-caryophyllene was 35.9 in *L. horrida* and 15.4 in *L. camara*. Identifying internal secretory formations for the first time in this genus was possible. Each species has distinct glandular and non-glandular trichomes and an abaxial surface. These results can help strategies to control *L. horrida* without affecting L. camara.

**Key words:** Idioblasts; *Lantana horrida*; *Lantana camara*; Glandular trichomes; Weed ornamental plant.

## Introduction

Verbenaceae belongs to the class of Dicotyledons. Verbenaceae are roughly 1000 species and 35 genera, and they are a relatively large family native to tropical and subtropical regions. They also reach temperate regions Cardoso *et al*., 2021. This group includes trees, shrubs, and herbs that are identified by their opposite leaves, heads, spikes, or clusters of small flowers, several of which smell aromatic (Stevens, 2001). It also includes flowers that have slightly bilateral corolla symmetry. Their fruits can have two or four seeds, often split into two or four segments. They can also be juicy or dry (Marx *et al*., 2010). The Verbenaceae family, including the Lantana genus, has been extensively studied. It is part of a larger family that comprises around 2600 species distributed in tropical and subtropical regions worldwide. Lantana, described by Linnaeus in 1753, has been widely researched for its biological and pharmacological properties (Ghisalberti, 2000). Since it was brought to many nations, lantana has taken up residence in various habitats across a large geographic area. Taxonomically, it poses challenges due to unstable species, widespread hybridization, and varying inflorescence and flower color with age and maturity. Native to the Americas, with some taxa originating from Asia and Africa, Lantana is grown for decorative purposes due to its beautiful flowers. Due to their anti-inflammatory qualities, various Lantana species are employed in traditional medicine. The leaves have crystals in the mesophyll and other tissues, contributing to their defensive role against herbivore attacks. Notable differences in morphology between species can contribute to taxonomic and systematic aspects. (Passos *et al*., 2012).

The species *Lantana horrida* and *Lantana camara* are included in the Verbenaceae family, known for their aroma and medicinal properties. *Lantana camara*, native to the American continent, is one of the species that has become invasive and is present in various locations worldwide. Invasiveness can be due to several characteristics, such as reproductive potential, dispersion, allelopathic potential, thick cuticle, and leaf thickness (Sanders 2012; Sultana & Rahman, 2020). *Lantana horrida* is a species of Verbena that has flowers. It can be found in subtropical South America, Mexico, and the West Indies. It inhabits many environments, such as montane, tropical savanna, forest, shrubland, and grassland. The perennial shrub *Lantana horrida* has a height of 61 to 122 cm and a width of 61 to 152 cm. From spring to fall, bright red, orange, and yellow blossoms draw birds and butterflies. Often found in forested areas, this shrub is extremely resistant to deer. (Wasowski & Wasowski, 1991).

The study of a plant's internal structure is generally referred to as phytotomy or plant anatomy. Plant anatomy is now only used to describe the inner structures of plants, although it formerly involved plant morphology, which describes the physical form and external structure of plants. This distinction was made around the middle of the 20th century. These days, cellular investigations of plant anatomy are common and frequently entail tissue sectioning and microscopy. Studies on anatomy are very important in many fields of study. The where, what, when, and how chemical component production can be explained by studying the anatomy of plants. The anatomical study explains the characteristics of wood. An investigation into the anatomical structure was conducted to address the taxonomy issues with important species and genera

(Duletić-Laušević *et al*., 1997). Numerous studies on the anatomy of various plant families, including the Asteraceae (Hadad *et al*., 2013), Cleomaceae (Thenmozhi *et al*., 2013), and Malvaceae (Garica *et al*., 2014). The commercial value of Verbenaceae plants lies in their ability to produce biochemicals, yield flowers, and be used as traditional medicinal plants. The wood from the trees is used to make paper, furniture, poles, etc (Rahmatullah, *et al*., 2011).

The leaf anatomy of *Lantana camara* has been studied independently, but information on *Lantana horrida* is scarce, mainly focusing on medicinal plant leaves. The present study aimed to investigate comparative studies on the morphological, chemical, anatomical, and molecular characteristics of *Lantana horrida* kunth and *L. camara* that is a relevant genus due to its ecological importance, although there are still no studies describing its anatomical features with emphasis on internal secretory cells. This research aimed also to characterize the internal secretory cells throughout the leaf blades of *Lantana camara* leaves. For this purpose, anatomical studies and histochemical assays were performed.

## Material and Methods

**Identification and collection of plant:** The botanical material came from plants grown in the greenhouse of the University of Shaqra campus. A specialist identified and herborized the plants, comparing each plant's morphological characteristics to those of the formerly recognized taxa published by Bailey (1951). Voucher specimens were deposited at the herbarium at the Department of Biological Sciences, Faculty of Science and Humanities, Shaqra University (PHI-V-LC-144), and (PHG-V-LH-145).

This research compares the anatomical aspects of the species found in Shaqra, Rhiad, Saudi Arabia herbals. Leaves and flowers were collected on 2nd March 2022 and 2023. The goal is to verify anatomical differences between the species used for the same purpose in folk medicine and contribute to the Verbenaceae family's taxonomy.

This work was based on the microscopic investigation of leaf organs and investigated the foliar anatomy of *Lantana horrida* and *Lantana camara*. The comparative study of the leaf blade, posterior nerve, and stem in these species serves as a diagnostic tool, as some characteristics of the leaves are shared with other Lantana species. Given the importance of macroscopic and microscopic features for the establishment of floral species, the objective of the present work was the morphological, chemical, anatomical, and molecular characteristics of the leaves for identification purposes.

### Anatomy of the plants

**Light microscopy (LM):** Samples measuring 1 cm2 that were taken from the center of the leaf blades were fixed in resin methacrylate (Gerrits 1991), dehydrated in an ethanol series, fixed in FAA 50 (Johansen 1940), and sectioned with a rotary microtome. O'Brien *et al*., 1964 stained leaf cross sections (6 μm thick) with 0.05% Toluidine blue at pH 4.3, and synthetic resin was used to mount permanent slides. Fresh samples were sectioned using a razor blade, and Safrablau was used to stain the sections (Bukatsch 1972). Glycerin jelly was used to mount the semi-permanent blades. (Santos Tozin, *et al*., 2016) Analysis was done on the non-glandular trichomes on both leaf surfaces. A digital image capture system was integrated with a Leica DMR microscope to document the findings.

**Scanning electron microscopy:** Trials were primed in a solution of 2.5% glutaraldehyde and 0.1 m phosphate buffer (pH 7.3) for an entire night at 4 degrees Celsius. They were then dried using a Leica EM CPD030 critical-point drier, placed on aluminum stubs, and covered with 20 nm gold using a BAL-TEC SCD 050 splatter coater (Robards, 1978). Finally, both leaf surfaces were inspected by a Fei Quanta 200 scanning electron microscope. The pertinent results were certified via an electron microscope linked to a digital image capture system.

**Molecular analysis:** Total DNA was extracted from leaves (Proebski *et al*., 1997) and digested by SaII enzyme according to enzyme instructions and conditions (bio rad). Gel electrophoresis 1% agarose gel, discolored with ethidium bromide, visualized by UV light, and fragment bands determined by DNA marker.

**GC-MS Analysis:** Methanol extract of *L. horrida* and *L. camara* was analyzed utilizing Model 7890A (Agilent Technologies) connected to a model of a mass selector detector 5975°C with a capillary column DB-1 (0.25 μm film × 0.25 mm i.d. × 30 m length). With an ionization voltage of 70 eV, the device operated in an electron impact mode, injector temperature of 230°C, and detector temperature of 280°C. Helium (99.9% purity) was employed as the gas carrier at a 1 mL/min flow rate, and 1 μL of the sample was inoculated. The oven temperature was planned to start at 80°C (isothermal for 5 min), then increase to 200°C at 5°C/min, and lastly to 280°C at 5°C/min (isothermal for 16 min). Mass spectral records were used to identify compounds from NIST and WILEY libraries.

## Results and Discussion

**Morphological characters:** Overall morphological characters: Habitat: All observed species are perennial (Fig. 1A). The shrub *Lantana camara* is a hardy evergreen. Stems have tiny prickles and a quadrangle profile. The leaves are paired off in opposing directions. They have coarse, broadly oval shapes, short hairs, and finely toothed edges. They smell when they are crushed. Flowers come in various colors, including cream, pink, or orange, with many small, rounded heads that are frequently red and yellow (Fig. 1A). (Girish, 2017).

Constant calyx, limbs 4–5 partite or toothed, aestivation typically valvate, enlarged or unaltered in fruits. In both species, it is membrane-bound, truncate, and dimly toothed *L. camara* (Fig. 1B) and *Lantana horrida* (Fig. 2B).

On the other hand, *Lantana horrida* is a colorful and abundant lantana species that is unavailable in the area. Its yellow-gold and orange-red blooms make it the most easily identifiable of the other species. It is a spectacle in a yard of any size, or even in the wilderness with thick undergrowth. Besides providing nectar for butterflies, its small black fruits feed certain birds and probably small mammals. It can get as tall as six feet and wide as equal segments, growing fairly woody at the base and main stems. (Griffith *et al*., 2007).

When crushed, the original scientific name of the leaves, horrida, came from their rather potent odor. Since blooms often sprout from new growth, an annual healthy trimming should be completed after the flowering season concludes in December. The robust, spreading *Lantana horrida* shrub can withstand severe droughts. As the stems age, very tiny thorns appear on them. Bright red, orange, and yellow flowers are tubular. From April to October, lantana flowers bloom. It produces round, meaty, dark blue to black fruits. For the majority of mammals, the berries are toxic. The leaves of lantanas are astringent and can produce a rash when handled. (Fig. 2A-D) (Wasowski & Wasowski, 1991).

The verbenaceous leaves under study were simple in taxa, petiolate, and unlobed and had hair on both the upper and lower surfaces. All of the species' leaves had pinnate veining. With lantana, the leaf apex was frequently acute. The leaf margin of *Lantana camara* was serrated (Fig. 1C). According to the findings of Tan *et al*., (2018) and Kadereit (2004), verbenaceous leaves had simple or three leaflets, were compound or palmate, petiolate or sessile, and had entire, dentate, crenate, lobed, or incised margins.

The petiole in the Lantana species, which is 1.5 to 2 cm long, carries the simple, opposite, decussate leaves. At 5 to 8 cm long and 3–4 cm wide, the leathery blade has an oval to broadly oval shape, is truncated to subcordate at the base, and is acute or acuminate at the top. Pubescent on the bottom, rough and hispid on the upper surface. Regular toothing is along the leaf edge (Fig. 1C) (Passos *et al*., 2009). An involucre of green, narrowly ovate bracts, ranging from 5 to 7 mm, surrounds the flowers. 6 to 12 mm long floral pedicel. With ascending hairs inside, the corolla tube curved along a length of 10 to 12 mm, opening at the top to reveal four rounded lobes spaced 6 to 8 mm apart. Often, the first flower is white, but it turns yellow or orange as it ages.

According to Puri (2018) and Hickey & King (1988), these results were consistent. The examined taxa had quadrangular, woody, erect, glabrous, and hairy stems, similar to those of hairy in *L. horrida*, and spiny, similar to *Lantana camara*. Boulos (2002), Kadereit (2004), Kumar (2009), and Iroka *et al*., (2016)'s findings corroborate these conclusions as well as Rahman (2016). They noticed the stems were either erect, hairy, prickly, terete, quadrangular, herbaceous, or woody (Miller, 2013).

Clear hand sections of the leaves of *Lantana camara* and *L. horrida* were analyzed and initiated to have similar characteristics. The stem's epidermis consisted of a single layer of barrel-shaped parenchyma cells, while the cortex comprised multiple layers of freely arranged parenchyma cells with noticeable intercellular spaces. In that order, xylem and phloem tissue materials were positioned above and below the meristematic cambium.

Anatomical investigations demonstrated distinct variations in the size and forms of stomata, epidermal cells, the presence of macro- and microhair, their size and kind, and other features. On both sides, the organelles had distinct sizes and shapes. The configuration of the secretory idioblasts and the vascular system, visible in *L. camara*'s cortex, are two distinguishing features of the petioles of the two lantana species. The distal segment of the petiole is the most considerable in terms of taxonomy, and the anatomy of the petiole frequently supports the identification of certain taxa (Park *et al*., 2021). It has been established that the petiole is a useful taxonomic feature for differentiating species within the Mesomorphaceae family (Reis *et al*., 2004) and differentiating Erythroxylum P species. Erythroxylaceae, or browne (Bieras & Sajo, 2004).

The petiole of *Lantana camara* is semi-circular on the abaxial surface and has a broad, multiple-furrowed shape on the adaxial surface. There are polygonal, oval, or circular-shaped collenchyma and parenchymal cells, with the latter found in both the cortex and the pith. The shape of the vascular bundles in *L. camara* is semi-arc, with two marginal traces at the center and a slightly invaginated end; the vascular pattern is arc-shaped. Only the adaxial side of the epidermis exhibits trichome; the abaxial side is devoid of it. *Lantana horrida*: This species has a deep arcing vascular system with marginal ribs, a centrally located vascular bundle, and an arcing vascular pattern. Some tissues are perivascular. On the two adaxial surfaces of the epidermis, trichomons are present. The outline of the petiole is semi-circular on the abaxial surface of the epidermis and widely and multiplely furrowed without dorsal and lateral traces on the adaxial surface (Fig. 4D-F).

Trichomes are the defining characteristic of the Verbenaceae family. Depending on the specific level, correct specimen identification and classification depend heavily on the most comprehensive hair type description, both glandular and non-glandular, and their distribution. Regarding the alterations to epidermal cells, both genera exhibit considerable variation. Whether thick or thin, the epidermal wall aids in identification to some degree. For morphological and ontogenetic classification, epidermal morphology and stomata ontogeny are distinctive characteristics of primitive and advanced sorts (Ramassamy, 1995). The internal secretory cells' ontogenesis in *L. camara* leaves was noted by Moura *et al*., 2005. Solereder (1908), Inamdar (1969), Raghavan & Arora (1960), Theobald *et al*., (1979), Gottlieb & Salatino (1987), Sinha & Sharma (1984), Rueda (1993), Judd *et al*., (1999) have documented the identification of glandular trichomes in Verbenaceae that secrete essential oil. Early synthesis like this offers a chemical barrier that acts as a deterrent against herbivores. The anti-herbivory substance's efficacy is significantly influenced by the sites of accumulation (Gershenzon & Croteau, 1991).

Trichomes on the abaxial surface of *L. camara* were found to have aggregates of 5 basal cells (Fig. 4A), whereas *L. horrida* only had one basal cell (Fig. 3A). The study observed transversal and longitudinal sections of three phases of the inflorescence, showing an immature development characterized by meristematic differentiation starting with a mass of cells. The unicellular non-glandular trichomes seen in Lantana are typical for plants of this genus, with a higher regularity on the adaxial surface of *L. camara,* possibly protecting contrary to excessive radiation and high temperatures.

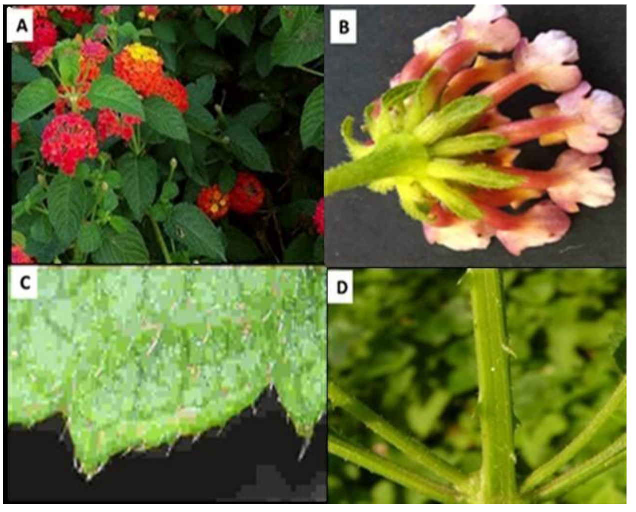


Fig. 1. Major morphological characters of *Lantana camara.*


Fig. 2. Major morphological characters of *Lantana horrida.*

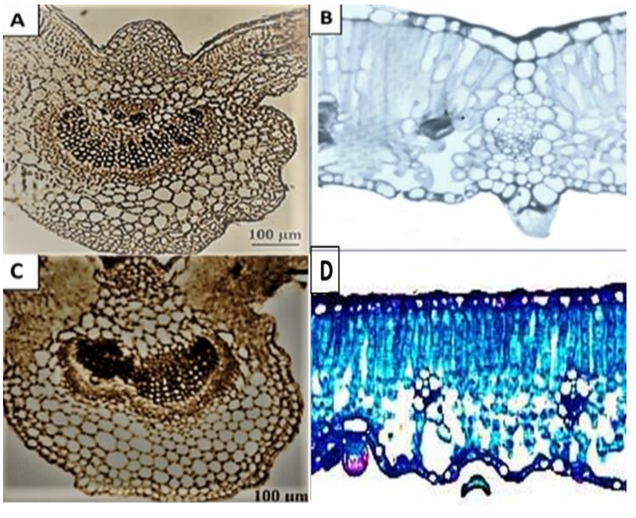


Fig. 3. Anatomy sections of *Lantana camara* and *Lantana horrida* leaf blades. A and B *L. camara*. C and D *L. horrida*. A and B - Transversal section of the leaf blade viewing mesophyll dorsiventral. B - Paradermal section of the leaf blade viewing the secretory idioblasts. (D) Light micrograph, with leaf cross-section stained with toluidine blue.

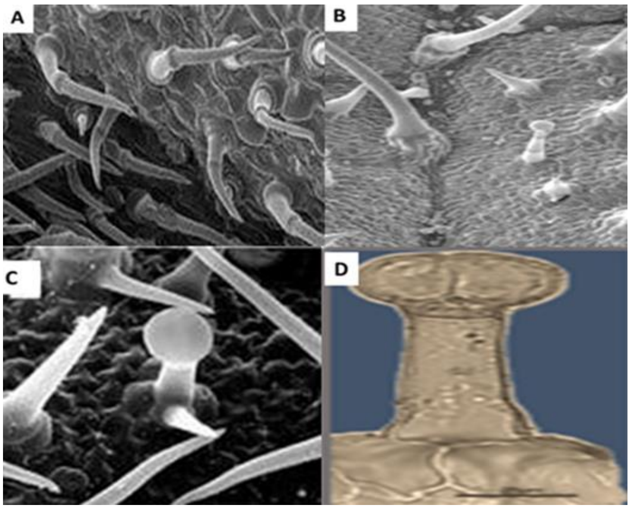


Fig. 4. Summary *of Lantana horrida* leaf blade*.* (A-B) Scanning electron micrographs of (A) adaxial and (B) abaxial leaf surface. Arrows designate glandular trichomes. Types of glandular trichomes in *Lantana horrida*. B, C -Scanning electron microscopy (SEM) C and D–Capitate trichomes of type I with two cells in the secretory head.

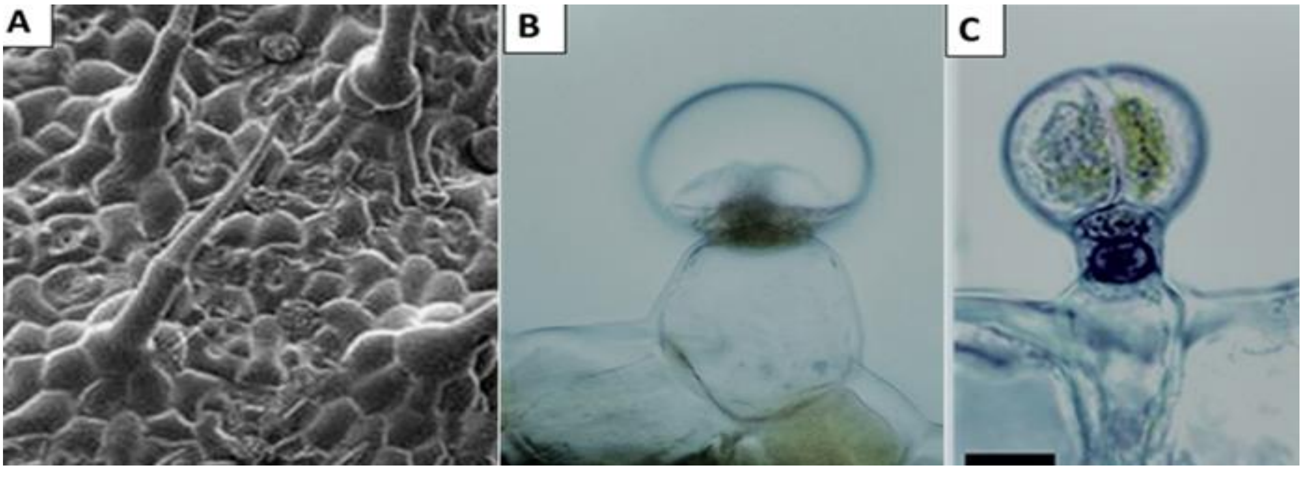


Fig. 5. Summary of *Lantana camara* leaf blade. (A-B) Scanning electron micrographs of (A) abaxial leaf surface. Arrows indicate glandular trichomes. Kinds of glandular trichomes in *Lantana camara*. B, C -Scanning electron microscopy (SEM) B and C–Capitate trichomes of type I with two cells in the secretory head.

**Table 1. Distribution and morphological characteristics of glandular trichomes located on above-ground organs of *Lantana camara* and *Lantana horrida*.**

| Plant organ | Type of trichomes | Number of trichome cells | Height (µm) | | | Diameter (µm) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | Min. | Max. | Mean |
| | | | | ***Lantana camara*** | | | | |
| Stem | Sesille | 6 ± 2 | 43.2 | 65.2 | 53.2 ± 0.2c | 29.3 | 39.1 | 35.4 ± 0.05a |
| | Stalk | 5 ± 1 | 51.5 | 109.3 | 76.4 ± 0.1a | 26.4 | 43.9 | 35.1 ± 0.2a |
| Leaf | Sesille | 7 ± 1 | 48.4 | 73.2 | 62.1 ± 0.05b | 25.5 | 37.4 | 32.2 ± 0.1b |
| | Stalk | 8 ± 2 | 53.1 | 98.2 | 75.4 ± 0.2a | 23.1 | 36.3 | 31.1 ± 0.01b |
| | | | | ***Lantana horrida*** | | | | |
| Stem | Sesille | 11 ± 1 | 54.2 | 71.2 | 63.2 ± 0.07b | 21.2 | 29.7 | 25.3 ± 0.05b |
| | Stalk | 13 ± 2 | 39.7 | 111.3 | 78.3 ± 0.3b | 26.2 | 35.5 | 31.3 ± 0.4a |
| Leaf | Sesille | 15 ± 2 | 47.2 | 81.3 | 64.5 ± 0.04c | 23.1 | 32.1 | 27.2 ± 0.07b |
| | Stalk | 18 ± 2 | 62.1 | 118.1 | 93.4 ± 0.06a | 19.3 | 38.3 | 29.1 ± 0.1a |

This means ± standard error within columns with the same letter is not significantly different according to Tukey's Honestly Significant Difference Test at the 0.05 level

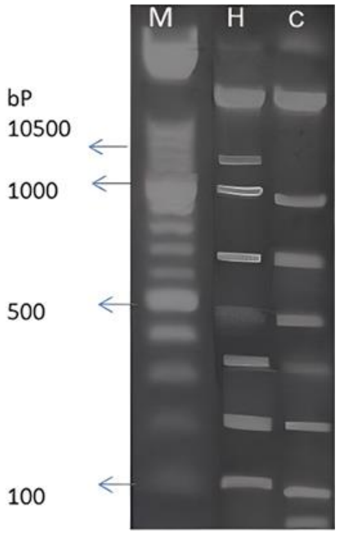


Fig. (6). Gel electrophoresis of *L. horrida* and *L. camara* DNA respectively, digested by SalI enzyme (M- DNA marker, H- *L. horrida* and C- *L. camara*).

In both species, the abaxial surface has a higher density of glandular trichomes (Table 1) than the adaxial. Sunlight is ideal for aromatic plant growth, and the abaxial surface of the trichomes provides protection, which prolongs the shelf life of the secretions within the plant. Essential oils abound in *L. camara* (Misra & Laatsch, 2000; Suryati, *et al*., 2021), and in high temperatures and low humidity, these substances typically evaporate and release. The trichomes (primarily type III) in *L. horrida* occur in depressions, supporting the protective hypothesis. The glandular trichomes have ecological significance since they are linked to how plants interact with their surroundings and effectively fend off pathogens and herbivore attacks (Werker, 1993). Based on the findings, it is possible to conclude that glandular trichome presence is a key difference between *L. camara* and *L. horrida* (Table 1).

Except for secreted material, internal secretory cells may have the same morphology as their neighbors or differ enough to be categorized as secretory idioblasts. They may contain phenolic derivatives, lipids, and carbohydrates. They can be spherical, ellipsoidal, or branching (Fahn, 1979; Moura, 2009). Comparative studies on leaf anatomy (Solereder, 1908; Raghavan & Arora, 1960; Sinha & Sharma, 1984; Herman, 1998) have not reported the occurrence of internal secretory cells, even though they are common in a diversity of angiosperm families (Moura, 2009). The sole secretory organs identified for *Lantana camara* L. According to Raghavan & Arora (1960), and Sinha & Sharma (1984), (Verbenaceae) leaves are glandular trichomes that yield a basic oil rich in mono- and sesquiterpenes (Arora & Kohli, 1993; Misra & Laatsch, 2000). According to Roy and Barua (1985) and Siddiqui *et al*., (1995), *L. camara*'s aerial parts are also known to contain several triterpenoids (Barre *et al*., 1997; Wollenweber *et al*., 1997).

The Verbenaceae family is notable for having various forms of glandular trichomes. Few studies describe these structures, which makes sense given the diversity of lantana species. Additionally, this characteristic's applicability to taxonomic studies is restricted by the absence of calibration in classifying these trichomes (Inamdar, 1969; Moura *et al*., 2005). A different kind of capitate trichomes is found in the Lamiaceae family, which is phylogenetically related to the Verbenaceae family. The different classifications of the trichomes confuse and make it hard to compare studies reported in the literature (Werker, 1993; Ascensão *et al*., 1999).

Within the epidermal cells of *L. camara* were thick-walled, square, hexagonal, and rectangular on the adaxial surface. Very noticeable macrohair with a broad five-celled (Fig. 5A) base and a somewhat acute tip; glandular hair is present. In *L. horrida* on the adaxial surface, with rectangular epidermal cells. The macrohairbit is swollen overhead at the base but tapering at the apex. The epidermal cells on the abaxial surface had rectangular, square, and somewhat polygonal shapes. There is macrohair and glandular hair. (Fig. 4A).

Based on the findings in this paper, two morphologically similar species of Lantana could be distinguished from one another by using specific leaf characteristics. We learn more about the Verbenaceae family with the first presentation of the anatomical description of *L. horrida*.

Gel electrophoresis of *L. horrida* and *L. camara* total DNA digested by SalI enzyme showed 10 bands. There was partial genetic variability between *L. horrida* and *L. camara* DNA. There was band no. 3 and absent band no. 10 in *L.*

*horrida* compared to *L. camara*. (Fig. 6). Neutral genetic diversity is typically measured at single loci, which are frequently randomly sampled throughout a genome, using genetic markers (biochemical markers like allozymes and, more recently, molecular markers like microsatellites). (Wenne, 2023). DNA sequence data of sufficient quantity and variability can be used to resolve their close relationships; however, due to the potential confounding effects of gene tree/species tree incongruence, care should be taken when interpreting the results (Lu-Irving, *et al*., 2021).

Thirty-nine compounds were identified using GC-MS, with 92.4% found in leaves. The compounds are illustrated in Figure 7 in order of elution from the TG-5 column, along with their percentage composition and retention index. The main constituents in the leaves oil included β-caryophyllene (10.6%). In line with Khan *et al*., 2015, which revealed enormous variances in the chemical conformation of *L. camara* essential oils from around the globe that have been researched up to this point. Nevertheless, it has been observed that the only compound identified as a major or significant amount in all of *L. camara* essential oils that have been investigated to date is b-caryophyllene, a naturally occurring bicyclic sesquiterpene. Thus, b-caryophyllene could be used as a chemical marker for the Lantana essential oils. Therefore, b-caryophyllene, a natural bicyclic sesquiterpene, was a distinguishing chemical that could help identify the extracts of the studied Lantana species. The b-caryophyllene was 35.9 in *L. horrida* and 15.4 in *L. camara*. (Fig. 7 A and B).

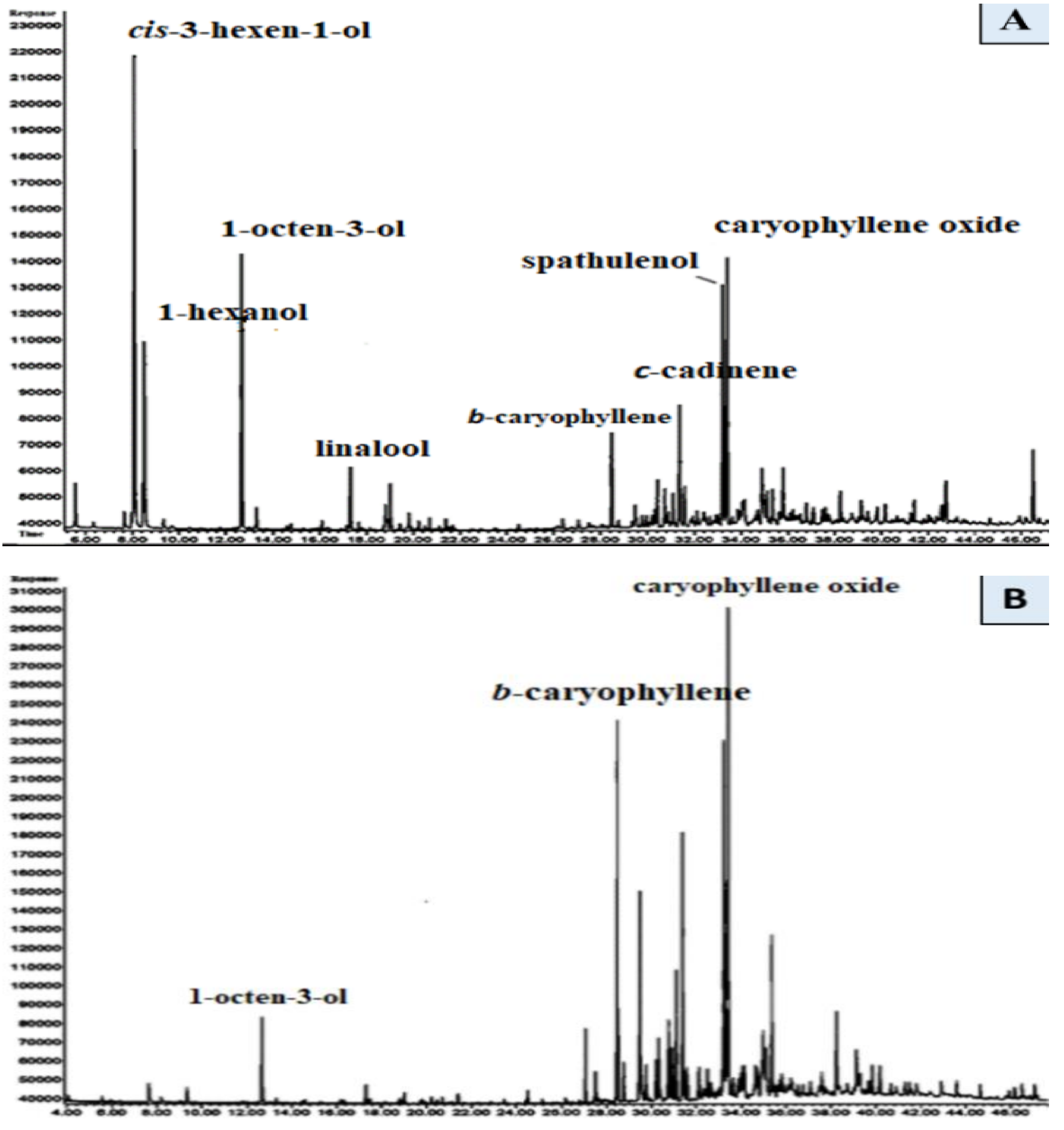


Fig. 7. GC-MS chromatograph of methanolic leaf extract of (A) *L. camara* and (B) *Lantana horrida*.

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