Morpho-anatomical and phytochemical evaluation of *Icacina trichantha* Oliver (*Icacinaeae*)

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**Abstract:** The leaf epidermis of *Icacina trichantha*, a quintessential medicinal tropical plant was investigated with the aid of light microscopy and the chemical constituents of its leaf and root were investigated using Gas Chromatography-Mass Spectrometry (GC-MS). Hitherto, leaf epidermis data is missing and similarly, the chemical analysis of the leaf and root of the plant is undertaken in a single study for the first time. The leaf epidermis characters with which the species can be defined include paracytic and diacytic stomatal types, irregular epidermal cell shape together with angular or curved anticlinal wall patterns. However, the quantitative data appeared to overlap thus providing the range of values of both the measured and counted features. N-hexane extract of the root is rich in reducing sugars, tannins, steroids, glycosides, terpenoids and flavonoids while GC-MS analysis revealed 11 and 3 significant esterified bioactive components in the leaf and tuberous root respectively with dodecenoic acid being most abundantly present (47.85%-54.10 %) but some chemical confined to specific areas are trichothece-9-en-8-one (47.73 %) in the root and 9-octadecenoic acid (25.05 %) in the leaf. The result of this study will assist in identifying the plant even if its parts are fragmentary and also be helpful in screening the plant for drug.

**Key words:** Microscopy, taxonomy, tropical plant

**Özet:** Önemli bir tıbbi tropik bitki olan *Icacina trichantha*’ın yaprak epidermisini ve kökünün kimyasal bileşenlerini de Gaz Kromatografisi-Kütle Spektrometresi (GC-MS) kullanılarak incelememiştir. Halihazırda bitkinin yaprak epidermis verileri eksik durumdadır ve bitkinin yaprak ve kökünün kimyasal analizi tek bir çalışmada ilk kez yapılmıştır. Parasitik ve diastik stomata tipleri, düzeniz epidermal hücre şekli ve köşeli veya kavisli antiklinal duvar desenleri, türün tanımlanmasına ve yaprak ve kök incelemesinde kullanılabilecek epidermis karakterleri arasında yer almaktadır. Bununla birlikte, hem ölçülen hem de sayılan özelliklerin değer aralığı sağlıyacak şekilde örtüşmüştür. N-heksan extract of the root is rich in reducing sugars, tannins, steroids, glycosides, terpenoids and flavonoids while GC-MS analysis revealed 11 and 3 significant esterified bioactive components in the leaf and tuberous root respectively with dodecenoic acid being most abundantly present (47.85%-54.10 %) but some chemical confined to specific areas are trichothece-9-en-8-one (47.73 %) in the root and 9-octadecenoic acid (25.05 %) in the leaf. The result of this study will assist in identifying the plant even if its parts are fragmentary and also be helpful in screening the plant for drug.

**Anahtar Kelimeler:** Mikroskopi, taksonomi, tropik bitki

**Citation:** Kadirii AB, Asekun TO, Asekunowo AK, Ayodele EA, Olowokudejo JD (2020). Morpho-anatomical and phytochemical evaluation of *Icacina trichantha* Oliver (*Icacinaeae*). Anatolian Journal of Botany 4(2): 100-105.
In this investigation, the phytochemical composition of hexane extract of leaf and root using preliminary screening and GC-MS analysis was carried out in a single study for the first time, in addition to the micro-morphological evaluation of the leaf epidermis which has not been studied before. Leaf epidermal characteristics are well known to offer useful identification criteria for plants, as well as chemical characters, which also can be used to define their pharmacological usefulness. These two studied character sources of the plants (anatomy and chemistry) are important data sources for species identification and utilization of the plant as a source of drug.

2. Materials and Method

Fresh samples of *I. trichantha* were collected from different locations across Southern Nigeria (Fig. 1), and the samples were dried and authenticated at the Lagos University Herbarium (LUH). Herbarium abbreviation follows Holmgren and Holmgren (2003). The leaves and roots were both used for the study. The former was mainly used for micro-morphological assessment, and 100 leaf samples obtained from all the individuals collected were investigated. However, the dried leaves and roots were pulverized and kept in ziplock for further investigation of chemical analysis and phytochemical screening.

For micro-morphological evaluation of the leaves, the study approach of Stace (1965), Kadiri et al. (2003), Kadiri and Olowokudejo (2016) and Ogundipe and Akinrinlade (1998) was followed while for phytochemistry, the method of Ajayi et al. (2011), Harborne (1991) and Trease and Evans (1998) was adopted.

2.1. Epidermal peel (leaf epidermis)

For the study, a light microscope was used. The acid soaking and counter-staining method which has proven useful for obtaining leaf epidermis from many African plants was adopted, following the approaches of Ogundipe and Akinrinlade (1998) and Kadiri and Olowokudejo (2016) with some modifications. Leaf portions of 2-3 cm² were cut from the standard median portion of the leaf lamina near the mid-rib, boiled in water for 30 minutes, and then soaked in concentrated nitric acid (HNO₃) in capped specimen bottles for two to four hours to macerate the mesophyll tissue. Tissue disintegration was indicated by air bubbles, the stage at which the leaf tissues were transferred into Petri dishes containing water for separation of the epidermis using a pair of forceps and mounting needle. Tissue debris was cleared off the epidermis with an artist’s fine-hair brush and washed in several changes of water. Two to three drops of sodium hypochlorite solution were dropped onto the epidermis on the slide to bleach opaque areas and allowed to soak for 30–120 seconds until a color change. The epidermis peel was mounted on the slide and then two to five drops of ethanol in a series of ascending concentrations (50%, 75%, and 100%) were added to harden the cell wall. Two to three drops of 10% aqueous Methylene Blue and one drop of 50% aqueous Safranin were later added for three to five minutes. One to two drops of glycerine were added, then the preparation was covered with a transparent coverslip and the edges were sealed with nail polish to prevent dehydration. Each slide was observed under magnifications of ×100 and ×400 so as to capture all the features of the epidermis. These features were recorded qualitatively, and basic statistical calculations were made to show means, standard error and ranges of variations. Photomicrographs were taken using an Olympus microscope with an attached camera.

Stomata index (SI) was calculated using the formula of Stace (1965).

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\text{Stomata index} = \frac{\text{Stomata number}}{\text{Cell number per unit area}} \times 100
\]

2.2. Extraction of plant material

The pulverized leaf and root sample (10 g) of *Icacina trichantha* was separately extracted using Soxhlet apparatus and hexane as solvent. The hexane solvent (250 ml) was put in a round bottom flask, attached to a Soxhlet extractor and condenser on an isomantle. The pulverized sample was loaded into the Soxhlet thimble and the side arm lagged with glass wool. As the hexane was heated via the isomantle, it evaporated, condensed then dripped into the reservoir containing the thimble. The solvent flows back into the flask once its level reaches the siphon it pours back and the cycle was repeated several times for a period of 9 hr. The non-volatile extract obtained from the plant was obtained and kept in a sterile vile and stored at 4 °C for phytochemical investigations.

2.3. Phytochemical screening

The preliminary phytochemical screening of the hexane root extract was carried out by physical observation of colour change. The following phytochemicals viz: alkaloid, saponin, flavonoid, anthraquinone, glycoside, tannins, steroid, protein and volatile were investigated using standard protocols (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1999).

2.4. GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) of both leaves and root hexane extracts were also carried out at the University of Lagos Central Research Laboratory, Nigeria. The model of the GC-MS used for mass spectral identification of the hexane extracts of the leaves and root of *I. trichantha* was an Agilent 6890 interfaced to a 5973 mass selective detector. The capillary column (30 m x 0.25 mm x 0.25 µm film thickness) was HP-5MS. The oven temperature of 50°C for 5 minutes was firstly maintained and then set to 250 °C at 5 °C min⁻¹. Helium (99.999%) was the carrier gas used at a flow rate of 1 ml/min, and 1 µl injection volume was employed (split ratio of 10:1). The electron-impact ionization mass spectrometry electron energy of 70 eV of was operated at an. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 40 to 450 Da. The total GC running time was 45 min. The spectra data obtained were compared with those of NIST library mass spectra.

3. Results

Detailed variations in the qualitative and quantitative characters of the leaf epidermis of *Icacina trichantha* are shown in Table 1. The leaf is hypostomate and the stomatal types are paracytic and diacytic (Fig. 1B, Table 1). The epidermal cell shape is irregular on both surfaces of the leaf (Fig. 1A, B; Table 1) but the anticlinal wall pattern is either angular on the adaxial surface or curved
Figure 1. Leaf epidermis features of *Icacina trichantha*. A: Adaxial surface, B-D: Abaxial surface, D: Typical unicellular trichome found on the abaxial surface of the leaf. Scale bar = 40µm.

on the abaxial surface of the epidermis (Fig. 1, Table 1). Both the non-stomatal and stomatal quantitative features reasonably overlap across the one hundred leaf samples that were investigated (Table 1).

The result of the phytochemical analysis from this study is as presented (Figure 2, Table 2). The hexane extract of the root of *I. trichantha* is rich in reducing sugars, tannins, steroids, glycoside, terpenoids and flavonoids, while saponins, alkaloid, anthraquinone and the volatile oil is absent. GC-MS analysis of n-hexane extracts of both the leaf and the root of *I. trichantha* were reported (Table 3). Eleven significant bioactive components were found in the leaf while the root revealed 3 bioactive components in high percentage concentration. Dodecanoic acid was revealed to be the major component in both extracts with the leaf (54.10 %) having a higher concentration than the root (47.85 %). The root in addition revealed the presence of Trichothecc-9-en-8-one (47.73 %). The leaf extract also revealed 9-Octadecenoic acid (25.05 %). Other prominent components in the leaf extract were acetamide (6.21 %) and methoxyacetic acid (5.58 %) (Table 3).

4. Discussions

A combined evaluation of the leaf epidermis and chemical features of *I. trichantha* carried out in the study has shown that the documented characters are useful for identification. They are also potentially suitable for differentiating the species from any other related species in the family Icacinaceae. Those features that seem to be good for defining the species include hypostomatic leaves, paracytic and diacytic stomatal types, and the presence of simple unicellular trichomes which are usually restricted to the abaxial surface of the leaf. However, the taxonomic relevance of epidermal features has been expounded by several workers, as being good for identification, delineation, classification and in resolving taxonomic intricacies (Davis and Heywood, 1963; Stace, 1965;

| Features                      | Adaxial surface | Abaxial surface |
|-------------------------------|-----------------|-----------------|
| Cell shape                    | Irregular       | Irregular       |
| Wall pattern                  | Angular         | Curved          |
| E. cell No./ field            | 36              | 68              |
| E. cell length (µm)           | 28.0(42.0±2.0)58.5 | 30.0(48.0±2.0)65.2 |
| E. cell width (µm)            | 12.0(15.0±2.0)25.3 | 10.5(12.0±2.0)16.0 |
| Stomata                       | Absent          | Present         |
| Stomata No./ field            | Absent          | 42              |
| Stomatal type                 | Absent          | Paracytic, diacytic |
| Stomatal length (µm)          | Absent          | 22.0(27.0±2.0)30.0 |
| Stomatal width (µm)           | Absent          | 8.5(10.0±2.0)12.0 |
| Stomatal index (%)            | Absent          | 38.2            |
| Trichome                      | Absent          | Long unicellular trichomes present |

**Table 1**: Qualitative and quantitative characteristics of the leaf epidermis of *Icacina trichantha*

**Table 2**: Phytochemical analysis of the tuberous root of *Icacina trichantha*

| Phytochemical      | Result |
|--------------------|--------|
| Reducing sugars    | +      |
| Tannins            | +      |
| Saponin            | -      |
| Alkaloids          | -      |
| Steroids           | +      |
| Glycoside          | +      |
| Flavonoids         | +      |
| Anthraquinone      | -      |
| Terpenoids         | +      |
| Volatile oils      | -      |
| Proteins           | -      |

Key: + = present, - = absent
Table 3. Chemical composition of leaf and root of *Icacina trichantha* as revealed by Gas Chromatography–Mass Spectrometry (GC-MS)

| S/N | Chemicals                                           | Concentration in leaf (%) | Chemicals                                                                 | Concentration in root (%) |
|-----|----------------------------------------------------|---------------------------|---------------------------------------------------------------------------|---------------------------|
| 1   | Carbonic acid, octadecyl vinyl ester,              | 0.362%-0.433%             | Dodecanoic acid, 1,2,3-propanetriyl ester,                                | 28.211%-83.84%            |
|     | Carbonic acid, octadecyl prop-1-en -2-yl ester     |                           | Trichothe-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-ester, monoacetate,   |                           |
|     |                                                    |                           | (3.alpha.,7.alpha.)-ester, Dodecanoic acid, 1-(hydroxymethyl) -1,2-ethanediyl ester |                           |
| 2   | Sulfurous acid, 2-propyl tetradecyl ester          | 0.366%-0.70%-             | Dodecanoic acid, 1,2,3-propanetriyl ester,                                | 19.642%-58.37%            |
| 3   | Octacosane, Octadecane, 3-Eicosene esters          | 0.616%-1.19%              | Trichothe-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-, monoacetate,        | 33.650%-100.00%           |
|     |                                                    |                           | (3.alpha.,7.alpha.)-ester                                                |                           |
| 4   | Heptacosane, 1-chloro-, Tritetracontane ester      | 0.756%-1.46%              | Trichothe-9-en-8-one, 12,13-epoxy-3,7,15-trihydroxy-, monoacetate,        | 14.086%-41.86%            |
|     |                                                    |                           | (3.alpha.,7.alpha.)-ester                                                |                           |
| 5   | Hexadecane, 1-iodo-, Docosane, 9-octyl- ester      | 0.835%-1.61%              | (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydroxynaphthalen-1(2H)-one ester | 4.411%-13.11%             |
| 6   | Tritetracontane ester                              | 0.698%-1.35%-             | -                                                                         | -                         |
| 7   | Oleic Acid, Dodecanoic acid, 2,3-dihydroxypropyl ester, Methyl nonyl ether | 2.197%-4.23%             | -                                                                         | -                         |
| 8   | Heptadecane, Docosane, 1,22-dibromo- ester         | 0.698%-1.35%-             | -                                                                         | -                         |
| 9   | Methoxyacetic acid, tridecyl ester, Diethylene glycol monododecyl ether, Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-cis-formyloxy-ester | 1.577%-3.04%             | -                                                                         | -                         |
| 10  | Cyclohexane, 1,1-(2-propyl-1,3-propanediyl)bis-ester, Cyclohexanol, 2-(2-propynylxylo)-trans-ester, Cyclohexanol, 2-(2-propynylxylo)-2-Dodecanol ester | 1.140%-2.20%             | -                                                                         | -                         |
| 11  | 9-Octadecenoic acid, Hexadecanoic acid, 2-hydroxy-, methyl ester, 9-Octadecenoic acid (Z)-, octadecyl ester | 5.038%-9.70%             | -                                                                         | -                         |
| 12  | 9-Octadecenoic acid (Z), 2-hydroxy-1-hydroxymethyl(methyl) ester, 2-Butoxyethyl oleate, Oleic acid, 3-hydroxypropyl ester | 1.077%-2.07%             | -                                                                         | -                         |
| 13  | 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester | 3.089%-5.95%             | -                                                                         | -                         |
| 14  | Cyclohexane, 1R-acetamido-2,3-cis-epoxy-4-cis-formyloxy-ester, Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl-ester, Cyclohexanone, 2-(2-propenyl)-ester | 0.292%-0.56%             | -                                                                         | -                         |
| 15  | 2-Methyltriacontane ester, Methoxyacetic acid, decyl ester | 1.280%-2.47%             | -                                                                         | -                         |
| 16  | 9-Octadecenoic acid (Z), 2,3-dihydroxypropyl ester, 9-Octadecenoic acid (Z), 2-hydroxy-1-hydroxymethyl(methyl) ester, Oleyl chloride | 15.838%-30.51%          | -                                                                         | -                         |
| 17  | Acetamide, 2-chloro-N-(2-cyanoethyl)-ester, 2-Dodecanol, 3-Heptadecanoyloxydodecane | 6.214%-11.97%            | -                                                                         | -                         |
| 18  | 1-Methoxy-3-hydroxypropylheptane, Cyclobutanone, oxime, Methoxyacetic acid, 2-tridecyl ester | 5.577%-10.74%            | -                                                                         | -                         |
| 19  | Dodecanoic acid, 1,2,3-propanetriyl ester, Dodecanoic acid, 1,2,3-propanetriyl ester, Piperidine, 3-(bromomethyl)-ester | 51.916%-100.00%          | -                                                                         | -                         |

Ogundipe and Akinrinlade, 1998; Kadiri, 2003; Kadiri and Olowokudejo, 2016. Characteristically, the quantitative data overlap significantly among the one hundred individuals investigated thus providing the value range of the data upon which the species can be defined or differentiated from another related species. Environmental factors have been implicated in influencing the expression of morphological characters both quantitatively and qualitatively (Stace, 1965).

*Iacina trichantha* incorporates interesting character constituents in line with Otun et al. (2015) who reported the presence of tannin, flavonoid, glycoside, terpenoid and steroid in hexane extract and the absence of saponin. These chemicals underlie their medicinal value. Phytochemicals are responsible for the biological activities of plants. Flavonoids are hydroxylated phenolic compounds known to be synthesized as a defense against microbial infection (Kumar and Pandey, 2013); consequently they exhibit pharmacological potentials such as: antimicrobial, cytotoxicity, anti-inflammatory and antitumor properties. Tannins have the ability to bind protein and metal ions from solution, hence, its benefit in the prevention of cancer activity and treatment of...
inflammatory conditions (Olajide et al. 2004. Okuda and Ito, 2011; Otun et al., 2015).

The GC-MS results of hexane leaf extracts of I. trichantha revealed the abundant presence of an ester 9-Octadecadienoic acid which have been reported to exhibit antiinflammatory and antiarthritic property (Lalitha Rani et al., 2009). It may also be useful as anti-cancer, hepatoprotective, nematicide, insectidiuge, anti-histaminic, antiinflammatory and anti-eczemic, anti-acne, 5-alpha reductase inhibitor and anti-androgenic agent (Vohra and Kaur, 2011). In addition, dodecanoic acid was also detected in both leaf and root hexane extracts and its antifungal activity has been reported which may attribute its use as an antimicrobial agent (Jagtap et al., 2009). Therefore, the chemical diversity in I. trichantha alludes to its quintessential medicinal uses in the folkloric health care system.

However, the information provided from the morphological and chemical assessment of the plant appears to be helpful in identifying and characterizing the species, and they would assist in crude drug research of the plant.

Acknowledgments
The authors are grateful to the local people of the communities where the plant materials were collected and Dr. (Mrs.) Ugbogu, Head of the herbarium of Forestry Research Institute, Ibadan, Nigeria for granting access to herbarium infrastructure and nomenclatural authentication.

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