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## PHYTOCHEMICAL CHARACTERIZATION OF COLD MACERATED METHANOLIC LEAF EXTRACT OF *CADABA INDICA* LAM. USING GC-MS

V. Thirumalai <sup>1</sup>, P. Nirmala <sup>\*1</sup> and R. Venkatanarayanan <sup>2</sup>

Department of Pharmacology <sup>1</sup>, Rajah Muthiah Medical College, Annamalai University, Chidambaram - 608002, Tamil Nadu, India.

Department of Pharmacognosy <sup>2</sup>, R. V. S. College of Pharmaceutical Sciences, Sulur, Coimbatore - 641402, Tamil Nadu, India.

### Keywords:

*Cadaba indica* Lam, Cold maceration, Gas chromatography, Linolenic acid, Phytochemical constituents, GC-MS analysis

### Correspondence to Author:

**Dr. P. Nirmala**

Professor,  
Department of Pharmacology, Rajah Muthiah Medical College, Annamalai University, Chidambaram - 608002, Tamil Nadu, India.

**E-mail:** tvthiru90@gmail.com

**ABSTRACT:** *Cadaba indica* Lam (Indian Cadaba), an Indian traditional medicinal plant has been used for various diseases. The cold macerated methanolic leaf extract of *Cadaba indica* was assessed using Agilent GC 7890A gas chromatography connected with an MS- 5975C mass spectrometer and the mass spectra were matched with NIST 14.0 – data library. Several chemical constituents were identified within 28 min of the entire GC-MS analysis. The GC chromatogram shows that presence of most abundant linolenic acids and its esters such as Hexa-decanoic acid, methyl ester, n-hexadecanoic acid, 10,13-octadecadienoic acid, methyl ester, 9, 12, 15-Octadecatrienoic acid, (Z, Z)-methyl ester, Octadecanoic acid, methyl ester, 9, 12-Octadecadienoic acid, (Z, Z)-, 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-, Octadecanoic acid, Hexadecanoic acid, 2,3-dihydroxy propyl ester and 9- Octadecanoic acid, (Z) – methyl ester. In contrast, compounds with diterpenes, phthalic esters, pyrrolidine, phenol, ketones, and myristic acid derivatives were also detected with average peak area percentage. The nature and chemical background of the detected phytochemical constituents may be responsible for the pharmacological effects of *Cadaba indica* Lam. in various diseases.

**INTRODUCTION:** Phytotherapy, a branch of medicine, deals with the application of plants and their products in the prevention and treatment of several diseases. Since the ancient days, several medicinal systems such as Ayurveda, Siddha, Chinese traditional medicine, Unani, naturopathy, anthroposophic medicine, and homeopathy are practicing phytomedicine to treat the various conditions <sup>1</sup>. Besides, as per the World Health Organization (WHO), about 2500 plants are being used for the treatment of various diseases <sup>2</sup>.

Traditional medicinal plants are mainly preferred due to their safety, accessibility, affordability, and faith. In this instance, validation and standardization of their pharmacological effects are essential to ensure the safety and efficacy of herbal medicines. Plants are naturally containing several phytochemical constituents which are responsible for their medicinal properties <sup>3-6</sup>.

Hence, the phytochemical profiling of secondary metabolites is primarily required to assess their pharmacological actions <sup>5, 6</sup>. Gas chromatography-mass spectrometry is one of the advanced techniques to determine the volatile phytochemical compounds of the herbal plant samples <sup>7</sup>. *Cadaba indica* Lam. is a tropical and subtropical region plant that belongs to the family Capparidaceae (Capparaceae). *Cadaba indica*, also known as Veezhi or vizhuthi in Tamil which was first

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described by Pulipani siddhar in his book “Jala thirattu”. The Ayurvedic and Siddha medicine system prescribed this leaf juice for the treatment of dysentery, fever, swelling, cough, lung problem and worm infestation. Also, this plant was known to medicine for treating menstrual irregularities, ovarian cysts, and other female infertility problems<sup>8-11</sup>. *Cadaba indica* Lam. methanolic leaf extract contains phenol, flavonoid, saponin, steroid, protein, and carbohydrate<sup>12</sup>. At the same time, the aerial parts have already been reported with a sensible amount of total phenol and flavonoids<sup>13</sup>. The antioxidant<sup>13</sup>, anti-inflammatory<sup>12, 14</sup>, analgesic<sup>14</sup>, anti-microbial<sup>15</sup>, and antipyretic<sup>16</sup> activities also established with various solvent extracts of *Cadaba indica* Lam. This present study aimed to detect and characterize the phytochemical constituents in cold macerated methanolic leaf extract of *Cadaba indica* Lam by gas chromatography-mass spectrometry method.

## MATERIALS AND METHODS:

**Collection of Plant Material:** The plant *Cadaba indica* was collected during the flowering season in February 2017 from, Melur, Madurai district, Tamil Nadu, India. The proposed plant was authenticated by Dr.V.Chelladurai, Formerly Research Officer of Botany, Central Council for Ayurveda and Siddha, Government of India. A voucher specimen (Dated 20/03/2017) was preserved in the laboratory for future reference.

**Preparation of Plant Leaf Extract:** In a cold maceration method, 100 g of *Cadaba indica* leaf powder was soaked in petroleum ether in a closed glass jar for 72 h. Such defatted material was subjected to methanol extraction. The extracted plant material was then filtered and dried under reduced pressure in Eyele Rotary evaporator (Japan) at room temperature to a viscous mass, weighed, and stored at 4 °C for further analysis.

**Procedure:** The chemical constituents of cold macerated methanolic leaf extract of *Cadaba indica* analyzed using Agilent GC 7890A gas chromatography connected with an MS- 5975C mass spectrometer instrument detector. Autosampler system-7693 (ALS 7693) was used in the sample injection process. Helium, a carrier gas used at 1ml/minute constant flow rate, and the splitless flow rate was 1 ml/min.

The capillary column used in this experiment was a DB-5MS non-polar capillary column (5% diphenyl, 95% dimethyl polysiloxane) with dimensions of 30 m length, 0.25 mm inner diameter, and 0.25 µm of film thickness. The initial oven temperature was kept as 50 °C for 1 min and programmed to reach 300 °C held for 2 min. The total run time of 28 min was programmed for the analysis, and the injection volume was 1 µl.

The detector operated in 50-550 mass range with 0.5s scan interval. The obtained chromatogram of plant extract was analyzed in mass spectrometry to identify the mass of detected fractions. Eluted chemical constituents were further identified based on the retention time and mass spectra. The comparison of eluted compounds made with standard mass spectra data library- National Institute of Science and Technology (NIST)-14.0 versions to determine the name, molecular weight, and structure of the eluted chemical constituents.

**RESULTS:** The cold macerated methanolic leaf extract of *Cadaba indica* (CICME) was analyzed in gas chromatography-mass spectrometry (GC-MS) to identify the bioactive compounds that responsible for its pharmacological actions. After 28 min of a complete run, 40 peaks were obtained, as shown in the chromatogram **Fig. 1**. The eluted bio-active compounds were characterized by retention time (RT), peak area, and peak area percentage (%). However, the mass spectrum was used to identify the structure of eluted chemical constituents by its database NIST-14.0 library.

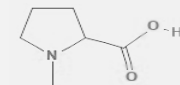
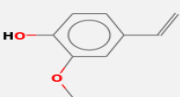
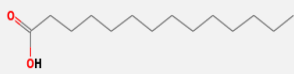
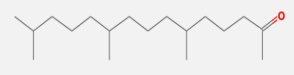

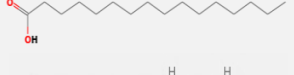

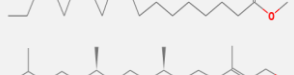




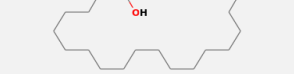

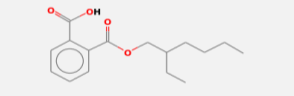

Among the several chemical constituents, sixteen peaks with moderate to higher concentrations were selected for the data analysis. The first compound obtained was 2-pentene, 3-methyl-, (E)- at 6.523 min of retention time.

Consecutively, 1,Methyl-pyrrolidine-2-carbo-xylic acid (5.10%), 2-Methoxy-4-vinyl phenol (0.72%), Tetradecanoic acid (1.06%), 2 – penta - decanone,6,10,14- trimethyl- (0.92%), Hexa-decanoic acid methyl ester (3.56%), n-Hexadecanoic acid (27.56%), 10,13-octa-decadienoic acid methyl ester (1.28%), 9,12,15-Octadecatrienoic acid, (Z, Z)-methyl ester (4.71%), Phytol (3.36%), Octadecanoic acid, methyl ester (0.88%), 9,12-Octadecadienoic acid, (Z, Z)-(5.34%), 9,12,15-

Octadectatronic acid, (Z,Z,Z)- (19.75%), Octadecanoic acid(4.77%), Hexa-decanoic acid, 2,3-dihydroxy propyl ester (1.94%), 1,2 Benzene dicarboxylic acid, mono (2-ethylhexyl) ester (1.35%), 9- Octadecanoic acid, (Z) – methyl ester (1.05%) were identified and the molecular formula,

molecular weight and nature of the compound were also tabulated in **Table 1** and **2**. The pharmacological activities of the identified compounds were tabulated in **Table 2** as per previously published phytochemical and ethnopharmacological studies.

**TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF COLD MACERATED METHANOLIC LEAF EXTRACT OF CADABA INDICA LAM.BY GC-MS SPECTRA**

S. no.	Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	Peak Area (%)	Chemical Structure
1	1,Methyl-pyrrolidine-2-carboxylic acid	C <sub>6</sub> H <sub>11</sub> NO <sub>2</sub>	129.16	9.009	5.10	
2	2-Methoxy-4-vinyl phenol	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	150.17	10.731	0.72	
3	Tetradecanoic acid	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47	16.130	1.06	
4	2-pentadecanone,6,10,14-trimethyl-	C <sub>18</sub> H <sub>36</sub> O	268.48	17.019	0.92	
5	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	17.863	3.56	
6	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.42	18.241	27.65	
7	10,13-octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47	19.496	1.28	
8	9,12,15-Octadecatrienoic acid, (Z, Z)-methyl ester	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292.46	19.552	4.71	
9	Phytol	C <sub>20</sub> H <sub>38</sub> O <sub>4</sub>	296.53	19.641	3.36	
10	Octadecanoic acid, methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298.50	19.785	0.88	
11	9,12-Octadecadienoic acid, (Z, Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.45	19.852	5.34	
12	9,12,15-Octadectatronic acid,(Z,Z,Z)-	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.43	19.907	19.75	
13	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.48	20.107	4.77	
14	Hexadecanoic acid, 2,3-dihydroxy propyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330.50	23.051	1.94	
15	1,2 Benzene dicarboxylic acid, mono (2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.34	23.207	1.35	
16	9- Octadecanoic acid, (Z) – methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49	24.462	1.05	

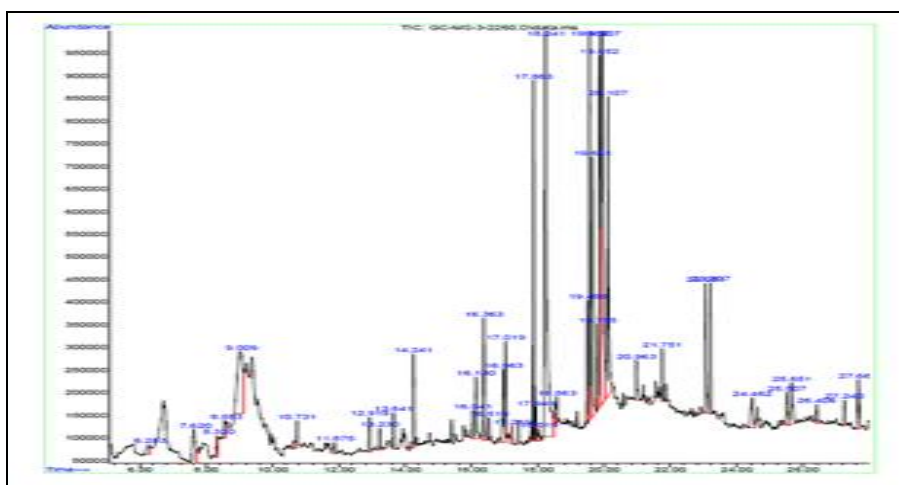


FIG. 1: GC-MS CHROMATOGRAM OF COLD MACERATED METHANOLIC LEAF EXTRACT OF *CADABA INDICA* LAM

TABLE 2: PHARMACOLOGICAL ACTIVITY AND NATURE OF IDENTIFIED PHYTOCHEMICAL CONSTITUENTS IN COLD MACERATED METHANOLIC LEAF EXTRACT OF *CADABA INDICA* LAM

S. no.	Compound Name	Nature of the Compound	Pharmacological Activity
1	1,Methyl-pyrrolidine-2-carboxylic acid	pyrrolidine	Antifungal and anti-bacterial <sup>17</sup>
2	2-Methoxy-4-vinyl phenol	Phenolic compound	Anti-inflammatory <sup>18</sup> and anti-cancer <sup>19</sup>
3	Tetradecanoic acid	Myristic acid	Antioxidant, anti-cancer, hypocholesterolemic, nematicide <sup>20</sup>
4	2-pentadecanone,6,10,14-trimethyl-	Ketone	Anti-inflammatory, wound healing, and anti-bacterial <sup>21</sup>
5	Hexadecanoic acid, methyl ester	Linolenic acid ester	Anti-inflammatory, anti-cancer, hepatoprotective, anti-arthritic, anti-androgenic and anti coronary activity <sup>20</sup>
6	n-Hexadecanoic acid	Linolenic acid	Anti-inflammatory, antioxidant, hypocholesterolemic and anti-androgenic <sup>20,22,23</sup>
7	10,13-octadecadienoic acid,methyl ester	Linolenic ester	Anti-inflammatory, anti-arthritic, hypocholesterolemic, hepatoprotective, antihistamine activity <sup>20</sup>
8	9,12,15-Octadecatrienoic acid, (Z, Z)-methyl ester	Linolenic acid ester	Anti-inflammatory, hypocholesterolemic, hepatoprotective, and anti-cancer <sup>24</sup>
9	Phytol	Diterpene	Anti-inflammatory, anti-cancer, antioxidant, diuretic, and anti-microbial <sup>25-28</sup>
10	Octadecanoic acid, methyl ester	Linolenic acid ester	Anti-tumor, cytotoxic and anti-microbial <sup>29,30</sup>
11	9,12-Octadecadienoic acid, (Z, Z)-	Linolenic acid	Anti-inflammatory, anti-cancer, anti-arthritic, antihistaminic, and hypocholesterolemic <sup>20,24</sup>
12	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-	Linolenic acid	Anti-arthritic, anti-inflammatory, anti-acne, hepatoprotective, hypocholesterolemic <sup>24,31</sup>
13	Octadecanoic acid	Linolenic acid	Antifungal, antibacterial and anti-tumor <sup>32</sup>
14	Hexadecanoic acid, 2,3-dihydroxy propyl ester	Linolenic acid ester	Anti-inflammatory and NF-κB inhibitory action <sup>33</sup>
15	1,2 Benzene dicarboxylic acid, mono (2-ethylhexyl) ester	Phthalic ester	Anti-cancer and cytotoxic activity <sup>34</sup>
16	9- Octadecanoic acid, (Z) – methyl ester	Linolenic acid ester	Anti-inflammatory, antiandrogenic, anti-cancer, antioxidant and anti-fungal <sup>20,35</sup>

**DISCUSSION:** *Cadaba indica* Lam. methanolic leaf extract was prepared by cold maceration method in this study. Cold maceration is a simple method to extract any raw materials without loss of thermolabile bioactive compounds due to low temperature<sup>36</sup>. Ramakrishnan *et al.* reported that

the availability of phytochemicals was higher in soxhlet methanolic extract of *Cadaba indica* leaves than other solvent extracts<sup>12</sup>. Mohan VR *et al.* reported the level of total phenol and flavonoid content in aerial parts of *Cadaba indica*. They found out that the level of phenol and flavonoids

were relatively higher in methanolic extract than other aromatic solvents<sup>13</sup>. Thirumalai et al. quantified the amount of rutin, gallic acid, and quercetin in various types of extracts of *Cadaba indica* leaf, and reported that quercetin, gallic acid, and rutin were found to be in higher quantities in cold macerated methanolic leaf extract than in hot percolation method<sup>37</sup>.

In this present study, the cold macerated methanolic extract was subjected to the Gas chromatography-Mass spectrometry (GC-MS) analysis to identify the secondary metabolites. The gas chromatogram exhibits the concentration of eluted compounds as a function of retention time (RT). The chromatogram peaks show the detected chemical constituents. The height of the peaks represented the concentrations of eluted chemical constituents. Mass spectrum of a compound is a graphical representation of ion distribution by their mass and charge ratio (m/z) which is essential in the identification of chemical structure and its characters as well.

Among the several chemical constituents identified, n-hexadecanoic acid has the highest peak area (concentration) of 27.65%. This compound is linolenic acid in nature and also called palmitic acid. However, it occurs in most natural sources and is responsible for their medicinal uses. Earlier studies reported that n-hexadecanoic acid has anti-inflammatory, antioxidant, anti-androgenic, and hypocholesterolemic activities<sup>20</sup>. n-hexadecanoic acid suppresses the inflammatory process by its inhibitory action of phospholipase A2 enzyme<sup>23</sup>. Palmitic acid inhibits the invasion of macrophages; hence this may reduce the accumulation of macrophages in the synovial fluid of the arthritic joint<sup>38</sup>.

Besides, *in-silico* cytotoxicity studies suggested that n-hexadecanoic acid interacts with DNA topoisomerase-1 enzyme and produces cytotoxic effects which are responsible for its anti-cancer activity<sup>22</sup>. 9, 12, 15- Octadectatrionic acid (Z, Z, Z)- is an alpha-linolenic acid compound with 19.75% peak area, which is the second major chemical constituent of *Cadaba indica* methanolic leaf extract. It has anti-inflammatory, antioxidant, anti-cancer, hepatoprotective, hypocholesterolemic, and anti-acne activities<sup>31</sup>. 9, 12, 15- Octa-

decatrionic acid (Z, Z, Z)- also inhibits the synthesis of prostaglandin and leukotrienes from an arachidonic acid pathway, which plays an essential role in inflammation<sup>24</sup>. Phytol is a diterpenoid, a notable bioactive compound in medicinal plants. Previous reports suggested that phytol has anti-inflammatory, antioxidant, anti-arthritic, anti-cancer, diuretic, and antimicrobial activities<sup>26, 27</sup>.

Phytol interacts with the nuclear factor kappa -B (NF-κB) signaling pathway and migration of neutrophils into inflammation sites that further causes the inhibition of pro-inflammatory cytokines such as TNF-α and interleukin -6, hence can regulate the inflammatory process of arthritis<sup>25, 28</sup>. 2-methoxy-4-vinyl phenol a phenolic compound that has anti-inflammatory and anti-cancer activity. The anti-inflammatory activity may be due to the suppression of NF-κB and mitogen-activated protein kinase (MAPK) signaling pathway. However, this effect results in the inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes<sup>18</sup>.

Tetradecanoic acid, a saturated fatty acid (myristic acid) that was identified in the extract, has antioxidant, anti-cancer activity, and hypocholesterolemic activities<sup>20</sup>. Whereas a ketone compound, 2-pentadecanone 6, 10, 14- trimethyl- with anti-inflammatory, wound healing, and antibacterial activity<sup>21</sup> was also identified with 0.92% peak area in this extract (CICME). A phthalic ester compound, 1, 2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester has previously been reported with anti-cancer and cytotoxic activities<sup>34</sup>. Some of the compounds identified in the extract such as 1-methyl-pyrrolidine-2-carboxylic acid<sup>17</sup>, Octadecanoic acid<sup>32</sup>, 9- Octadecanoic acid, (Z) – methyl ester, have been reported to have antifungal and antibacterial activities<sup>20, 35</sup>.

Linolenic acid and its esters are the most common chemical constituents detected in the methanolic leaf extract of *Cadaba indica* (CICME). Earlier reports revealed that these compounds have potent anti-inflammatory, anti-cancer, and antioxidant activity<sup>24</sup>. Linolenic compounds such as n-hexadecanoic acid (27.65%), 9, 12-Octa-decadienoic acid, (Z, Z) - (5.34%), 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)- (19.75%) and Octadecanoic acid (4.77%) are more common in several medicinal

plants. However, these secondary metabolites are responsible for their pharmacological actions such as anti-inflammatory, antioxidant, and anti-cancer activities<sup>20, 22, 38</sup>. Additionally, octadecanoic acid has antibacterial and antifungal activities<sup>32</sup>. Whereas 9, 12-Octadecadienoic acid, (Z, Z) - possess anti-histaminic activity<sup>20</sup> and the compound, 9,12,15-Octadecatrienoic acid,(Z, Z, Z)- has hepato-protective activity<sup>31</sup>.

Hexadecanoic acid, methyl ester (3.56%), 10,13-octadecadienoic acid, methyl ester (1.28%), 9,12,15-Octadecatrienoic acid, (Z, Z)-methyl ester (4.71%), Octadecanoic acid, methyl ester (0.88%), Hexadecanoic acid, 2,3-dihydroxy propyl ester (1.94%),9- Octadecanoic acid, (Z) – methyl ester (1.05%) are the linolenic acid esters identified in this extract (CICME). Esters of linolenic acid also have anti-inflammatory, anti-cancer, and hypo-cholesterolemic activities with additional hepato-protective and anti-androgenic effects<sup>24, 35</sup>. Among these ester compounds, octadecanoic acid, methyl ester has a cytotoxic activity as reported by the previous studies<sup>30</sup>. Whereas hexadecanoic acid, 2, 3-dihydroxy propyl ester interacts with NF- $\kappa$ B signaling pathway and inhibits the production of pro-inflammatory mediators<sup>33</sup>.

9, 12, 15-Octadecatrienoic acid, (Z, Z, Z); n-hexadecanoic acid; phytol; 1, methyl pyrrolidine-2-carboxylic acid and 1, 2-benzene dicarboxylic acid – mono (2- Ethylhexyl) ester are such bioactive compounds which were identified in this methanolic leaf extract of *Cadaba indica* and are also found in the various plants of *Capparidaceae* family such as *Cadaba trifoliata*<sup>39, 40</sup>, *Cadaba fruticosa*<sup>27</sup> and *Capparis spinosa*<sup>41</sup>.

The gas chromatogram of the methanolic leaf extract of the proposed plant revealed that more than ten bioactive compounds are having anti-inflammatory, anti-arthritis and antioxidant activities. These compounds are present in higher concentrations (peak area percentage) and may contribute to the anti-inflammatory and anti-arthritis activity of *Cadaba indica*.

**CONCLUSION:** Gas chromatography-Mass spectrometry is one of the standard analytical techniques to characterize the phytoconstituents present in herbal plants. In this present study, the results reveal that the detected active compounds in

cold macerated methanolic leaf extract of *Cadaba indica* Lam. are responsible for its pharmacological effects. Notably, detected compounds with anti-inflammatory, anti-arthritis, and antioxidant activities might be responsible for their folklore medicinal uses in chronic inflammatory conditions. Further investigations are required to assess the safety and efficacy of this traditional herbal plant in various disorders.

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**CONFLICTS OF INTEREST:** The authors declare that they have no conflicts of interest.

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