TRADITIONAL MEDICINAL PLANT LEUCAS ASPERA LINN.: GC-MS ANALYSIS, CHEMICAL EXAMINATION, ANTIDIABETIC, AND ANTI-OBESITY ACTIVITIES

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Summary

Objectives: Leucas aspera Linn. commonly known as Thumbai, is a medicinal plant with a rich history of traditional use in various systems of medicine. This research article presents a comprehensive study aimed at exploring the potential antidiabetic and anti-obesity activities of L. aspera, along with a detailed chemical examination.
Methodology: Chemical examination was carried out by using GC-MS and column chromatographic analysis. Antidiabetic activity was assessed by using α-amylase and α-glucosidase inhibitorymethods, while anti-obesity activity was performed through porcine pancreatic lipase inhibitory test.
Results: The results of the GC-MS analysis of methanolic extract of L. aspera (La) revealed a diverse array of 18 known major bioactive compounds. The chromatographic analysis of La revealed the presence of five known compounds, which upon analysing the spectral data identified as clovanediol (1), lupeol (2), stilbenoid (3), chrysoeriol (4), and 2-isoprenyl emodin (5). The plant extract and isolated metabolites (4 and 5) exhibited significant antidiabetic and anti-obesity effects by enhancing regulating key enzymes involved in glucose metabolism and inhibiting adipogenesis, respectively.
Conclusions: Overall, this investigation offers a scientific basis for the traditional use of L. asperaas a potential treatment option for diabetes and obesity.

Keywords: Leucas aspera, GC-MS analysis, phytochemical analysis, antidiabetic, anti-obesity.

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1. Introduction

In recent years, there has been growing interest in exploring natural products for their potential therapeutic applications [1]. Medicinal plants, in particular, have been a rich source of bioactive compounds with diverse pharmacological properties [2]. Leucas aspera Linn. commonly known as Thumbai, is one such plant that has long been recognized for its traditional medicinal uses[3,4]. L. aspera belongs to the family Lamiaceae and is widely distributed in various regions of the world, including Asia, Africa, and Australia. The plant is characterized by its aromatic nature, with leaves that possess distinct odours and flavours. Throughout history, this herb has been employed in traditional systems of medicine, such as Ayurveda and Siddha, for the treatment of various ailments, including diabetes and obesity [5–10]. GC-MS analysis is a widely used technique for the identification and quantification of chemical constituents in complex plant extracts [11]. By employing this technique, researchers can gain a deeper understanding of the chemical composition of L. aspera, thereby facilitating the discovery of potential bioactive compounds responsible for its therapeutic effects. Diabetes mellitus and obesity are two prevalent chronic metabolic disorders that have reached alarming levels globally. These conditions pose significant health challenges and contribute to the development of various complications, leading to increased morbidity and mortality [12]. Current therapeutic approaches for diabetes and obesity often involve synthetic drugs, which may be associated with undesirable side effects [13]. Therefore, the exploration of natural alternatives, such as L. aspera, holds immense promise. The present research article aims to shed light on the potential antidiabetic and anti-obesity activities of L. aspera. Furthermore, the study incorporates a comprehensive chemical examination of the plant using GC-MS analysis. This article presents the findings of the GC-MS analysis and chemical investigation conducted on methanolic extract of L. aspera, followed by an evaluation of its antidiabetic and anti-obesity activities. The study aims to bridge the gap between traditional knowledge and modern scientific exploration, providing evidence for the folklore usage of L. asperain managing diabetes and obesity.

2. Methodology

Collection of L. aspera: The whole plant Leucas aspera Linn. (family Lamiaceae) was collected from local areas of Visakhapatnam, Andhra Pradesh, India, during July 2018. The specimens of this species were authenticated (accession number 1004) and deposited at the Department of Botany, Andhra University, India.

Extraction ofL. Aspera

The whole plant was shade dried (1.0 kg) extracted three times with methanol (90%) and concentration with rotapavorto obtain a methanolic extract of the whole plant L. aspera (**La,** 18.0 g, 1.8%w/w). The La (10.0 g) was subjected to column chromatography (45 cm × 20 cm with 10 mm Sinter diameters) by using silica gel (#100-200), and hexane and AcOEt as solvent system resulted in five fractions. These fractions were subjected to re-column chromatography (45 cm × 10 cm with 10 mm Sinter diameters) by using silica gel (#230-400) yielded five compounds. These compounds were characterized by using elemental analysis, FT-IR and NMR spectroscopy.

GC-MS analysis of La

The GC-MS profiling of La was performed using the following parameters. The column oven temperature (temp) was set at

50.0 °C, while the injection temp was maintained at 250.00 °C. The injection mode employed was split injection, and the flow control mode was pressure-based, with a pressure of 49.5 kPa. The total flow rate was set to 8.7 mL/min, with a linear velocity of 35.3 cm/sec, corresponding to acolumn flow rate of 0.95 mL/min. The split ratio was set at 5.0. The oven temp program consisted of a rate of 10.00 °C/min, starting at 50.0 °C and held for 2.00 min, subsequently an increase to 300.0 °C with a hold time of 10.0 min. The solvent cut time of 4.00 min was implemented, with a detector gain of 0.97 kV + 0.20 kV. The threshold was set to 0, and the analysis was initiated at 4.00 min, continuing until 37.00 min. The acquisition mode was scan, with an event time of 0.30 seconds and a scan speed of 3333. The scan range started from 50.00 m/z and extended up to 1000.00 m/z. Finally, the sample inlet unit utilized for the analysis was the GC sample inlet unit.

In vitro α-amylase inhibition assay

The α-amylase inhibitory assay was conducted (n = 3) using the DNSA (3,5-dinitrosalicylic acid) method[14]. Acarbose (positive control), La and its isolated compounds were dissolved in 10% DMSO and further dissolved in a sodium phosphate buffer solution (pH 6.9, 0.02 M) to achieve 25-100 µg/mLconcentrations. A mixture of α-amylase solution (2 U/mL,200 µL,CAS: 9000-85-5, Sigma-Aldrich, USA) and acarbose/La/isolated metabolites(200 µL) was incubated for 10 min at 30 °C. Following this, added starch solution (200 µL, 1% w/v in water) and incubated for 3 min. The reaction was ended by adding DNSA reagent (200 µL) and boiling the mixture at 85–90 °C for 10 min, cooled and diluted. Finally, absorbance was measured at 540 nm using a spectrophotometer (BioTek Synergy HT, USA). The percentage of α-amylase inhibition was plotted against the concentration of the acarbose/La/isolated compounds, and the IC_{50} values were obtained from the resulting graph.

In vitro α-glucosidase inhibition assay

The test was conducted (n = 3) following the protocol developed by Tatipamula et al. 2021 [15]. To prepare the samples, 50 µL of acarbose/**La**/isolated compounds was used at 25 to 100 µg/mLconcentrations. The samples were then incubated with 10 µL α-glucosidase (1 U/mL,CAS: 9001-42-7,Sigma-Aldrich, USA) and 0.1 M phosphate buffer (pH 6.8, 125 µL) for 20 min at 37 °C. Added4-Nitrophenyl-β-D-glucopyranoside (20 µL, 1 M) as the substrate, followed by a 30 min incubation. To stop the reactionadded Na_2CO_3 (50 µL, 0.1 N), and absorbance was calculated at 405 nm.

In vitro anti-obesity activity

The anti-obesity activity was assessed by utilizing porcine pancreatic lipase (PPL) and substrate:p-nitrophenyl butyrate (p-NPB) [16]. 1 mg/mL PPL stock solutions were and stored at −20 °C. Orlistat(positive control)/**La**/isolated compounds (at final concentrations of 25-100) was pre-incubated by PPL for 60 min at 30 °C. The reaction was started by adding p-NPB (0.1 µL) as the substrate and incubation at 30 °C for 5 min and quantified using a spectrophotometer at 405 nm.

3. Results and Discussion

GC-MS analysis of La: Many peaks were identified during the GC-MS analysis of **La** (Figure I), which visualized presence of 18 known major compounds. Each peak was characterized by its retention time, area percentage, A/H ratio, and name, providing information about the specific compounds present in the analyzed sample (Table I). At a retention time of 4.404 min, a peak with an area percentage of 0.70% and an A/H ratio of 9.70 was identified as clovanediol. Another peak appeared at a

retention time of 7.678 min, exhibiting an area percentage of 0.61%, an A/H ratio of 9.96, and identified as 2-isoprenyl emodin. At a retention time of 13.272 min, a peak with an area percentage of 0.92% and an A/H ratio of 11.20 was observed and identified as lupeol. Another peak appeared at a retention time of 13.364 min, exhibiting an area percentage of 0.98%, an A/H ratio of 11.20, and identified as stilbenoid. At a retention time of 15.599 min, a peak with an area percentage of 8.01% and an A/H ratio of 4.07 was identified as 1-Methoxy-4-[[[[2-(4-hydroxyphenyl)ethyl]amino]methyl]-2-benzenol. Another peak appeared at a retention time of 16.142 min, exhibiting an area percentage of 9.14%, an A/H ratio of 5.49, and identified as 4-Ethoxy-3-methoxybenzyl alcohol. At a retention time of 17.623 min, a peak with an area percentage of 6.61% and an A/H ratio of 25.47 was observed and identified as Methyl-alpha-D-glucopyranoside. Another peak appeared at a retention time of 17.975 min, exhibiting an area percentage of 0.88%, an A/H ratio of 6.53, and identified as chrysoeriol. Similarly, at a retention time of 18.263 min, a peak was detected with an area percentage of 4.4%, an A/H ratio of 6.54, and identified as 2-Methyl ester-(4-ethyl-2-acetoxy-5-methoxyphenyl)acetic acid. Other peaks included 2-Methylpropyl ether-4-hydroxy-3-methoxybenzyl alcohol at 18.623 min (area percentage: 6.81%, A/H ratio: 5.64), 5-Pentyl-1,3-benzenediol at 18.915 min (area percentage: 3.41%, A/H ratio: 12.94), and Ethyl 2-hydroxy-4-methoxy-6-propylbenzoate at 19.185 min (area percentage: 3.75%, A/H ratio: 12.17).

Furthermore, a significant peak was observed at a retention time of 19.799 min, accounting for 18.29% of the total peak area with an A/H ratio of 42.25. This peak was identified as L-Arabinitol. Another notable peak appeared at 20.687 min, exhibiting an area percentage of 5.76% and an A/H ratio of 28.2, identified as 3-Deoxy-d-mannitol. Additional peaks detected included n-Heptadecanol-1 at 22.166 min (area percentage: 1.23%, A/H ratio: 8.05), 1-(7-Acetyl-4,6-dihydroxy-3,5-dimethyl-2-benzofuranyl)-2,4-pentanedione at 25.067 min (area percentage: 1.51%, A/H ratio: 6.96), and 3-Carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione at 26 min (area percentage: 1.53%, A/H ratio: 27.8). Finally, a peak was observed at 27.578 min, accounting for 1.14% of the total peak area with an A/H ratio of 30.2. This peak was identified as 2-Hydroxy-3-[(9E)-9-octadecenoyloxy] propyl (9E)-9-octadecenoate. These peaks provide valuable information about the presence and composition of various compounds in the analyzed sample La.

Chemistry examination of La: The chemical examination of La yielded five known compounds, which upon analysing the spectral data identified as clovanediol (1), lupeol (2), stilbenoid (3), chrysoeriol (4), and 2-isoprenyl emodin (5) (Figure II). To the best of our knowledge, except 4, all the isolated compounds were first time reported from L. aspera. Clovanediol (1): Colorless liquid; R_f: 0.8 (Hexane: AcOEt, 4:1); Mol. Formula: $C_{15}H_{26}O_2$; FT-IR (KBr): 575.56, 603.83, 666.35, 715.97, 758.95, 855.44, 929.25, 1016.80, 1082.69, 1156.81, 1203.34, 1242.72, 1279.56, 1338.80, 1453.50, 1530.41, 1639.53, 1843.71, 2119.31, 2931.28, 3410.61 cm^{-1}. ^1H (400 MHz, $CDCl_3$): 0.99 (s, 1H, 5-CH), 1.06 (s, 3H, 13-CH_3), 1.07 (s, 3H, 14-CH_3), 1.09 (s, 1H, 12-CH), 1.19-1.34 (m, 4H, 7,11-CH), 1.20 (s, 3H, 15-CH_3), 1.40-1.46 (m, 4H, 3,10-CH), 1.65-1.73 (m, 2H, 3-CH), 3.32-3.35 (t, J = 4 Hz, 1H, 9-CH), 3.96 (s, 1H, 9-OH), 3.40-3.42 (t, J = 4 Hz, 1H, 2-CH), 3.96 (s, 1H, 2-OH). ^{13}C (400 MHz, $CDCl_3$): 19.16 (C-6), 23.45 (C-15), 27.36 (C-10), 30.07 (C-13/14), 33.57 (C-11), 36.31 (C-7), 38.79 (C-4), 39.26 (C-8), 40.83 (C-12), 42.77 (C-1), 50.37 (C-3), 52.22 (C-5), 75.41 (C-2), 79.58 (C-9).

Lupeol (2): Pale yellowish powder; R_f: 0.6 (Hexane: AcOEt, 4:1); Mol. Formula: $C_{30}H_{50}O$; m.p.: 217-218 ºC; Elemental analysis: anal. C-84.59, H-11.32(%); calcd. C-84.44, H-11.81(%). FT-IR (KBr): 842.55, 959.43, 1060.87, 1108.53, 1148.08, 1241.03, 1281. 95, 1345.77, 1412.50, 1469.32, 1634.58, 1786.83, 1967.04, 2164.85, 2238.04, 2507.57, 2580.86, 2697.63, 2741.50, 2887.02, 3444.20 cm^{-1}.^{1}H (400 MHz, $CDCl_3$): 0.86 (s, 1H, 12-CH_2), 0.98 (s, 3H, 28-CH_3), 1.00 (s, 6H, 26,27-CH_3), 1.01 (s, 9H, 23,24,25-CH_3), 1.04 (s, 1H, 13-CH), 1.07 (s, 1H, 18-CH), 1.14-1.18 (m, 3H, 11,16-CH_2), 1.27 (s, 1H, 6-CH_2), 1.35-1.41 (m, 4H, 1,15-CH_2), 1.39 (s, 1H, 21-CH_2), 1.44-1.49 (m, 4H, 3,9,12,22-CH_2), 1.59-1.72 (m, 8H, 2,6,7,16,21,22-CH_2), 1.78 (s, 3H, 30-CH_3), 1.91-1.98 (m, 1H, 5-CH), 1.98-2.10 (m, 1H, 19-CH), 3.46-3.48 (t, 1H, 3-OH), 4.54 (s, 1H, 29-CH2), 4.79 (s, 1H, 29-CH_2).^{13}C (400 MHz, $CDCl_3$):16.62 (C-27), 17.45 (C-25), 17.81 (C-23), 17.95 (C-26), 20.01 (C-6), 20.58 (C-28), 22.33 (C-30), 22.59 (C-11), 26.63 (C-24), 27.35 (C-12), 28.16 (C-2), 30.59 (C-15), 31.84 (C-21), 35.67 (C-7), 37.33 (C-16), 38.47 (C-10), 38.93 (C-13), 39.31 (C-1), 40.31 (C-4), 41.10 (C-22), 42.37 (C-8), 43.70 (C-14), 44.16 (C-17), 49.37 (C-19), 51.13 (C-18), 51.43 (C-9), 55.38 (C-5), 79.89 (C-3), 110.99 (C-20), 152.14 (C-29).

Stilbenoid (3): Pink powder; R_f: 0.4 (Hexane: AcOEt, 4:1); Mol. Formula: $C_{14}H_{12}O_3$; m.p.: 212-213 ºC; Elemental analysis: anal. C-73.77, H-5.32(%); calcd. C-73.67, H-5.30(%). FT-IR (KBr): 601.74, 665.67, 756.30, 848.78, 929.78, 1018.40, 1043.20, 1083.31, 1129.02, 1204.17, 1243.49, 1279.21, 1330.77, 1399.70, 1455.36, 1477.44, 1530.90, 1637.66, 1842.58, 2126.72, 2722.08, 2851.82, 2925.09, 3087.99, 3141.21, 3175.79, 3411.33, 3483.93, 3769.67 cm^{-1}. ^{1}H (400 MHz, $CDCl_3$): 3.81 (s, 2H, 3,5-OH), 4.08 (s, 1H, 4'-OH), 6.25-6.26 (d, J = 4 Hz, 1H, 4-Ar-H), 6.58 (s, 2H, 2,6-Ar-H), 6.84-6.86 (d, J = 8 Hz, 2H, 3',5'-Ar-H), 7.18-7.21 (d, J = 12 Hz, 1H, 7-CH), 7.26-7.29 (d, J = 12 Hz, 1H, 8-CH), 7.32-7.34 (d, J = 8Hz, 2H, 2',6'-CH).^{13}C (400 MHz, $CDCl_3$): 104.87 (C-4), 107.81 (C-2/6), 117.14 (C-3'/5'), 127.70 (C-7), 128.40 (C-1'), 130.01 (C-8), 130.76 (C-2'/6'), 140.38 (C-1), 159.62 (C-3/5), 160.07 (C-4'). Chrysoeriol (4): Pale yellowish powder; R_f: 0.8 (dichloromethane: AcOEt, 3:2); Mol. Formula: $C_{16}H_{12}O_6$; m.p.: 332-333 ºC; Elemental analysis: anal. C-63.96, H-4.05(%); calcd. C-64.00, H-4.03(%). FT-IR (KBr): 602.15, 668.30, 754.91, 849.16, 930.59, 1043.90, 1086.13, 1163.13, 1241.81, 1278.06, 1329.77, 1395.64, 1455.75, 1530.79, 1638.01, 1745.22, 2123.27, 2316.98, 2855.31, 2926.71, 3009.95, 3177.24, 3771.13 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 3.83 (s, 3H, 10-OCH_3), 4.16 (s, 1H, 4'-OH), 4.71 (s, 1H, 7-OH), 4.94 (s, 1H, 5-OH), 6.10-6.11 (d, J = 4 Hz, 1H, 6-Ar-H), 6.15 (s, 1H, 8-Ar-H), 6.29 (s, 1H, 2-Ar-H), 6.85-6.87 (d, J = 8 Hz, 1H, 5'-Ar-H), 7.03-7.04 (d, J = 4 Hz, 2H, 2',6'-Ar-H). ^{13}C (400 MHz, DMSO-d_6): 57.36 (C-10), 95.32 (C-8), 99.82 (C-6), 104.92 (C-4), 106.03 (C-2), 111.57 (C-2'), 115.88 (C-5'), 121.30 (C-6'), 123.49 (C-1'), 149.06 (C-3'), 152.27 (C-4'), 159.69 (C-9), 161.10 (C-5), 164.37 (C-1), 165.07 (C-7), 182.86 (C-3).

2-Isoprenyl emodin (5): Pale yellowish powder; R_f: 0.8 (dichloromethane: AcOEt, 3:2); Mol. Formula: $C_{20}H_{18}O_5$; m.p.: 236-237 ºC; Elemental analysis: anal. C-70.70, H-5.40(%); calcd. C-71.00, H-5.36(%). FT-IR (KBr): 601.88, 625.97, 665.60, 756.64, 849.06, 929.39, 952.32, 1016.62, 1042.74, 1083.68, 1128.76, 1177.13, 1205.64, 1242.99, 1279.06, 1328.74, 1398.25, 1457.16, 1477.62, 1531.03, 1638.70, 1843.89, 1956.65, 2132.00, 2721.44, 2777.11, 2811.08, 2849.16, 2910.37, 2978.18, 3086.94, 3140.61, 3175.32, 3410.46, 3483.41, 3766.57 cm^{-1}. ^{1}H (400 MHz, DMSO-d_6): 1.88 (s, 3H, 18,19-CH_3), 2.73 (s, 3H, 20-CH_3), 3.55-

3.56 (d, J = 4 Hz, 2H, 15-CH_2), 4.52 (s, 1H, 9-OH), 4.61 (s, 1H, 11-OH), 4.99 (s, 1H, 1-OH), 5.48-5.51 (m, 1H, 16-CH), 6.63 (s, 1H, 10-Ar-H), 7.32 (s, 1H, 8-Ar-H), 7.71 (s, 1H, 4-Ar-H). ^{13}C (400 MHz, DMSO-d_6): 18.52 (C-19), 20.86 (C-20), 25.38 (C-18), 25.79 (C-15), 107.68 (C-8), 110.30 (C-12), 110.34 (C-10), 122.11 (C-14), 123.45 (C-4), 123.55 (C-16), 131.68 (C-5), 132.14 (C-17), 136.01 (C-7), 138.55 (C-2), 148.15 (C-3), 162.56 (C-1), 164.95 (C-9), 166.45 (C-11), 181.58 (C-6), 188.79 (C-13).

In vitro α-amylase and α-glucosidase inhibition assays

The evaluation of the antidiabetic activity of L. asperathrough in vitro α-amylase and α-glucosidase inhibition studies revealed promising results. Figure IIIA provides information on the percentage of inhibition and IC_{50} values of various samples from L. asperaagainst α-amylase, which is an enzyme involved in the breakdown of starch [17]. Whereas Figure IIIB provides information on the percentage of inhibition and IC_{50} values of various samples from L. asperaagainst α-glucosidase, which is an enzyme involved in the breakdown of complex carbohydrates into simple sugars [13]. The IC_{50} value represents the concentration at which the samples inhibit α-amylase and α-glucosidase activities by 50%. The reported IC_{50} value for standard drug, acabrose is an average value (mean) based on three measurements, and it also provides the standard deviation (SD) of the measurements. The samples 1, 2, and 3 derived from L. aspera, the IC_{50} values are reported as greater than 100 µg/mL. This suggests that these samples have less inhibitory effects on α-amylase and α-glucosidase activities or require higher concentrations to achieve 50% inhibition. On the other hand, the samples 4, 5, and La derived from L. aspera have specific IC_{50} values of 57.76±3.22 µg/mL, 65.09±2.55 µg/mL, and 42.27±2.01 µg/mL, respectively. These values indicate that these samples have a moderate inhibitory effect on α-amylase activity. Comparatively, the reference sample acabrose has an IC_{50} value of 27.28±1.03 µg/mL, indicating that it has a stronger inhibitory effect on α-amylase compared to the samples from L. aspera.

Instead, the samples 4, 5, and La derived from L. aspera have specific IC_{50} values of 57.83±3.24 µg/mL, 59.66±3.14 µg/mL, and 53.52±3.87 µg/mL, respectively. These values indicate that these samples have a moderate inhibitory effect on α-glucosidase activity. Relatively, acabrose has an IC_{50} value of 31.67±2.21 µg/mL, indicating that it has a stronger inhibitory effect on α-glucosidase compared to the samples from L. aspera. Overall, the superior antidiabetic activity of 4, 5, and La may be due to the presence of freely available oxygenated substances like phenolics, etc. in their chemical structure [18].

In vitro anti-obesity activity

Figure IV provides information on the IC_{50} values of various samples from L. aspera against PPL, which is an enzyme involved in the digestion of dietary fats [19]. The IC_{50} value represents the concentration at which the samples inhibit PPL activity by 50%. The reported IC_{50} values for the samples and Orlistat are average values (mean) based on three measurements, and it also provides the standard deviation (SD) of the measurements.The samples 1, 2, and 3 derived from L. aspera, the IC_{50} values are reported as greater than 100 µg/mL. The sample 4, 5, and La derived from L. aspera has an IC_{50} value of 96.55±6.57 µg/mL, 83.29±5.14 µg/mL, and 81.04±5.54 µg/mL, respectively, indicating a moderate inhibitory effect on PPL activity. Comparatively, the reference sample Orlistat has an IC_{50} value of 75.19±5.68 µg/mL, indicating a similar inhibitory effect on PPL as the samples from L. aspera. Taken together, based on

the available literature, this is the first study on the anti-obesity effect ofL. Aspera. The significant PPL inhibitory effect exhibited by 4, 5, and La ofL. Asperaimply that this species could play a major role in protecting against or treating obesity.

4 Conclusion

In conclusion, the research conducted on L. asperahas provided valuable insights into its potential as a therapeutic agent for the management of diabetes and obesity. The comprehensive chemical examination using GC-MS analysis unveiled a diverse range of 18 known bioactive compounds present in the plant, including clovanediol (1), lupeol (2), stilbenoid (3), chrysoeriol (4), and 2-isoprenyl emodin (5). These compounds are known for their beneficial effects on human health, and their presence in L. asperasuggests its pharmacological potential. The plant extract and isolated metabolites (4 and 5) exhibited significant antidiabetic effects by enhancing glucose uptake and regulating key enzymes involved in glucose metabolism. Additionally, they demonstrated anti-obesity activities by inhibiting adipogenesis and promoting lipolysis. These findings validate the traditional use of L. asperain managing diabetes and obesity.However, further research is warranted to elucidate the precise mechanisms of action and to identify the specific bioactive compounds responsible for the observed effects. In-depth studies exploring the pharmacokinetics, pharmacodynamics, and toxicological aspects of L. asperawill be crucial for its development as a therapeutic agent.

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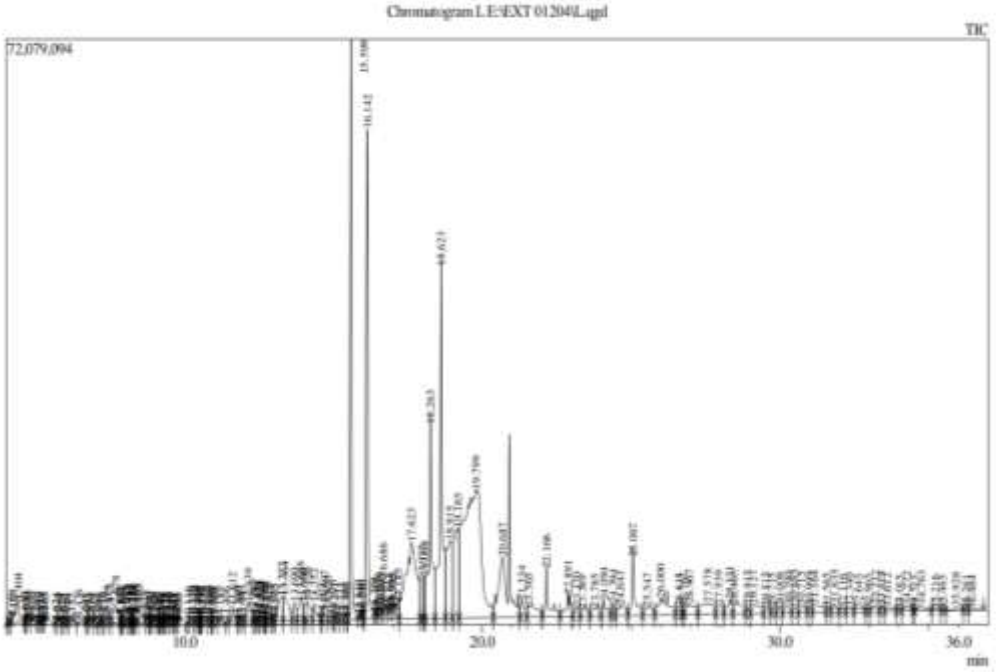

Figure I. GC-MS chromatogram of La.

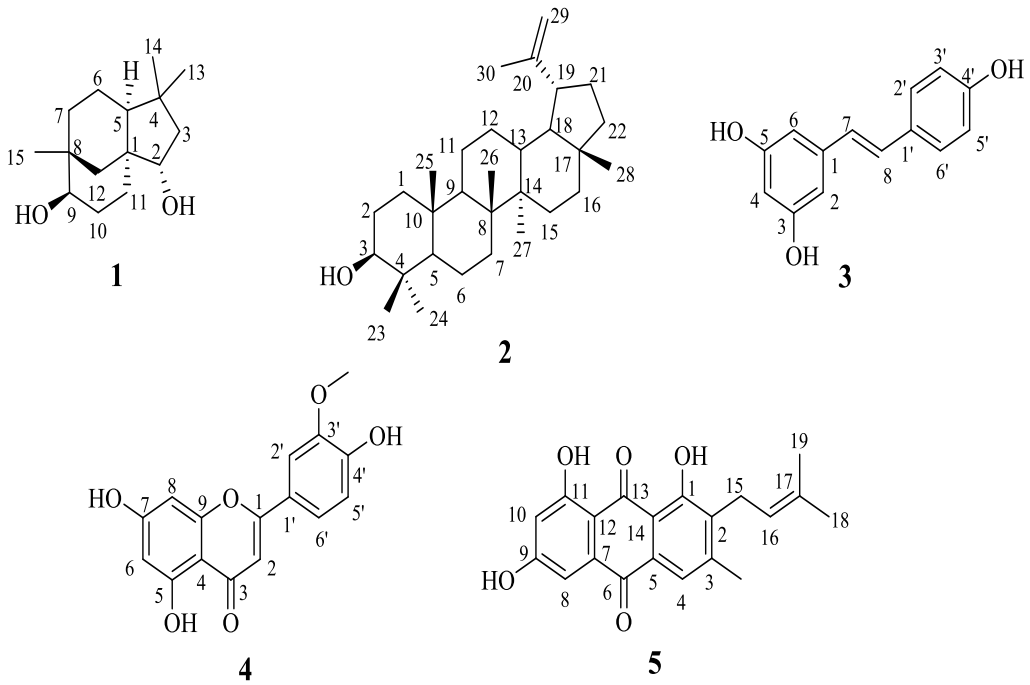

Figure II. Phytochemicals isolated from methanolic extract of L. aspera.

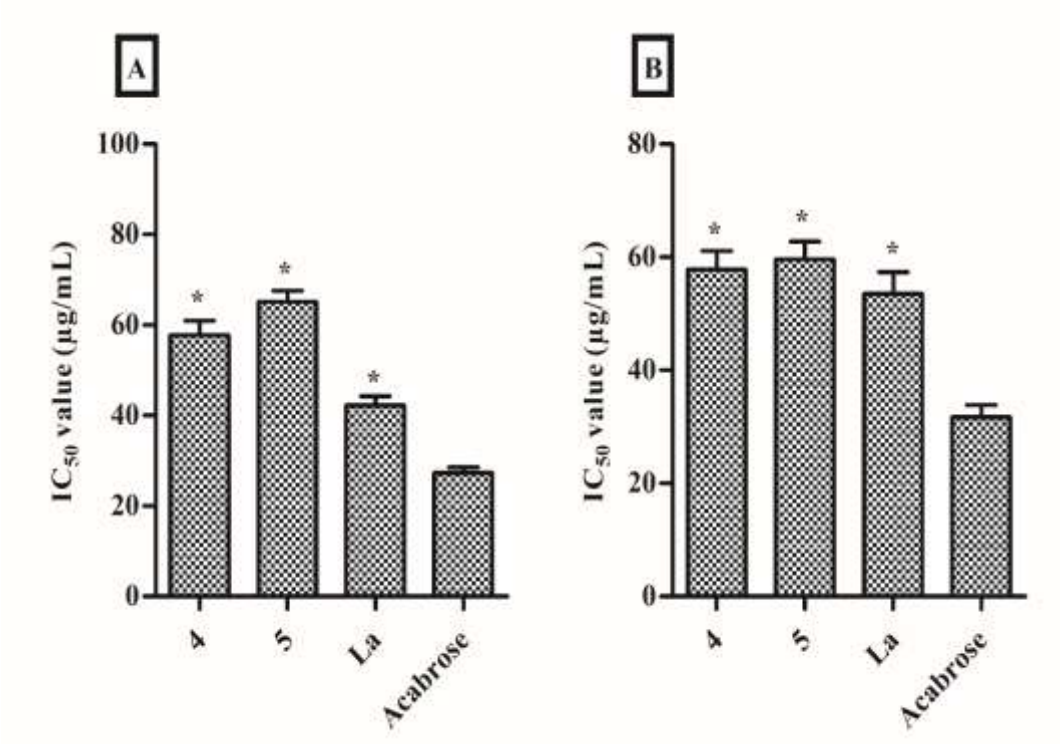

Figure III. IC_{50} values of all samples from L. asperaagainst (A) α-Amylase and (B) α-Glucosidase. Where $^{*}P \leq 0.05$ statistically significant from the standard using ANNOVA followed by Tukey's test.

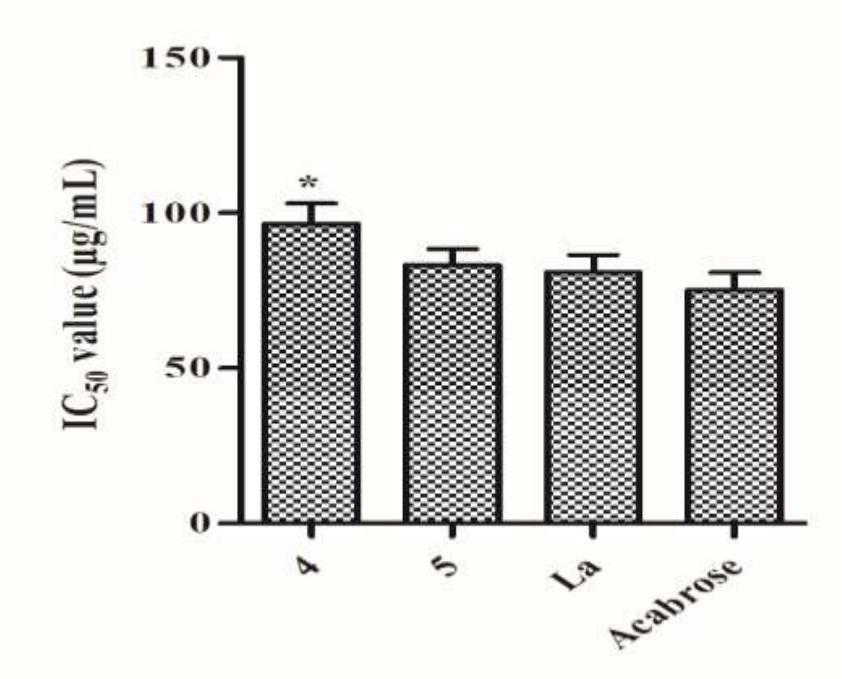

Figure IV. IC_{50} values of all samples from L. asperaagainst porcine pancreatic lipase. Where $^{*}P \leq 0.05$ statistically significant from the standard using ANNOVA followed by Tukey's test.

Table I. Compounds identified from GC-MS analysis of La.

R. Time	Area%	A/H	Compound name
4.404	0.70	9.70	Clovanediol
7.678	0.61	9.96	2-Isoprenyl emodin
13.272	0.92	11.20	Lupeol
13.364	0.98	11.20	Stilbenoid
15.599	8.01	4.07	1-Methoxy-4-[[[[2-(4-hydroxyphenyl)ethyl]-amino]methyl]-2-benzenol
16.142	9.14	5.49	4-Ethoxy-3-methoxybenzyl alcohol
17.623	6.61	25.47	Methyl-alpha-D-glucopyranoside
17.975	0.88	6.53	Chrysoeriol
18.263	4.4	6.54	2-Methyl ester-(4-ethyl-2-acetoxy-5-methoxyphenyl)acetic acid
18.623	6.81	5.64	2-Methylpropyl ether-4-hydroxy-3-methoxybenzyl alcohol
18.915	3.41	12.94	5-Pentyl-1,3-benzenediol
19.185	3.75	12.17	Ethyl 2-hydroxy-4-methoxy-6-propylbenzoate
19.799	18.29	42.25	L-Arabinitol
20.687	5.76	28.2	3-Deoxy-d-mannitol
22.166	1.23	8.05	n-Heptadecanol-1
25.067	1.51	6.96	1-(7-Acetyl-4,6-dihydroxy-3,5-dimethyl-2-benzofuranyl)-2,4-pentanedione
26	1.53	27.8	3-Carboethoxy-4-hydroxybenzo[g]quinoline-5,10-dione
27.578	1.14	30.2	2-Hydroxy-3-[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate