GC-MS ANALYSIS, PHYTOCHEMICAL PROFILING, ANTIDIABETIC, AND ANTI-OBESITY ACTIVITIES OF A TRADITIONAL MEDICINAL PLANT, ACHYRANTHES ASPERA LINN.

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Abstract

The occurrence of metabolic disorders, such as diabetes and obesity, continues to rise globally, necessitating the exploration of alternative and effective therapeutic options. Achyranthes aspera, a traditional medicinal plant, has gained attention due to its potential pharmacological properties and minimal adverse effects. This research article aims to evaluate the phytochemical analysis, antidiabetic, and anti-obesity activities of A. aspera. The GC-MS analysis of methanolic extract of A. aspera (**Aa**) visualized presence of 21 known major compounds. The phytochemical analysis of **Aa** revealed the presence of six known compounds, which upon analysing the spectral data identified as syringic acid (**1**), sinapic acid (**2**), caffeic acid (**3**), luteolin (**4**), apigein (**5**), and 6-prenyl apigenin (**6**). Among all tested samples, **4, 5, 6,** and **Aa** showed superior antidiabetic activity may be due to the existence of freely existing oxygenated constituents like phenolics. The antidiabetic activity of A. aspera was demonstrated through inhibition of carbohydrate-digesting enzymes. On the other hand, **4,6** and **Aa** derived from A. aspera has an IC_{50} value of 84.57±5.44 μgmL^{-1}, 88.24±5.58 μgmL^{-1}, and 72.95±4.13 μgmL^{-1}, respectively, indicating a moderate inhibitory effect on porcine pancreatic lipase activity.These findings highlight its potential as a natural remedy for managing diabetes and obesity-related complications. In conclusion, utilizing A. aspera as a natural source for the development of novel therapeutic interventions offers promising prospects in addressing the management of diabetes and obesity.

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

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1. INTRODUCTION

In recent years, there has been a significant rise in the prevalence of metabolic disorders such as diabetes and obesity, posing a global health challenge. The conventional therapeutic approaches for managing these conditions often come with various side effects, warranting the need for alternative, safe, and effective treatments[1]. In this context, traditional herbal medicine has gained significant attention due to its potential therapeutic properties and minimal adverse effects[2].

Achyranthes aspera, commonly known as "Apamarga" or "Prickly Chaff Flower," is a medicinal plant widely used in traditional systems of medicine. It is native to tropical and subtropical regions and has been extensively utilized for its diverse pharmacological properties[3–10]. Several studies have reported the presence of various bioactive compounds in A. aspera, including alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds. These phytochemical constituents have been attributed to its potential therapeutic effects[11].

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to impaired insulin secretion or action[12,13]. Obesity, on the other hand, is a complex condition associated with excessive fat accumulation and increased body weight. It is a significant risk factor for the development of several chronic ailments, including diabetes[1,14].

Given the traditional use of A. asperain various medicinal preparations and its reported antidiabetic and anti-obesity properties, there is a need for comprehensive scientific evaluation. Overall, the findings from this research can potentially bridge the gap between traditional knowledge and modern medicine, paving the way for the utilization of A. aspera as a valuable source for the development of novel therapeutic interventions against diabetes and obesity. This research article aims to provide a systematic evaluation of the phytochemical analysis of A. aspera, along with its potential antidiabetic and anti-obesity activities. By elucidating the underlying mechanisms and identifying the active constituents responsible for these therapeutic effects, this study seeks to contribute valuable insights into the development of natural remedies for the management of diabetes and obesity.

2. MATERIALS AND METHODS

Collection of A. aspera

The whole plant Achyranthes aspera L. (family Amaranthaceae) was collected from local areas of Visakhapatnam, Andhra Pradesh, India, during October 2018. The specimens of this species were authenticated and deposited at the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Extraction ofA. aspera

The whole plant was shade dried (1.0 kg) extracted thrice with methanol (90%) and concentration at a reduced pressure to obtain a methanolic extract of the whole plant A. aspera (**Aa,** 13.0 g, 1.3%w/w). The **Aa** (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 ethyl acetate 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 six compounds. These compounds were characterized by using elemental analysis, FT-IR and NMR spectroscopy.

GC-MS analysis of Aa

The GC-MS analysis of **Aa** was performed using the following parameters. The column oven temperature was set at

50.0 °C, while the injection temperature 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 $mLmin^{-1}$, with a column flow rate of 0.95 $mLmin^{-1}$, corresponding to a linear velocity of 35.3 $cmsec^{-1}$. A purge flow of 3.0 $mLmin^{-1}$ was used, and the split ratio was set at 5.0. High pressure injection, carrier gas saver, and splitter hold were all turned off. The oven temperature program consisted of a rate of 10.00 °$Cmin^{-1}$, starting at 50.0 °C and held for 2.00 min, followed by an increase to 300.0 °C with a hold time of 10.0 min.

The ion source temperature was set at 200.00 °C, and the interface temperature was maintained at 250.00 °C. A solvent cut time of 4.00 min was implemented. The detector gain mode was relative to the tuning result, 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 [15]. Acarbose (positive control), **Aa** and its isolated compounds were dissolved in a minimal amount of 10% DMSO and further dissolved in a sodium phosphatebuffer solution (0.02 M, pH 6.9) to achieve concentrations ranging from 25-100 μgmL^{-1}. A mixture of 200 µL α-amylase solution (2 $unitsmL^{-1}$,CAS: 9000-85-5, Sigma-Aldrich, USA) and 200 µL of the acarbose/**Aa**/isolated compounds was incubated for 10 min at 30 °C. Following this, added starch solution (200 µL, 1% w/v in water) to each tube and incubated for 3 min. The reaction was terminated by adding DNSA reagent (200 µL) and boiling the mixture at 85–90 °C in a water bath for 10 min. Later, the cooled mixture was diluted with distilled water (5 mL), and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer(BioTek Synergy HT, USA). The percentage of α-amylase inhibition was plotted against the concentration of the acarbose/**Aa**/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 Mechchate et al., 2021b [16]. To prepare the samples, 50 µL of acarbose/**Aa**/isolated compounds was used at concentrations ranging from 25 to 100 μgmL^{-1}. The samples were then incubated with 10 µL α-glucosidase (CAS: 9001-42-7,Sigma-Aldrich, USA) at a concentration of 1 UmL^{-1} and phosphate buffer (125 µL, 0.1 M, pH 6.8) at 37 °C for 20 min. The reaction was initiated by adding pNPG (4-Nitrophenyl-β-D-glucopyranoside, 20 µL, 1 M) as the substrate, followed by a 30 min incubation. To stop the reaction, Na_2CO_3(50 µL, 0.1 N) was added. The absorbance was measured at 405 nm using a spectrophotometer. After determining the α-glucosidase inhibitory activity at various concentrations, the IC_{50} values were determined for acarbose/**Aa**/isolated compounds.

In vitro anti-obesity activity

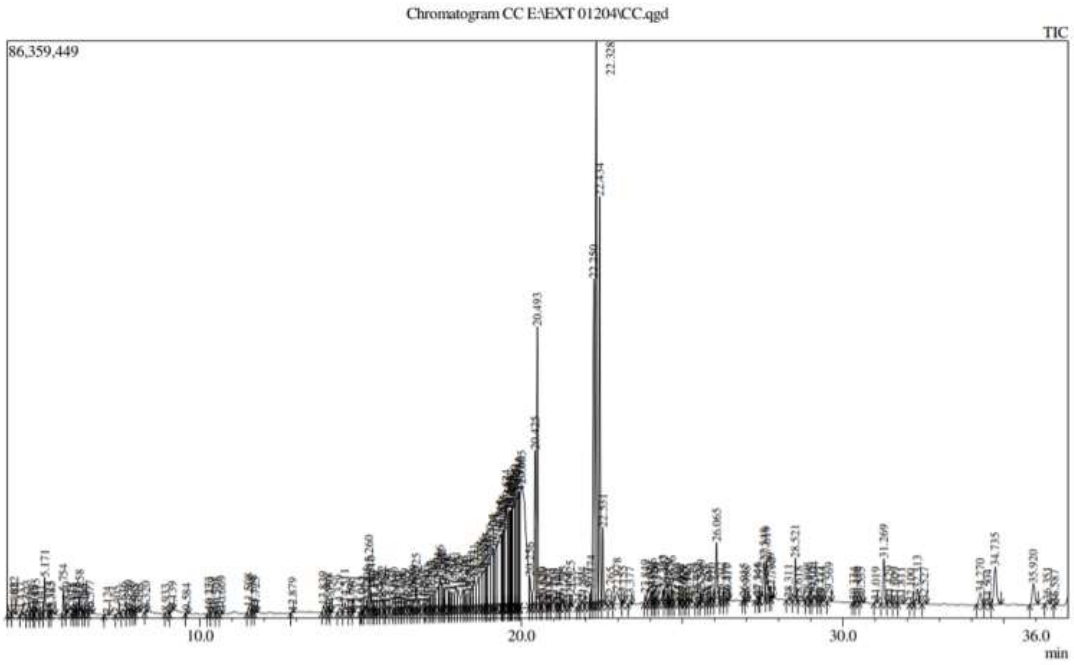
The anti-obesity activity was assessed by utilizing porcine pancreatic lipase (PPL, type II) and p-nitrophenyl butyrate (p-NPB) as the substrate [17]. PPL stock solutions were prepared in a potassium phosphate buffer (0.1 mM, pH 6.0) at a concentration of 1 $mgmL^{-1}$ and stored at −20 °C. For the determination of lipase inhibitory activity, Orlistat(positive control)/**Aa**/isolated compounds(at final concentrations of 25-100) was pre-

incubated with PPL for 1 h in a 0.1 mM potassium phosphate buffer (pH 7.2) at 30 °C. The reaction was initiated by adding NPB (0.1 μL) as the substrate and incubation for 5 min at 30 °C.The release of p-nitrophenol in the reaction was quantified using a spectrophotometer at 405 nm. IC_{50} values were determined for Orlistat/**Aa**/isolated compounds by plotting percentage inhibition vs concentration.

Table 1. Compounds identified from GC-MS analysis of Aa.

R. Time	**Area%**	**A/H**	**Compound names**
5.171	0.82	4.74	Syringic acid
5.260	0.80	3.36	Sinapic acid
7.404	0.75	5.66	Caffeic acid
17.466	0.68	3.93	6-Prenyl apigenin
17.662	0.77	6.14	Luteolin
18.659	0.95	5.20	Apigein
18.755	1.04	5.4	(1R,7S,E)-7-Isopropyl-4,10-dimethylene-cyclodec-5-enol
19.02	1.5	5.34	10-Undecenyl hexofuranoside
19.104	1.57	4.7	2-Ethyl-norbornan-2-ol
19.345	3.19	7.78	Mome inositol
19.524	3.05	5.27	Neophytadiene
19.594	1.43	2.62	6,10,14-Trimethyl-2-pentadecanone
19.78	2.97	4.93	6-Dodecanol acetate
19.914	2.07	3.23	2-O-Methyl-D-mannopyranosa
19.95	1.35	2.09	2-Methyl-hexanoic acid
20.425	2.76	3.18	Methyl ester hexadecanoic acid
22.25	4.88	2.71	Methyl ester-9,12-octadecadienoic acid
22.328	8.14	2.61	Cis-methyl ester-9,12,15-octadecatrienoic acid
22.434	5.52	2.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26.065	1.14	3.47	2-Hydroxy-hexadecanoic acid
34.735	1.19	5.71	Stigmasta-5,22-dien-3-ol

Figure 1. GC-MS chromatogram of Aa.



3. RESULTS AND DISCUSSION

GC-MS analysis of Aa

During the GC-MS analysis of **Aa**, multiple peaks were detected (Figure 1) thatvisualized presence of 21 known major compounds. Each compound characterized by its retention time, area percentage, A/H ratio, and name (Table 1). At a retention time of 5.171 min, a peak with an area percentage of 0.82% and an A/H ratio of 4.74 was identified as syringic acid. Another peak appeared at a retention time of 5.260 min, exhibiting an area percentage of 0.80%, an A/H ratio of 3.36, and identified as sinapic acid. At a retention time of 7.404 min, a peak with an area percentage of 0.75% and an A/H ratio of 5.66 was observed and identified as caffeic acid. Similarly, at a retention time of 17.466 min, a peak was detected with an area percentage of 0.68%, an A/H ratio of 6.14, and identified as 6-prenyl apigenin. Other peaks included luteolin at 17.662 min (area percentage: 0.77%, A/H ratio: 6.14) and 6-apigein at 18.659 min (area percentage: 0.95%, A/H ratio: 5.20).

At a retention time of 18.755 min, a peak with an area percentage of 1.04% and an A/H ratio of 5.4 was identified as (1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol. Another peak appeared at a retention time of 19.02 min, exhibiting an area percentage of 1.5%, an A/H ratio of 5.34, and identified as 10-Undecenyl hexofuranoside. At a retention time of 19.104 min, a peak with an area percentage of 1.57% and an A/H ratio of 4.7 was observed and identified as 2-Ethyl-norbornan-2-ol.

Similarly, at a retention time of 19.345 min, a peak was detected with an area percentage of 3.19%, an A/H ratio of 7.78, and identified as Mome inositol. Other peaks included Neophytadiene at 19.524 min (area percentage: 3.05%, A/H ratio: 5.27), 6,10,14-Trimethyl-2-pentadecanone at 19.594 min (area percentage: 1.43%, A/H ratio: 2.62), and 6-Dodecanol acetate at 19.78 min (area percentage: 2.97%, A/H ratio: 4.93). Furthermore, a peak was observed at a retention time of 19.914 min, accounting for 2.07% of the total peak area with an A/H ratio of 3.23. This peak was identified as 2-O-Methyl-D-mannopyranosa.

Additionally, 2-Methyl-hexanoic acid was detected at 19.95 min (area

percentage: 1.35%, A/H ratio: 2.09), Methyl ester hexadecanoic acid at 20.425 min (area percentage: 2.76%, A/H ratio: 3.18), Methyl ester-9,12-octadecadienoic acid at 22.25 min (area percentage: 4.88%, A/H ratio: 2.71), and Cis-methyl ester-9,12,15-octadecatrienoic acid at 22.328 min (area percentage: 8.14%, A/H ratio: 2.61). Furthermore, a peak was observed at 22.434 min, accounting for 5.52% of the total peak area with an A/H ratio of 2.45. This peak was identified as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol.

Lastly, 2-Hydroxy-hexadecanoic acid was detected at 26.065 min (area percentage: 1.14%, A/H ratio: 3.47), and Stigmasta-5,22-dien-3-ol was observed at 34.735 min (area percentage: 1.19%, A/H ratio: 5.71). These peaks provide valuable information about the presence and composition of various compounds in the analyzed sample **Aa**.

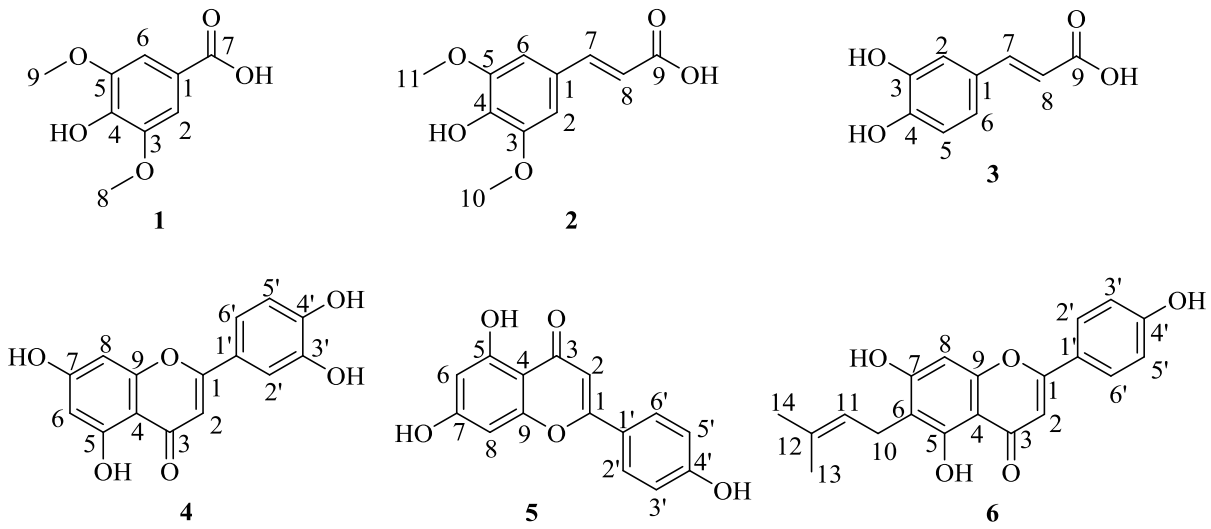


Figure 2. Phytochemical isolated from methanolic extract of A. aspera.

Phytochemistry

The chemical examination of **Aa** yielded six known compounds, which upon analysing the spectral dataidentified as syringic acid (**1**), sinapic acid (**2**), caffeic acid (**3**), luteolin (**4**), apigein (**5**), and 6-prenyl apigenin (**6**) (Figure 2). To the best of our knowledge, except **Aa-6**, all the isolated compounds were first time reported from A. aspera.

Syringic acid (**1**): Pale yellow powder; R_f: 0.8 (Hexane:ethyl acetate); Mol. Formula: $C_9H_{10}O_5$; m.p.: 195-196 ºC; Elemental analysis: anal. C-54.96, H-5.02(%), calcd. C-54.55, H-5.09(%). FT-IR (KBr): 579.78, 653.72, 730.01, 788.11, 837.67, 896.22, 951.03, 1032.64, 1086.87, 1157.87, 1289.66, 1378.06, 1425.53, 1464.30, 1497.29, 1657.71, 1759.06, 2930.01, 3424.73. ^{1}H (400 MHz, $CDCl_3$): 3.82 (s, 6H, 8,9-OCH_3), 4.05 (s, 1H, 4-OH), 7.07 (s, 2H, 2,6-Ar-H), 8.08 (s, 1H, 7-COOH). ^{13}C (400 MHz, $CDCl_3$): 57.55 (C-8/9), 106.87 (C-2/6), 122.17 (C-1), 142.55 (C-4), 149.97 (C-3/5), 168.27 (C-7).

Sinapic acid (**2**): Pale yellow powder; R_f: 0.6 (Hexane:ethyl acetate); Mol. Formula: $C_{11}H_{12}O_5$; m.p.: 207-208 ºC; Elemental analysis: anal. C-58.99, H-5.32(%); calcd. C-58.93, H-5.39(%). FT-IR (KBr): 575.85, 654.01, 732.62, 788.55, 836.93, 895.56, 951.28, 1031.21, 1101.00, 1158.72, 1276.76, 1376.14, 1424.51, 1463.27, 1496.89, 1663.48, 1759.29, 2317.79, 2927.48, 3435.16. ^{1}H (400 MHz, $CDCl_3$): 3.82 (s, 6H, 10,11-OCH_3), 4.49 (s, 1H, 4-OH), 6.49-52 (d, J = 12 Hz, 1H, 8-CH), 6.74 (s, 2H, 2,6-Ar-H), 7.80-7.83 (d, J = 12 Hz, 1H, 7-CH), 8.21 (s, 1H, 9-

COOH). ^{13}C (400 MHz, $CDCl_3$): 56.70 (C-10/11), 106.75 (C-2/6), 115.30 (C-8), 127.70 (C-1), 138.88 (C-4), 144.83 (C-7), 147.80 (C-3/5), 168.86 (C-9).

Caffeic acid (**3**): Pale yellow powder; R_f: 0.4 (Hexane:ethyl acetate); Mol. Formula: $C_9H_8O_4$; m.p.: 212-213 ºC; Elemental analysis: anal. C-59.99, H-4.44(%); calcd. C-60.00, H-4.48(%). FT-IR (KBr): 576.74, 602.73, 653.82, 707.25, 789.20, 832.73, 895.30, 951.15, 990.61, 1032.36, 1099.75, 1156.37, 1269.72, 1378.65, 1421.51, 1467.05, 1506.44, 1679.51, 1758.89, 2932.13, 2985.52, 3415.61. ^{1}H (400 MHz, $CDCl_3$): 3.45 (s, 1H, 3-OH), 4.35 (s, 1H, 4-OH), 6.53-56 (d, J = 12 Hz, 1H, 8-CH), 6.78-6.79 (d, J = 4 Hz, 1H, 5-Ar-H), 6.96 (s, 1H, 2-Ar-H), 7.01-7.02 (d, J = 4 Hz, 1H, 6-Ar-H), 7.81-7.84 (d, J = 12 Hz, 1H, 7-CH), 8.53 (s, 1H, 9-COOH). ^{13}C (400 MHz, $CDCl_3$): 114.54 (C-2), 115.82 (C-5), 116.13 (C-8), 121.75 (C-6), 128.06 (C-1), 145.31 (C-7), 145.99 (C-3), 148.25 (C-4), 168.85 (C-9).

Luteolin (**4**): Yellow powder; R_f: 0.8 (dichloromethane:ethyl acetate); Mol. Formula: $C_{15}H_{10}O_6$; m.p.: 332-333ºC; Elemental analysis: anal. C-62.96, H-3.55(%); calcd. C-62.94, H-3.52(%). FT-IR (KBr): 574.84, 599.56, 654.58, 727.88, 789.24, 840.44, 895.02, 955.27, 986.11, 1035.21, 1060.81, 1106.30, 1149.47, 1278.58, 1344.56, 1419.58, 1468.52, 1506.00, 1676.90, 1759.01, 1959.56, 2697.13, 2887.79, 3423.52. ^{1}H (400 MHz, DMSO-d_6): 3.07 (s, 1H, 4’-OH), 3.66 (s, 1H, 3’-OH), 4.72 (s, 1H, 7-OH), 4.92 (s, 1H, 5-OH), 6.09-6.10 (d, J = 4 Hz, 1H, 6-Ar-H), 6.21 (s, 1H, 8-Ar-H), 6.46 (s, 1H, 2-Ar-H), 6.72 (s, 2H, 2’,5’-Ar-H), 6.98 (s, 1H, 6’-Ar-H). ^{13}C (400 MHz, DMSO-d_6): 95.27 (C-8), 99.77 (C-6), 104.87 (C-4), 105.98 (C-2), 114.66 (C-2’), 116.84 (C-5’), 119.92 (C-6’), 123.12 (C-1’), 146.96 (C-3’), 150.56 (C-4’), 159.64 (C-9), 161.05 (C-5), 164.32 (C-1), 165.02 (C-7), 182.81 (C-3).

Apigein (**5**): Yellow powder; R_f: 0.6 (dichloromethane:ethyl acetate); Mol. Formula: $C_{15}H_{10}O_5$; m.p.: 350-351ºC; Elemental analysis: anal. C-66.70, H-3.70(%); calcd. C-66.67, H-3.73(%). FT-IR (KBr): 577.60, 602.56, 654.09, 716.92, 789.99, 838.64, 896.04, 951.26, 1033.27, 1087.61, 1155.09, 1268.45, 1332.32, 1376.73, 1421.75, 1421.75, 1465.04, 1635.74, 1757.97, 2931.18, 3426.89, 3862.65. ^{1}H (400 MHz, DMSO-d_6):3.64 (s, 1H, 4’-OH), 4.73 (s, 1H, 7’-OH), 5.28 (s, 1H, 5-OH), 6.12 (s, 1H, 6-Ar-H), 6.22 (s, 1H, 8-Ar-H), 6.46 (s, 1H, 2-Ar-H), 6.84-6.85 (d, J = 4 Hz, 2H, 3’,5’-Ar-H); 7.30-7.32 (d, J = 8 Hz, 2H, 2’,6’-Ar-H). ^{13}C (400 MHz, DMSO-d_6): 95.29 (C-8), 99.79 (C-6), 104.89 (C-4), 106.24 (C-2), 116.53 (C-3’/5’), 122.87 (C-1’), 129.27 (C-2’/6’), 159.66 (C-9), 161.08 (C-5), 162.23 (C-4’), 164.30 (C-1), 165.04 (C-7), 182.84 (C-3).

6-Prenyl apigenin (**6**): Yellow powder; R_f: 0.5 (dichloromethane:ethyl acetate) Mol. Formula: $C_{20}H_{18}O_5$; m.p.: 230-231ºC; Elemental analysis: anal. C-70.98, H-5.32(%); calcd. C-71.00, H-5.36(%). FT-IR (KBr): 573.34, 606.58, 717.52, 801.79, 842.88, 896.68, 945.27, 973.50, 1024.65, 1108.02, 1196.58, 1242.18, 1373.54, 1445.24, 1481.05, 1633.79, 1736.42, 2316.08, 2466.25, 2860.28, 2929.08, 3455.97, 3770.09, 3853.70. ^{1}H (400 MHz, DMSO-d_6): 1.88 (s, 6H, 13,14-CH_3), 3.49-3.51 (d, J = 12 Hz, 2H, 10-CH_2), 3.99 (s, 1H, 4’-OH), 4.96 (s, 1H, 7-OH), 5.56-5.58 (m, 1H, 11-CH), 5.90 (s, 1H, 5-OH), 6.27 (s, 1H, 8-Ar-H), 6.53 (s, 1H, 2-Ar-H), 6.94-6.95 (d, J = 4 Hz, 2H, 3’,5’-Ar-H), 7.47-7.48 (d, J = 4 Hz, 2H, 6’-Ar-H). ^{13}C (400 MHz, DMSO-d_6): 18.52 (C-14), 23.00 (C-10), 25.39 (C-13), 95.81 (C-8), 106.22 (C-2), 106.94 (C-4), 111.71 (C-6), 116.50 (C-3’/5’), 122.86 (C-1’), 123.55 (C-11), 129.25 (C-2’/6’), 132.14 (C-12), 159.01 (C-5), 159.71 (C-9), 162.21 (C-4’), 164.28 (C-1), 164.78 (C-7), 182.23 (C-3).

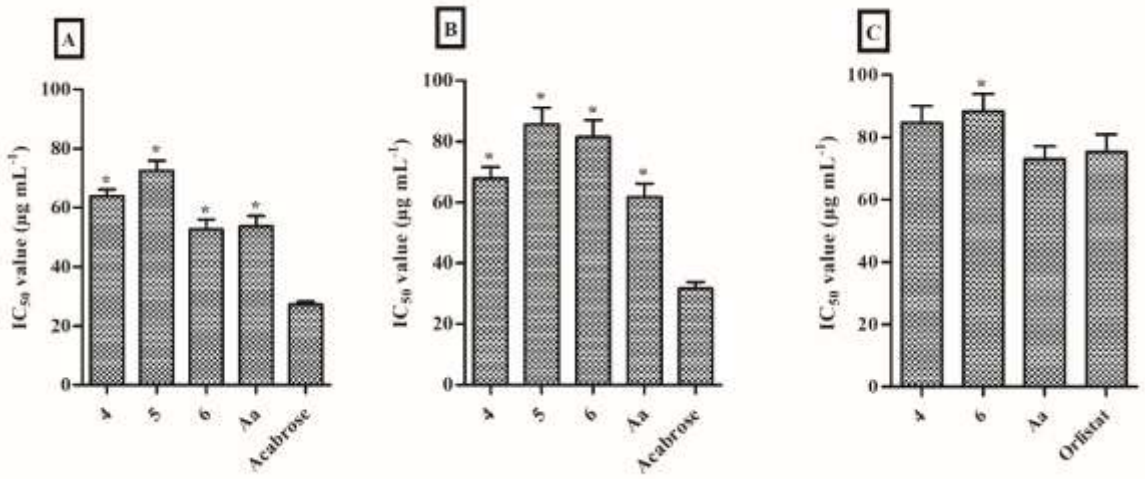


Figure 3. IC_{50} values of all samples from A. asperaagainst (A) α-Amylase, (B) α-Glucosidase, and (C) Porcine pancreatic lipase. Where $^{*}P \leq 0.05$ statistically significant from the standard using ANNOVA followed by Tukey's test.

In vitro α-amylase and α-glucosidase inhibition assays

Figure 3a provides information on the percentage of inhibition and IC_{50} values of various samples from A. asperaagainst α-amylase, which is an enzyme involved in the breakdown of starch[18]. Whereas Figure 3b provides information on the percentage of inhibition and IC_{50} values of various samples from A. asperaagainst α-glucosidase, which is an enzyme involved in the breakdown of complex carbohydrates into simple sugars[19]. 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.

In both assays, the compounds **1, 2,** and **3** derived from A. aspera, the IC_{50} values are reported as greater than 100 μgmL^{-1}. 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 compounds **4, 5, 6,** and **Aa**, also derived from A. aspera, have specific IC_{50} values of 63.88±2.33 μgmL^{-1}, 72.42±3.50 μgmL^{-1}, 52.71±3.33 μgmL^{-1}, and 53.74±3.50 μgmL^{-1}, respectively, whereas the reference sample acabrose has a stronger inhibitory effect on α-amylase with an IC_{50} value of 27.28±1.03 μgmL^{-1}. In contrast, the compounds **4, 5, 6,** and **Aa**, also derived from A. aspera, have specific IC_{50} values of 67.97±3.56 μgmL^{-1}, 85.54±5.50 μgmL^{-1}, 81.44±5.57 μgmL^{-1}, and 61.70±4.50 μgmL^{-1}, respectively, while acabrose has a stronger inhibitory effect on α-glucosidase with an IC_{50} value of 31.67±2.21 μgmL^{-1}. Overall, the superior antidiabetic activity of **4, 5, 6,**and **Aa**may be due to the presence of freely available oxygenated substances like phenolics, etc. in their chemical structure.

In vitro anti-obesity activity

Figure 3c provides information on the IC_{50} values of various samples from A. aspera against porcine pancreatic lipase, which is an enzyme involved in the digestion of dietary fats. The IC_{50} value represents the concentration at which the samples inhibit porcine pancreatic lipase 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.

For the compounds **1, 2, 3,** and **5** derived from A. aspera, the IC_{50} values are reported as greater than 100 μgmL^{-1}. This suggests that these samples have less inhibitory effects on porcine pancreatic lipase activity or require higher concentrations to achieve 50% inhibition. The compounds **4**and**6** derived from A. aspera has an IC_{50} value of 84.57±5.44 $\mu gmL^{-1}$and 88.24±5.58 μgmL^{-1}, respectively, indicating a moderate inhibitory effect on porcine pancreatic lipase activity. Comparatively, the reference sample Orlistat has an IC_{50} value of 75.19±5.68 μgmL^{-1}, indicating a similar inhibitory effect on porcine pancreatic lipase as the sample **Aa**(IC_{50} value = 72.95±4.13 μgmL^{-1}). These findings highlight A. aspera potential as a natural remedy for managing obesity-related complications.

4. CONCLUSIONS

In conclusion, this research article has provided a comprehensive evaluation of the phytochemical analysis, antidiabetic, and anti-obesity activities of A. aspera, a medicinal plant with significant therapeutic potential. The findings highlight the rich bioactive profile of A. aspera, including syringic acid (**1**), sinapic acid (**2**), caffeic acid (**3**), luteolin (**4**), apigenin (**5**), and 6-prenyl apigenin (**6**), which contribute to its diverse pharmacological properties. The antidiabetic effects of **4, 5, 6,** and **Aa** have been demonstrated through inhibition of carbohydrate-digesting enzymes mechanism, suggest A. aspera potential as a natural remedy for managing diabetes mellitus. Moreover, the anti-obesity properties of **4,6** and **Aa**offer promise in combating the growing epidemic of obesity and its associated complications. Hence, A. asperaability to inhibit adipocyte differentiation, reduce lipid accumulation, and suppress appetite provides a foundation for further exploration of A. asperaas a potential therapeutic intervention for obesity management.

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HVSanthoshi Allu: Researcher who carried out Isolation and Biological evaluations
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Arun Kumar KS: Major contributor in drafting the Manuscript.

5. REFERENCES

1. Bagnall, A. M., Radley, D., Jones, R., Gately, P., Nobles, J., Van Dijk, M., Blackshaw, J., Montel, S., Sahota, P.,BMC Public Health,2019, 19 (1), 8.
2. Tatipamula, V. B., Annam, S. S. P.,J.Ethnopharmacol.,2022, 282, 114641.
3. Priya, C. L., Kumar, G., Karthik, L., Rao, K. V. B.,Pharmacologyonline,2010, 2, 228–237.
4. Upadhya, V.,Ankad, G. M., Pai, S. R., Hegde, H. V.,Plant Science Today,2015, 2 (1), 7–10.

5. Somagari, D. R.,Basappa, K., Rolla, S.,Jithendar, P.,Rajashekar, B.,J.Pharmacogn.Phytochem., 2014, 3 (1), 190–193.
6. Rama, P., Vignesh, A., Lakshmanan, G., Murugesan, K.,International Journal of Medicine and Pharmaceutical Sciences,2013, 3 (2), 67–78.
7. Mishra, D,The Pharma Innovation Journal,2018, 7 (6), 147–153.
8. Das, A. K.,Bigoniya, P., Verma, N. K., Rana, A. C.,Asian Pac. J. Trop. Med.,2012, 5 (3), 197–201.
9. Londonkar, R., Reddy, C. V., Kumar, A. K.,Recent Research in Science and Technology,2011, 3 (4), 53–57.
10. Sukumaran, V. K., Sankar, P.,Varatharajan, R.,Pharm. Biol.,2009, 47 (10), 973–975.
11. Nargatti, P., Patil, S.,Wadkar, K.,J. Pharm. Res. Int.,2021, 33 (34B), 187–206.
12. Tatipamula, V. B., Annam, S. S. P., Nguyen, H. T.,Polimati, H.,Yejella, R. P.,Nat. Prod. Res.,2021, 35 (23), 5420–5424.
13. Killari, K. N.,Polimati, H., Prasanth, D. S. N. B. K., Singh, G., Panda, S. P., Vedula, G. S., Tatipamula, V. B.,RSC Adv.,2023, 13 (19), 12991–13005.
14. Manna, P., Jain, S. K.,Metab.Syndr.Relat.Disord., 2015, 13 (10), 423–444.
15. Wickramaratne, M. N.,Punchihewa, J. C.,Wickramaratne, D. B. M.,BMC Complement. Altern. Med.,2016, 16 (1), 466.
16. Mechchate, H., Es-Safi, I.,Louba, A.,Alqahtani, A. S., Nasr, F. A., Noman, O. M., Farooq, M., Alharbi, M. S.,Alqahtani, A., Bari, A.,Bekkari, H.,Bousta, D.,Molecules,2021, 26 (2), 293.
17. Roh, C., Jung, U., International Journal of Molecular Sciences,2012, 13 (2), 1710–1719.
18. Tatipamula, V. B., Kolli, M. K., Lagu, S. B., Paidi, K. R., Reddy, P. R., Yejella, R. P., Pharmacological Reports,2019, 71 (2), 233–242.
19. Tatipamula, V. B.,Brazilian Journal of Pharmaceutical Sciences,2022, 58, e19323.