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Research Article

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-ULTRAVIOLET PROFILING OF BIOPHARMACEUTICALS AND *IN VITRO* ANTIDIABETIC PROPERTIES OF ETHANOL EXTRACT OF *ANNONA MURICATA* LEAVES

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ABSTRACT

Objectives: This study aimed to identify and quantify the phytochemical compounds and vitamins present in the ethanol extract of *Annona muricata* leaves and investigate on its antidiabetic potential.

Methods: The plant bioactive compounds and vitamin contents of the extract were analyzed using high-performance liquid chromatography-ultraviolet (HPLC-UV) analytical methods. The antidiabetic potential of the extract was evaluated through *in vitro* assays of α-amylase and α-glucosidase inhibitory activities.

Results: The HPLC-UV profiling revealed the presence of 19 bioactive compounds in the extract. Resveratrol (20.68 ppm) was the most abundant followed by steroids (14.65 ppm) and spartein (10.92 ppm). Flavan-3-ol (7.56 ppm) and proanthocyanidins (7.29 ppm) were moderately abundant. The extract also contained significant ($p<0.05$) quantities of water-soluble and fats-soluble vitamins. Among the water-soluble vitamins were B9 (0.0471 ppm), B1 (0.0470 ppm), B12 (0.0272 ppm), B6 (0.0198 ppm), B2 (0.0194 ppm), and the least in concentration was the B3 (0.0144 ppm). The most significant ($p<0.05$) in the order of abundance of the fats-soluble vitamins was calciferol (2.47 ppm), followed by tocopherol (0.63 ppm) and retinol (0.42 ppm) while the least was carotenoids (0.19 ppm). The extract also exhibited significant ($p<0.05$) percent of α-amylase inhibitory activity, but the effect was not concentration-dependent. However, the α-glucosidase inhibitory activity of the extract was concentration-dependent.

Conclusion: The ethanol extract of *A. muricata* leaves has shown a significant ($p<0.05$) bioactive profile, with rich vitamin compositions, and exhibited a considerable percentage of antidiabetic activities. These findings suggest the therapeutic potential of the extract for management of diabetes and other disorders.

Keywords: *Annona muricata*, Antidiabetics, Resveratol, Proanthocyanidins, Vitamins, Chromatography.

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INTRODUCTION

Plants have been utilized for a variety of purposes for thousands of years, including for medicinal uses. The application of plants for health benefits can be traced back to ancient societies, such as Sumeria, Mesopotamia, Egypt, Greece, and Islamic cultures [1]. The earliest documented evidence of the use of plants for the treatment of diseases is approximately 5000 years old and was found on a Sumerian clay tablet from Nagpur. Traditional medicine includes a vast array of knowledge, practices, and skills that different societies have developed and employed over time for health maintenance, as well as for the diagnosis, treatment, and prevention of both physical and mental health issues [2]. The use of medicinal plants plays a key role in traditional medicine across many cultures, and it includes the identification of beneficial plants through trial-and-error approaches, which is then followed by the refinement of their applications over numerous generations.

Medicinal plants generate a wide variety of secondary metabolites, which are chemical compounds that are not crucial for the plant's growth and development but possess possible therapeutic properties; such secondary metabolites have been utilized as foundational elements for numerous pharmaceutical drugs and herbal treatments [3]. Plants are an important source of medicines and play an essential role in global health. In contemporary medicine, plants are employed as sources of direct therapeutic agents, as models for innovative synthetic compounds, and as taxonomic markers for the discovery of new compounds. Various parts of plants, including roots, leaves, stem bark, and seeds, contain active components that have therapeutic significance, thus making them beneficial in the treatment of diseases such as cancer, coronary heart disease, diabetes, and infectious diseases [4].

Annona muricata Linn. (Annonaceae) has been used as a therapeutic remedy for many years, drawing numerous scientists to study this plant. *A. muricata* L. is a tropical fruit-bearing tree found in lowland areas and belongs to the Annonaceae family. Graviola, soursop, durian belanda, and guanabana are some of the well-known local names for *A. muricata* in Malaysia. The genus name *Annona* may have originated from the Latin term "anon," which translates to "yearly produce." It can be explained as "the fruit production characteristics of the various species within this genus." The term soursop relates to the sweet and sour taste of the fruit [5]. Numerous studies have highlighted the therapeutic properties of *A. muricata*, including anti-tumor, anti-helminth, anti-fungal, anti-bacterial, hypotensive, anti-viral, and anti-inflammatory properties [5]. Different parts of *A. muricata*, such as its leaves and bark, have been utilized for medicinal purposes. More than 200 chemical compounds have been identified and extracted, which include phenolics, acetogenins, and alkaloids [6]. Given its medicinal and pharmacological properties, this plant is regarded as a promising alternative therapy for diabetes mellitus (DM), hypertension, cancer, and bacterial infections [6]. Moreover, it is also cost-effective, readily available, and environmentally friendly in comparison to existing commercial medications, making it a valuable candidate for new potential treatments [6].

The application of *A. muricata* extracts as a treatment for DM has not been thoroughly investigated. DM is a disorder in which the body does

not produce adequate insulin or fails to respond to it, resulting in a relatively elevated level of glucose in the bloodstream. A deficiency of glucose in the tissues has resulted in increased thirst, frequent urination, and sensations of hunger [7]. Individuals with DM also often experience weight loss due to a lack of sufficient energy. In 2013, there were 382 million instances of diabetes globally, but this number is expected to rise to 592 million by 2035. Most diabetes cases are located in low and middle-income nations [8]. The excessive use of diabetes medications may result in specific side effects and complications. Therefore, it is vital to create alternative treatments from natural sources that have fewer side effects. Despite the advancement of new synthetic medications and their scientific validation, ongoing research in the worldwide scientific community is still investigating the anti-diabetic effects of natural sources with minimal side effects, whether in their raw or processed forms [8].

Phytochemical investigations have shown the existence of bioactive substances in *A. muricata* leaves, such as alkaloids, flavonoids, phenolic acids, and vitamins, which could play a role in their potential antidiabetic properties. The discovery and analysis of phytochemicals found in medicinal plants can offer important insights into their possible therapeutic uses and operational mechanisms [9]. In spite of the existence of numerous conventional treatments, there is growing interests in alternative and complementary therapies sourced from nature. The analysis of the bioactive substances in *A. muricata* leaf extracts will enhance the comprehension of their nutraceutical benefits and possible health advantages. Furthermore, the research will aid in the scientific validation of the traditional uses of *A. muricata* leaf extracts in managing diabetes, fostering their incorporation into both traditional and modern healthcare approaches.

METHODS

Sample collection and preparation of the extract

The fresh leaves of *A. muricata* (Fig. 1) were gathered from a local farm located in Abakaliki Local Government Area of Ebonyi State. A taxonomist from the Department of Applied Biology of Ebonyi State University confirmed the identification of the leaves. The leaves were air-dried at 25°C, and the dried material was ground into a fine powder. Precisely 200 g of the sample was measured into a white sterile container, and 1,000 mL of absolute ethanol was introduced. The mixture was maintained and stirred properly at intervals for 3 days, after which it was filtered using cheesecloth, and the filtrate was concentrated with a rotary evaporator to yield the extract.

Extraction of phytochemicals

Precisely 1 g of the extract was measured into a test tube and 25 mL of ethanol was included. The test tube was left to react on a hotplate at 60°C for 90 min. Following the reaction period, the product formed in the test tube was moved to a separating funnel. The tube was thoroughly rinsed with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water, and 3 mL of hexane, all of which were added to the funnel. These extracts were combined and washed 3 times with 10 mL of 10% v/v ethanol aqueous solution. The solution was dried using anhydrous sodium sulfate and the solvent was removed by evaporation. The sample was dissolved in 1,000 µL of pyridine, with 200 µL being transferred to a vial for analysis.

Quantificationby high-performance liquid chromatography (HPLC)

The HPLC setup included a Spectra-Physics (San Jose, CA) HPLC system featuring an 8700 XR ternary µL Rheodyne (Cotati, CA) injection loop, an SP8792 column pump, a 20 heater, an 8440 XR ultraviolet (UV)-vis detector, and a 4290 integrator connected through Labnet to a computer operating WINner 8086 software (operating system, MS. DOS version 3.2). For the separation, a 250×4.6 mm column filled with 5 µm Spherisorb C18 (Sugelabor, Madrid, Spain) was utilized.

Preparation of samples and standards

Before derivatization, the proteins in the sample were hydrolyzed as described below. A 0.1 g lyophilized sample was placed into a 16×125 mm screwcap Pyrex (Barcelona, Spain) tube, 15 mL of 6 N hydrochloric acid was introduced, and the tube was purged with N_2, promptly sealed, and placed in an oven at 110°C for 24 h. Following hydrolysis, the contents of the tube were vacuum filtered (Whatman 541, Maidstone, England) to eliminate solids, the filtrate was adjusted to 25 mL with water, and a portion of this solution was further filtered through a membrane with a pore size of 0.5 µm (Millipore, Madrid, Spain). A standard solution featuring 1.25 µmol/mL of each bioactive compound was prepared in 0.1 N hydrochloric acid.

Identification of biochemical constituents

Bioactive compounds extracted from various extracts were identified according to their gas chromatography (GC) retention time on the HP-5MS column and by comparing the spectra with data from computer software of standards (Replib and Mainlab data from GC-mass spectrometry systems).

Quantification of vitamin B contents

The Vitamin B group was extracted utilizing the standard method. The sample powder (2 g) was dissolved in 25 mL of H_2SO_4 (0.1 N) solution and incubated for a duration of 30 min at 121°C. Following this, the contents were cooled and the pH was adjusted to 4.5 using 2.5 M sodium acetate, and 50 mg of takadiastase enzyme was incorporated. The mixture was kept at 35°C for 12 h. Subsequently, the blend was filtered using a Whatman No. 4 filter, and the resulting filtrate was diluted with 50 mL of distilled water before being filtered again through a micropore filter (0.45 µm). Twenty microliters of the filtrate were injected into the HPLC system. The quantification of Vitamin B content was executed by comparing it to vitamin B standards. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, cobalamin, and other B vitamins were formulated. Chromatographic separation was performed on a reversed phase HPLC column (Agilent ZORBAX Eclipse plus C18; 250×4.6 mm i. d., 5 µm) using an isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH=3.54) at a flow rate of 0.5 mL/min. UV absorbance was measured at 270 nm at room temperature.

Determination of fat-soluble vitamins in the extract

In a 10 g powdered sample, 1 g of pyrogallic acid, 70 mL ethanol, and 30 mL (50%) KOH were added, mixed, and refluxed for 40 min in a water bath maintained at 50°C. Extracts were obtained three separate times employing different ether volumes (50 mL, 30 mL, and 20 mL). The extract was neutralized using double-distilled water, which was then dehydrated with anhydrous sodium sulfate. In addition, the extract was concentrated to around 5 mL via a water bath at 50°C, diluted to 10 mL using methanol, filtered through a 0.45 µm membrane, and subsequently analyzed via HPLC. HPLC analysis was conducted using the Agilent 1,100 series HPLC system (Agilent; USA), which featured a diode array detector. The column used was constructed from stainless steel. For carotene quantification, the Agilent TC-C18 column was employed (5 µm, 4.6×250 mm) with a solvent mixture of acetonitrile-methyl alcohol-ethyl acetate (88: 10: 2), and UV absorbance measurements were taken at 453 nm. For fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was utilized (5 µm, 4.6 × 150 mm), with methanol as the solvent, and UV detection was performed at 325 nm for vitamin A, 265 nm for vitamin D3, 290 nm for Vitamin E, and 244 nm for Vitamin K3. The separation of all vitamins was carried out using isocratic elution, maintaining the solvent flow rate at 1 mL/min. Fat-soluble vitamins were identified by matching their retention times with those of authentic standards.

Alpha-amylase inhibitory assay on the extract

The α-amylase inhibitory potential of the *A. muricata* ethanol leaf-extract was evaluated using a modified spectrophotometric method [10]. A quantity of 250 µL of the extract (300 mg/mL) was combined with 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase at a concentration of 0.5 mg/mL. The resulting mixture was pre-incubated for 10 min. Afterward, 250 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was introduced

and incubated at 25°C for an additional 10 min. The reaction was halted by the addition of 500 µL of dinitrosalicylic acid. The tubes were subsequently incubated in a water bath at 95°C for 5 min and then cooled to room temperature, followed by dilution with 5 mL of distilled water. The optical density was assessed at 540 nm. The inhibitory effect on α-amylase was determined as a percent inhibition using the subsequent formula:

$$\text{inhibition (\%)} = \frac{\text{OD control} - \text{OD extract}}{\text{OD Control}} \times 100$$

Alpha-glucosidase inhibitory assay on the extract

The capability of the extract to hinder the function of α-glucosidase was evaluated using the standard procedure [11]. The α-glucosidase (1 U/mL) sourced from *Saccharomyces cerevisiae* was pre-incubated with 250 µL of the extract for a duration of 10 min. The reaction was initiated by adding a p-nitrophenylglucopyranoside substrate solution (pNPG, 3 mM), formulated in a 20 mM phosphate buffer (pH 6.9) containing 2 mg/mL Bovine Serum Albumin. The mixture was incubated at 37°C for 20 min and then the reaction was halted with 1 mL of Na_2CO_3 (1M). Alpha-glucosidase activity was assessed by quantifying the paranitrophenol released from pNPG at a wavelength of 405 nm. The percentage inhibition was calculated using the formula:

$$\text{inhibition (\%)} = \frac{\text{OD control} - \text{OD sample}}{\text{OD Control}} \times 100$$

Statistical analysis

The results were subjected to appropriate statistical analysis. The averages were compared using one-way analysis of variance and considerable variations among sets determined by Ducan multiple range test using the Statistical Packages for the Social Sciences for window version 20. The degree of significance was set at $p<0.05$

RESULTS

Bioactive compounds of *A. muricata* leaves ethanol extract profiled with HPLC

The findings indicated the existence of 19 bioactive compounds within the extract. The plant bioactive metabolites that exhibited the highest concentrations were resveratrol (20.68 ppm), steroids (14.65 ppm), spartein (10.92 ppm), flavan-3-ol (7.56 ppm), and proanthocyanin (7.29 ppm). On the other hand, additional bioactive compounds identified in the extract comprise oxalate (27.53 µg/mL), sapogenin (21.77 µg/mL), epicatechin (20.59 µg/mL), ribalinidine (13.16 µg/mL), phytate (12.25 µg/mL), cardiac glycoside (11.33 µg/mL), rutin (9.01 µg/mL), and lunamarin (8.62 µg/mL), whereas anthocyanin, cyanogenic glycoside, kaempferol, and catechin were detected at very minute concentrations ranging from 1.13 to 5.0 µg/mL as illustrated in Table 1 and the HPLC-UV chromatogram in Fig. 2.

Vitamin B complex components of ethanol extract from *A. muricata* leaves

The findings from the vitamin analysis of the extract indicated the presence of B complex vitamins. The vitamins present in the highest amounts were B9 (0.0471 ppm) and B1 (0.0470 ppm), followed by B12 (0.0272 ppm), B6 (0.0198 ppm), B2 (0.0194 ppm), and the lowest concentration was B3 (0. 0144 ppm), as illustrated in Fig. 3 and the HPLC-UV chromatogram representing the vitamin elution profile in Fig. 4.

Fats soluble vitamin content of the extract

The findings indicated that out of the four fat soluble vitamins identified, namely retinol, calciferol, tocopherol, and carotenoids, the predominant one in the sample was calciferol (2.47 ppm), followed by tocopherol (0.63 ppm) and retinol (0.42 ppm), whereas the lowest concentration was found in carotenoids (0.19 ppm), as illustrated in Fig. 5 the HPLC chromatogram displaying the elution profile, retention time, and peak in Fig. 6.

Antidiabetic activities of the extract

Alpha-amylase inhibitory activities of the extract

The findings indicated that the alpha-amylase activities of the extract varied significantly ($p<0.05$), ranging from 57.61% to 48.69% (at 5 mg/mL and 10 mg/mL extract concentration). The impact of the extract was not dependent on the concentration, as illustrated in Fig. 7.

Alpha-glucosidase inhibitory activities of the extract

The outcome of the alpha-glucosidase inhibitory activity (AGIA) of the extract indicated that the activity varied from 41.48 to 63.67% at 5 and 100 mg/mL extract concentration. The impact of the extract was dependent on the concentration (Fig. 8). There was a significant difference ($p<0.05$) in the alpha-glucosidase activities among the extract concentrations.

DISCUSSION

The research examined the profile of bioactive compounds and vitamin content in the ethanol extract of *A. muricata* leaves and also assessed its potential as an antidiabetic agent. The results offer important

Fig. 1: Picture of *Annona muricata* L plant

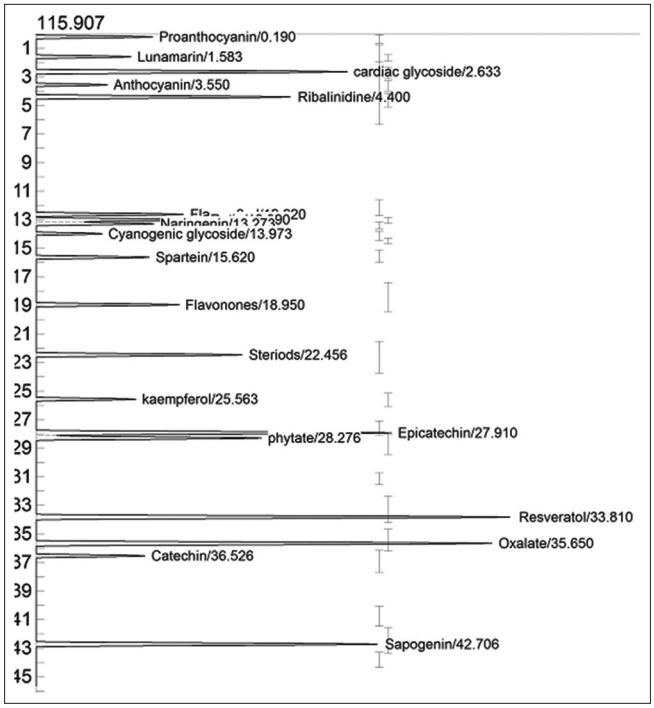

Fig. 2: High-performance liquid chromatography-ultraviolet Chromatogram of ethanol extract of *Annona muricata* leaves

Table 1: High-performance liquid chromatography-ultraviolet bioactive profile of ethanol extract of *Annona muricata* leaves

Component	Retention	Area	Height	External	Units
Proanthocyanin	0.190	5184.3944	427.802	7.2917	ppm
Lunamarin	1.583	4708.7496	369.572	8.6196	ug/ml
Cardiac glycoside	2.633	12170.5138	945.575	11.3284	ug/ml
Anthocyanin	3.550	3903.4112	306.580	5.0215	ug/ml
Ribalinidine	4.400	10229.5051	797.090	13.1597	ug/ml
Flavan-3-ol	12.620	6505.2012	510.587	7.5576	ppm
Rutin	12.990	7261.1404	564.292	9.0117	ug/ml
Naringenin	13.273	5414.6802	430.677	2.2881	ug/ml
Cyanogenic glycoside	13.973	3725.9862	292.760	5.0080	ppm
Spartein	15.620	5351.2845	419.380	9.5901	ug/ml
Flavanones	18.950	6368.0202	498.244	10.9228	ppm
Steroids	22.456	8539.7226	666.846	14.6479	ppm
Kaempferol	25.563	4875.0349	382.357	3.3761	ug/ml
Epicatechin	27.910	13725.1531	1063.342	20.5907	ug/g
Phytate	28.276	9186.5206	716.488	12.3475	ug/ml
Resveratol	33.810	18147.5364	1384.596	20.6849	ppm
Oxalate	35.650	17427.5578	1329.989	27.5258	ug/ml
Catechin	36.526	5159.9954	404.908	1.1328	ug/ml
Sapogenin	42.706	13247.6644	1026.936	21.7710	ug/ml
	161133.0720		211.8759		

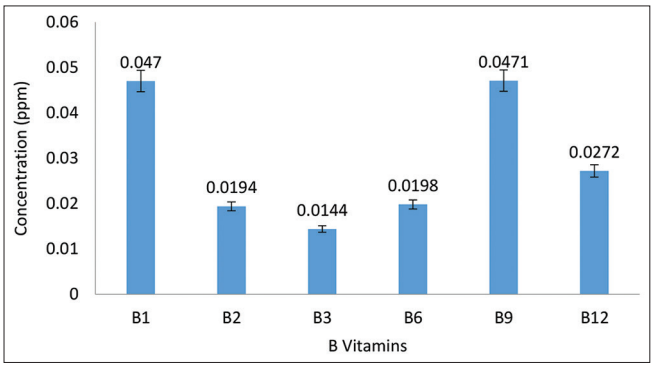

Fig. 3: Vitamin content of ethanol extract of *Annona muricata* leaves

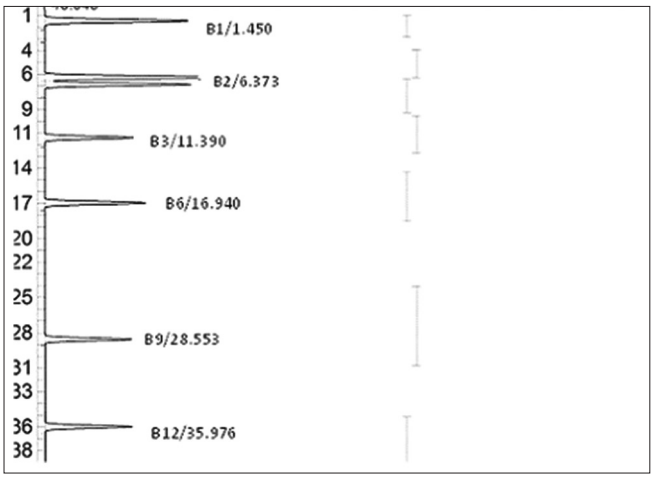

Fig. 4: High-performance liquid chromatography-ultraviolet elution profile chromatogram of the vitamin B complex content of ethanol extract of *Annona muricata* leaves

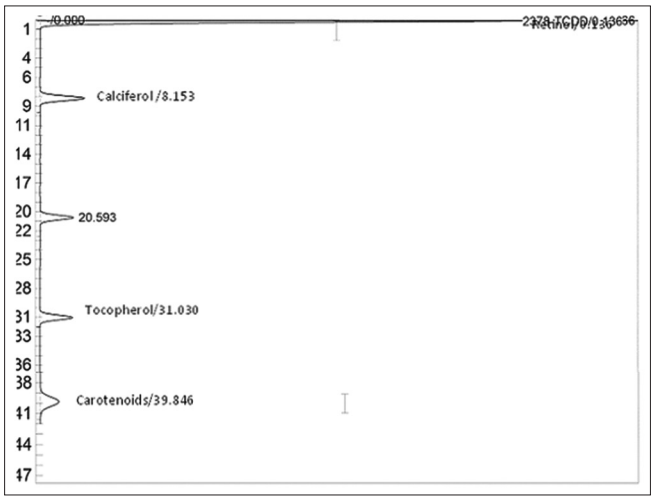

Fig. 6: High-performance liquid chromatography chromatogram showing the elution profile of fat soluble vitamin content of the extract

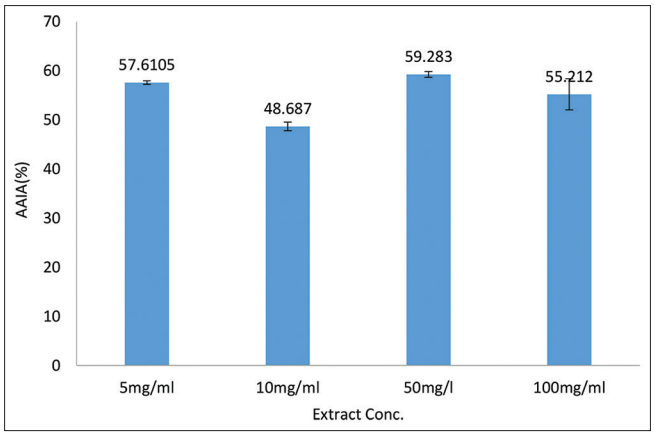

Fig. 7: Alpha-amylase inhibitory activities of the extract

perspectives on the phytochemical makeup and possible therapeutic uses of this medicinal plant. The HPLC-UV chromatogram of the ethanol extract of *A. muricata* leaves demonstrated the existence of 19 peaks, signifying the presence of various bioactive compounds at different concentrations, which encompass resveratrol, steroids, spartein, flavan-3-ol, proanthocyanins, oxalate, sapogenins, epicatechin, ribalinidine, phytate, cyanogenic glycosides, rutin, and lunamarin (Fig. 2). The identification of these bioactive compounds aligns with the observations

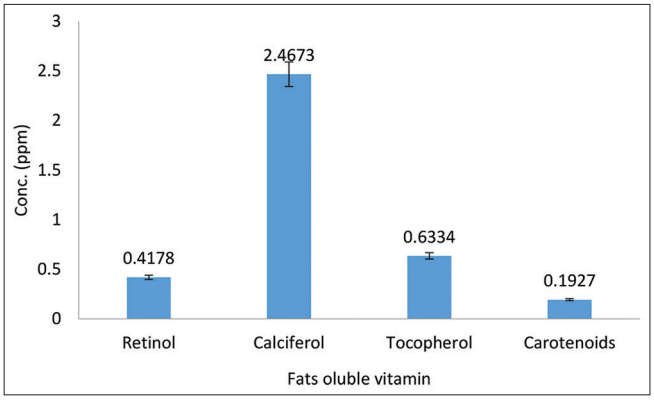

Fig. 5: Fat soluble vitamin content of the extract

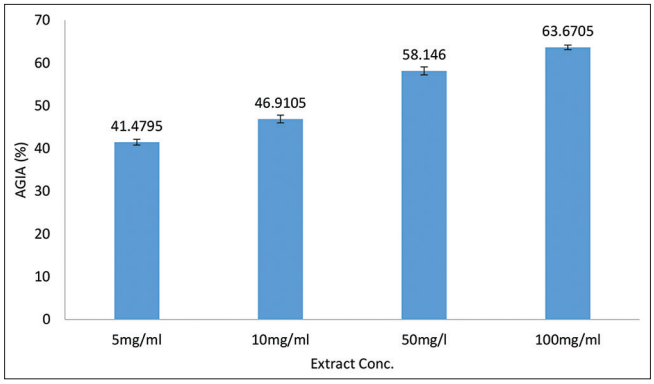

Fig. 8: Alpha-glucosidase inhibitory activity of the extract

made by a researcher [12], who found similar phytochemicals in their research on *A. muricata* leaf extracts.

The significant ($p<0.05$) level of resveratrol in the extract is remarkable for a medicinal plant. Resveratrol acts as a powerful antioxidant and has been widely researched for its possible therapeutic uses in a range of diseases, such as cancer, cardiovascular issues, and neurodegenerative conditions [13]. The concentrations of steroids and spartein were also significant ($p<0.05$), as these substances have been documented to exhibit anti-inflammatory and antioxidant effects [14]. The detection of significant ($p<0.05$) amount of flavan-3-ol and proanthocyanins in the extract aligns with the findings of another study [15], which noted the existence of these substances in their investigation on *A. muricata* leaf extracts. These substances are recognized for their antioxidant, anti-inflammatory, and anticancer properties [16]. Other phytocompounds such as oxalate, sapogenins, epicatechin, ribalinidine, phytate, cyanogenic glycosides, rutin, and lunamarin were also found in considerable amounts, and this report is consistent with the results presented previously [17], which also observed a similar outcome in their research on *A. muricata* leaf extract. These substances have shown various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [13]. The detection of low levels of anthocyanins, cyanogenic glycosides, kaempferol, and catechin in the extract corroborates with other findings, who made similar observations in their work on the plant. These substances were also recognized for their antioxidant, anti-inflammatory, and anticancer effects [18].

The HPLC-UV analysis showed the existence of different B complex vitamins from the ethanol extract of *A. muricata* leaves. The highest levels were noted for vitamins B9 and B1, followed by vitamins B12, B6, B2, and B3 in descending order (Figs. 3 and 4). These vitamins are vital for multiple metabolic processes and serve important functions in preserving overall health [19]. Vitamin B9 (folate) is critical for DNA synthesis, cell division, and growth, and a deficiency in this vitamin can result in anemia and neural tube defects in expectant mothers [20]. Vitamin B1 (thiamine) plays a role in energy metabolism and is crucial for the proper operation of the nervous system, muscles, and heart. Vitamin B12 (cobalamin) is essential for the formation of red blood cells, DNA synthesis, and appropriate neurological functioning [19]. Vitamin B6 (pyridoxine) plays a part in protein metabolism, with its deficiency leading to anemia, neurological issues, and skin lesions. Vitamin B2 (riboflavin) is fundamental for energy generation, growth, and development, and its deficiency can result in various problems, including anemia, skin conditions, and stunted growth [6]. Vitamin B3 (niacin) is involved in energy metabolism and is crucial for the maintenance of healthy skin, the digestive system, and nervous system function. These results confirm earlier findings regarding the vitamin composition of *A. muricata* extracts. For example, a research [19] noted the presence of several water-soluble vitamins, such as vitamins B1, B2, B3, and B6, in the aqueous extract of the plant. The bioactive compound profile and vitamin composition of *A. muricata* extracts have been studied by various researchers, and the results of this investigation are mainly consistent with prior findings and these results are indications of biopharmaceutical agents in the extract.

The examination of the fat-soluble vitamin composition of *A. muricata* ethanol leaf extract unveiled the existence of four significant vitamins: Retinol, Vitamins A, D, E, and carotenoids (Figs. 5 and 6). These vitamins are crucial for a variety of physiological processes, such as antioxidant protection, immune assistance, and bone strength [21]. The experimental results provide valuable information regarding the plant's possible health advantages and serve as a foundation for comparison with other research on fat-soluble vitamins in medicinal plants. Calciferol is the most plentiful fat-soluble vitamin found in the extract. It is vital for calcium metabolism and bone well-being. Its relatively elevated levels imply that *A. muricata* might serve as a treatment for osteoporosis and disorders related to Vitamin D deficiency [22]. Tocopherol functions as a powerful antioxidant, shielding cells from oxidative harm. Its presence signifies potential anti-inflammatory and cardioprotective attributes, which correspond with the traditional application of *A. muricata* in treating chronic diseases [23]. Retinol is crucial for vision, immune health, and skin wellness. Although found in moderate quantities, it enhances the plant's potential to promote overall health. Carotenoids, which are precursors for Vitamin A, display antioxidant properties and aid in diminishing oxidative stress [24]. Despite their lower concentrations, their existence adds significance to the plant's comprehensive nutritional and medicinal profile. The fat-soluble vitamin composition of *A. muricata* ethanol leaf extract underscores its promise as a medicinal plant with potential uses in enhancing bone health, antioxidant protection, and immune support. While calciferol predominates the profile, tocopherol, retinol, and carotenoids offer supplementary advantages. Comparisons with other plant sources indicate that *A. muricata* provides a distinct composition, especially as a prospective source of Vitamin D.

DM is the consequence of an imbalance in the metabolism of carbohydrates, lipids, and proteins, which is followed by impaired insulin secretion, insulin activity, or both. Its control using plant extracts has recently gained popularity [25]. *In vitro* assessment of α-amylase and α-glucosidase inhibitory activities of the plant extract was employed in this investigation. The α-amylase inhibitory activity of the *A. muricata* leaf extract ranged from 48.69% to 57.61% at concentrations of 10 mg/mL and 5 mg/mL, respectively. Nonetheless, the effect was not dependent on concentration, which contrasts with the earlier report [26], which documented a concentration-dependent alpha-amylase inhibitory activity in their investigation of plant extracts. The extract's ability to inhibit α-amylase, an enzyme that plays a role in the breakdown of starch and glycogen, indicates its potential for managing postprandial hyperglycemia in diabetic individuals [26].

The AGIA of the *A. muricata* leaf extract demonstrated a concentration-dependent impact, ranging from 41.48% to 63.67% at concentrations

of 5 mg/mL and 100 mg/mL respectively. This finding aligns with another result [27,28], which observed a concentration-dependent AGIA in their examination of the plant extracts. Alpha-glucosidase is an enzyme that aids in carbohydrate digestion, and inhibiting this enzyme can significantly lessen postprandial hyperglycemia in diabetic patients [29]. The concentration-dependent AGIA of the *A. muricata* leaf extract indicates its potential in managing diabetes by modulating glucose absorption.

CONCLUSION

The analysis of the ethanol extract of *A. muricata* leaves using HPLC-UV demonstrated the existence of multiple bioactive compounds. Moreover, the extract also showed considerable levels of water-soluble vitamins from the B complex and fat-soluble vitamins. The antidiabetic properties of the extract were tested using *in vitro* assays. Although the extract displayed α-amylase inhibitory activity, the effect was not dependent on the concentrations. On the other hand, the AGIA of the extract was dependent on the concentrations, indicating its potential role in controlling postprandial hyperglycemia in diabetic individuals. The presence of these bioactive compounds and vitamins, together with the antidiabetic characteristics of the extract, suggest its possible therapeutic uses in various diseases and conditions.

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AUTHOR'S CONTRIBUTION

The research author contributed all aspects of the research procedure.

CONFLICT OF INTEREST

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