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RESEARCH PAPER

Antioxidants, Anti-inflammation, Anti-hyperglycemia and Chemical Evaluation of the whole plant extracts of *Anisopus mannii* N.E.Br.

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A B S T R A C T

Anisopus mannii from the family Asclepiadaceae is a perennial herb that is currently utilized in the traditional Northern Nigerian medicinal system. The study document comprehensive *In Vitro* biological evaluation and chemical profiling of *A. mannii* whole plant crude extracts. Qualitative phytochemical, antioxidants, anti-inflammation, anti-hyperglycemia and chemical profiling was determined. The results revealed the presence of phenols, alkaloids, tannins, glycosides flavonoids, and saponins. The plant has the inhibition capacity to convert Fe^{3+} to Fe^{2} at 18.9 mmol/gm, scavenging at 75.5, injured tissues 28.4 75.5, -amylase 62.7 and α-Glucosidase 89.5 µg /mL respectively. 9-Octadecenoic acid, methyl and Lup-20(29)-en-3-ol, acetate, (3. beta) are the major compounds found in the whole plant extract. The current groundbreaking research revealed ethanolic extract from the whole plant is an effective medicinal agent. It provides the possibility for different therapy regimens to be developed from it. Research is needed to discover and purify the active chemicals that are responsible for therapeutic efficacy.

KEY WORDS: Africa, Antioxidant, Medicinal plants.
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1.INTRODUCTION

Plants are gaining relevance because of their potentials to produce natural products and also to cure several ailments for decades (Dogara *et al*., 2021; Mahmoud & Abba, 2021). Today, despite developments of synthetic drugs by the developed countries, Significant number of the world population still relies on traditional medicinal plants (Abdulrahman *et al*., 2021). From archeological records human beings used herbs and plants for medicinal purposes, spices, and food plants.

Several reports also prove that mankind has been using plants and herbs for medicinal purposes all over the world (Abdulrahman, 2021). Thus, still used as alternative medicine in the modern world. Plants and herbs are, in fact, mankind's oldest companions. According to estimates, 70-80 percent of people around the world rely on traditional herbal medicine to meet their primary health care needs (Kunwar *et al*., 2009; Abdulrahman *et al*., 2012; Dogara, 2021). They aided humanity not just by providing food and shelter, but also by healing numerous illnesses. Historians from all across the world have discovered evidence that all prehistoric peoples employed plants in some form or another, often in complex ways (Gilani, 2005).

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Medicinal plants are widely used for food, medicine, and trade, and they help to relieve human suffering (Kunwar *et al*., 2009). Plants have long been recognized for their therapeutic properties, and people from all over the world have used them to treat a variety of ailments. Nigeria is known for its biodiversity and is regarded as a natural repository of medicinal plants, which are found in various geographical regions around the country. Due to its tough topography and diverse climatic circumstances, the country is blessed with genetic diversity of flora.

A. mannii from the family Apocynaceae is a perennial herb that is currently utilized in the traditional Northern Nigerian medicinal system. Various parts of the plant are used to treat diabetes, malarial, diarrhea, pile, and other infectious diseases. The plant is known locally as "***kashe zaki***" by ***Hausa*** people in Northern Nigeria. Anti-nutritional agents, proximate and mineral composition, anti-inflammatory studies of methanol leaf extract and antibacterial screening of the stem aqueous extract were reported. Traditionally the whole plants have been reported to be more effective in the treatment and management of diabetes and other ailments in Kaduna State, Northern Nigeria. Nigeria now has 1.6 million individuals living with diabetes, making it the third most affected country in Africa (Aladeniyi *et al*., 2017). Therefore, the study aimed at a comprehensive study on *in-vitro* biological evaluation of *A. mannii* and the chemical analysis of the most active crude extract using Gas chromatography-mass.

2.MATERIALS AND METHODS

2.1. MATERIALS

2.1 Taxonomic Identification:

A botanist (Sunusi Namadi) in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, identified and thoroughly authenticated herbarium specimens before depositing them in the School herbarium. ABU0489 was assigned as a voucher number

2.2 Collection of Sample and Extraction

In ***Dakace***, Zaria, Kaduna State, Nigeria, a fresh sample was taken from the wild. The powder form (100 g) was weighed separately for four different solvent concentrations 100 % ethanol, 50 % ethanol, 30 % ethanol and 100 % aqueous using Soxhlet extraction. The extraction product was filtered through Whatman filter no. 2 and subsequently evaporate the solvents (Mahmoud Abdulrahman, 2021; Abdulrahman *et al*., 2019). **%Yield of Extract** = Dried extract (g) / weight of dried material (g) x 100 (Abdulrahman *et al*., 2019).

2.3 Analysis of Phytochemical

100 % ethanol, 50 % ethanol, 30 % ethanol and 100 % aqueous extracts were dissolved in DMSO, they were subjected to phytochemical examination. Using normal techniques, the extracts were screened for alkaloids, flavonoids, saponins, tannins, carbohydrates, terpenoids, protein, glycosides, diterpenes, and steroids (Gupta *et al*., 2016).

2.4 Ferric Reducing Antioxidant Power (FRAP) Assay

Acetate buffer (B 300 mM), 2,4,6-tri (2-Pyridyl) –S-triazine (10 mM) TPTZ in HCl (40 mM), and $FeCl_3$ (20 mM) mixed in a 10:1:1 ratio and heated at 37 °C in a water bath for 10 minutes. 285 μL of FRAP working solution (100 g/mL concentrations) were introduced to 15 μL of whole plant samples (100 % ethanol, 50 % ethanol, 30 % ethanol, and 100 % aqueous) and incubated at room temperature for 30 minutes in the dark (Mahmoud Abdulrahman, 2021). At 517 nm, the absorbance was measured. The findings were reported in micromoles of Fe^{2+} equivalents per gram of dry extract (mmol Fe^{2+}/g), with FeSO4.7H2O at concentrations ranging from 125 to 1000 M used as a reference.

2.5 DPPH (Scavenging Radical Activity) Assay

200 µL of DPPH Methanolic solution (0.004% w/v) was added to 100 µL of leaves, bark, root (100, 50, 30 of ethanolic and 100 %aqueous) and Essential oil at 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/mL concentration respectively. The mixture was allowed for 30 min in the dark at room temperature and reduction of DPPH was measured at 517 nm. The percentage

of scavenging activity was evaluated by comparing with the control (100 µL methanol + 200 µL DPPH). Quercetin was used as reference standards (Mahmoud Abdulrahman, 2021). A reduction in DPPH was seen at 517 nm. By comparing the scavenging % activity to the control, the scavenging percentage activity was assessed. (DPPH (200 μL) + methanol (100 μL). Quercetin was utilized as a reference standard. The formula was used to calculate the activity of radical scavenging:

Inhibition (%) = [(Ao - A1)/ Ao)] × 100

Where, Ao is the absorbance of the control reaction and A1 is the absorbance of the sample itself. The inhibitory concentration at 50% (IC_{50}) values (extract concentration that cause 50% scavenging of DPPH radical) were determined from the graph of scavenging percentage against the extract concentrations (1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/mL).

2.6 Lipoxygenase assay

The whole plant extract in % 100 ethanol, 50 % ethanol, 30 % ethanol, and 100 % aqueous was screened at eight different concentrations (Singh *et al*., 2012). The extract and the compound were evaluated for dose-dependent inhibition after attaining the active concentration range. At 254 nm, absorbance measurements were taken (Singh *et al*., 2012).The % inhibition was calculated using the formula:

The absorbance of blank − absorbance of test/absorbance of test × 100.

2.7. α-Amylase Inhibitory Effects

250 μL extract (1.56–100 mg/mL) and 250 μL buffer sodium phosphate of pH 6.9 (0.02 M) containing solution of α -amylase (0.5 mg/mL) mixed in a tube. 250 μL solution of 1% starch in 0.02M buffer (sodium phosphate) was added at regular intervals after a pre-incubation period of 10-minute at 25 ° C and re incubated for 10 minutes at the same degree. By introducing 500 μL of dinitro salicylic acid (DNS) reagent, the process was halted. The tubes were cooled to room temperature after boiling for 5 minutes (Mahmoud Abdulrahman, 2021). The absorbance was measured at 540nm with a spectrophotometer after diluting the reaction liquid with 5mL distilled water. The same process was used to make a control, except instead of extract, distilled water was used. -amylase's inhibitory effect was estimated as a % inhibition:

%Inhibition = [Abs control– Abs extracts / Abs control] × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined (Abdulrahman *et al.,* 2019).

2.8 α-Glucosidase Inhibitory Effects

In a well plate, 10 µL of whole plant samples (1.56–100 mg/mL) were added to 50 µL phosphate buffer of 0.1 M at pH 7.0, 25 µL (α-glucosidase (buffer 0.2 U/mL)) and heated for 10 minutes at 37°C. A 25 µL of 0.5 mM 4-nitrophenyl alpha-D-glucopyranoside (pNPG) substrate was mixed to complete the reaction and incubated at 37°C for 30 minutes stoppage of the reaction was initiated by introducing a 100 µL solution of sodium carbonate (0.2 M). At 410 nm, the absorbance of the released p- nitrophenols was measured. The same process was used to make a control, except instead of extract, distilled water was used. The inhibition % was determined according to (Konaté *et al*., 2014).

2.9 Gas Chromatography-Mass Spectrometry Analysis

Crude extracts were analyzed with the aid of gas chromatography-mass spectrometry (GC-MS-2010 with Shimadzu). The chemical mixtures were separated using the HP-5MS column. Temperature programmed: 30 m 0.25 mm, film thickness of 0.25 mm. Begin by heating to 60°C for 10 minutes, then to 230°C for 1 minute at 3°C/min with a 1-minute hold. The injector temperature will be set at 245°C, with a 1 mL/min carrier helium gas flow rate. The MS was operated at 70 volts, with the ion source and analyzer set to 260°C. The composition of the crude extract was calculated based on the fraction using the mass spectra of computer assessment through the NIST (National Institute of Standards and Technology). Software-based automatic mass spectra deconvolution identification is connected to the GC-MS. The data was interpreted using mass

spectral matching with compounds from the NIST Libraries. Only compounds with an 80 percent or higher similarity index are considered.

2.10 Statistical Analysis

Results of all analysis are presented as means of triplicate ± standard deviation (SD) and/or standard error (n = 3). The mean of the concentrations was compared using analysis of variance (ANOVA) and statistical analysis system (SAS) (version 20.0). ANOVA and Duncan's multiple comparisons test. was used to determine significant differences. Statistical significance was defined as a probability level of less than 5% (P>0.05).

2. RESULTS AND DISCUSSION

3.1. Extraction Yield and Qualitative Phytochemical Analysis

Table 1 shows the percentages for the yield of extracts recovered. The results show that 100 % ethanol extract has a much higher percentage yield than other extracts, followed by 50% ethanol at 12.39 and 9.02 % respectively (Table 1). The results revealed a considerable difference between the treatments.

Table 1: Percentage yield of whole plant extracts of *A. mannii*.

S/N	Solvent Concentrations	%Yield
1	100% Ethanol	12.39
2	50% Ethanol	9.02
3	30% Ethanol	7.71
4	100% Aqueous	5.88

A phytochemical study of the whole plant extracts in 100 % ethanol, 50 % ethanol, 30 % ethanol, and 100 % aqueous revealed the presence of primary and secondary metabolites responsible for their diverse biological activities. Phytochemical screening revealed the presence of alkaloids, phenols, tannins, flavonoids, glycosides and saponins in 100% ethanol and missing of few compounds in the other extract (Table 2).

Table 2: Phytochemical constituents

Phytochemicals	100% Ethanol	50% Ethanol	30% Ethanol	100 % Aqueous
Alkaloids	++	++	++	++
Phenols	++	++	++	++
Tannins	++	-	-	++
Flavonoids	++	++	++	++
Glycosides	++	++	-	++
Saponins	++	++	-	-

++present -absent

3.2 FRAP, DPPH and Anti-inflammatory

Two methods were adopted for the antioxidant determination, FRAP and DPPH. The following methods were used due to their effectiveness (Mahmoud Abdulrahman, 2021; Ruiz *et al*., 2013). The capacity to convert Fe^{3+} to $Fe^{2+}$was found to be highest in the 100% ethanolic extract at 18.9 mmol/gm (Table 3). Similarly, the maximum DPPH scavenging activities were observed at 100 % ethanolic extract at 75.5 µg/mL (Table 3). The whole plant extract of *A. mannii* was discovered to be a very good source of antioxidants against free radicals produced in the human body. The whole plant extract of *A. mannii* shows good anti-inflammatory potentials except for the 30 % ethanolic extract (Table 3). The established activity of the whole plant extracts might be due to the synergistic effect of different compounds on the different parts of the plant.

Table 3: Antioxidant and Anti-inflammatory Determination

S/N	Sample	% of inhibition FRAP Fe2+/ (mmol/gm)	% of inhibition of DPPH (µg/mL)	% of inhibition of Lipoxygenase (µg/mL)
1	% 100 ethanol	$18.9^{a} \pm 0.1$	$75.5^{a,b} \pm 0.8$	28.4 ± 0.2^{a}
2	50% Ethanol	$7.4^{d, e} \pm 0.4$	$60.2^{c,d} \pm 0.1$	$18.3 \pm 0.7^{b, c}$
3	30% Ethanol	$6.9^{e} \pm 0.9$	$27.8^{e} \pm 0.7$	ND
4	100% Aqueous	2.1f ± 0.6	$58.3^{d} \pm 0.2$	14 ± 0.3^{d}
5	Acarbose	$9.8^{c} \pm 0.3$	$73.1^{b} \pm 0.4$	17.9 ± 0.6^{c}

Note: Values are expressed as means ± SD. Means have no significant difference in the vertical column with the same letter

3.3 The therapeutic action of α-amylase and α-glucosidase.

The α-amylase inhibitory activity of the ethanolic and aqueous extracts of the whole plant shows the extracts have activity against the tested enzyme with 100 % ethanolic extracts showing a good activity when compared to the other extracts at 39.10 µg /mL (Table 4). Similarly, the 100% ethanolic extract inhibits the α-glucosidase enzyme 28.34 µg /mL (Table 4). The presence of many phytochemicals from various parts of the plant could explain the significant effect of the whole plant extracts.

Table 4: Inhibition activity (%) and IC_{50} values of α-amylase and α-Glucosidase

Sample	α-amylase		α-Glucosidase	
	% Inhibition	IC_{50} (µg /mL)	% Inhibition	IC_{50} (µg /mL)
100% Ethanol	62.7± 0.1b	39.10±0.4d	89.5± 0.3^{a}	28.34±0.1^{d}
50% Ethanol	45.0± 0.2c	66.24±0.5b	88.2±0.5^{ba}	36.74±1.4^{c}
30% Ethanol	81.3± 0.4e	430.4±1.4a	55.1 ± 1.4^{c}	41.46±1.2^{b}
100% Aqueous	72.6± 0.2d	64.83±0.7c	28.3± 0.8^{d}	68.20±0.2^{a}
Acarbose	87.3± 0.9a	5.28±0.2e	87.3± 0.2^{b}	5.26±0.2^{e}

Note: Values are expressed as means ± SD. Means have no significant difference in the vertical column with the same letter

3.4 Chemical Composition of Whole Plant 100 and 50 % Ethanol Extracts of *Anisopus mannii*.

The GC-MS results of most active plant extract are 100 and 50% ethanolic extract (Figure 1 and Figure 2). Only compounds with a 100 % match similarity index on the NIST library were considered. The whole plant extract revealed the presence of various pharmacognostic volatile (Figure 1 and Figure 2). 100% ethanolic extract showed thirteen main phytoconstituents (Table 5). With the dominance of 9-Octadecenoic acid, methyl ester 28.48, Hexadecenoic acid, methyl ester 19.74 (Figure 3). While the 50% ethanolic exhibited only five volatile compounds with 100 % recovery (Table 5 and Figure 3). Lup-20(29)-en-3-ol, acetate, (3. beta) was the major compounds in the extract with a total account of 62.67 % of the total recovery.

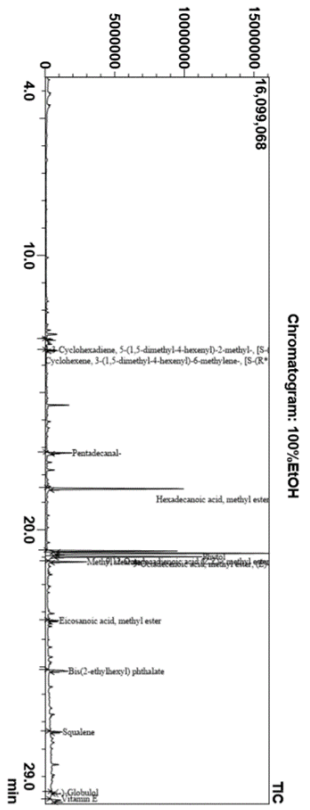


Figure 2: GC-MS chromatogram for whole plant 100% ethanolic extract of *Anisopus mannii.*

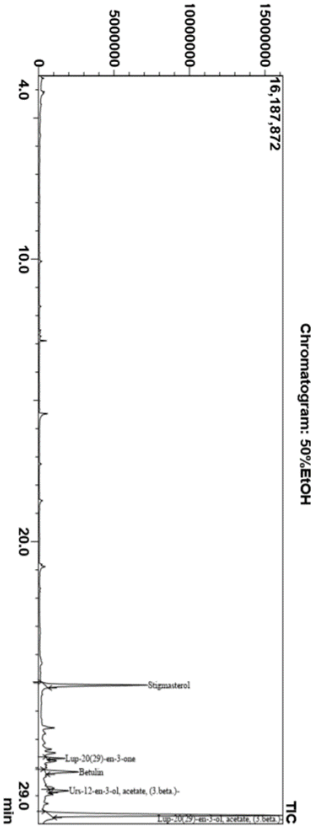
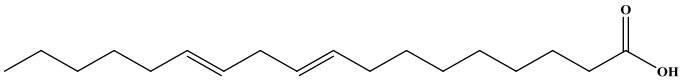


Figure 3: GC-MS chromatogram for whole plant 50 % ethanolic extract of *Anisopus mannii.*

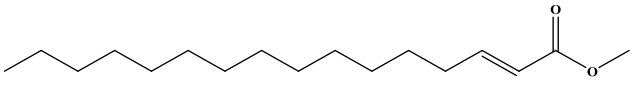
Table 5: Chemical Profiling of 100% and 50 % Whole plant Ethanolic Extracts

RT	Compound	PA %	RT	Compound	PA %
13.057	1,3-Cyclohexadiene, 5-(1,5-dimethyl)	0.76			
13.456	Cyclohexene, 3-(1,5-dimethyl-4-hexe	1.10			
17.202	Pentadecanal-	2.84			
18.509	Hexadecenoic acid, methyl ester	19.74			
20.779	9,12-Octadecadienoic acid	15.05			
20.863	9-Octadecenoic acid, methyl ester	28.48			
20.986	Phytol	17.89			
21.173	Methyl stearate	4.54			
23.360	Eicosanoic acid, methyl ester	1.31	25.079	Stigmasterol	21.78
25.200	Bis(2-ethylhexyl) phthalate	3.87	27.675	Lup-20(29)-en-3-one	2.68
27.420	Squalene	1.57	28.154	Betulin	8.71
29.635	(-)-Globulol	0.60	28.821	Urs-12-en-3-ol, acetate, (3.beta.)	4.16
29.940	Vitamin E	2.23	29.695	Lup-20(29)-en-3-ol, acetate, (3.beta)	62.67
	Total		**99.98**		**100**

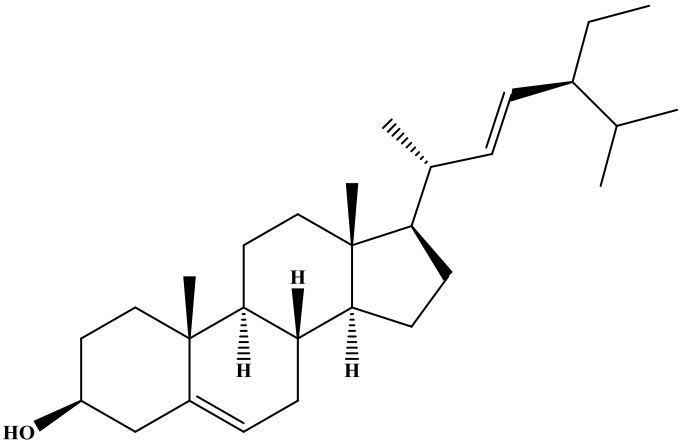
Note: S/N = Serial Number, RT = Retention time, PA = Peak Area



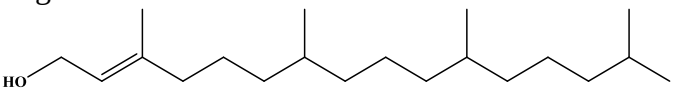
9,12-Octadecadienoic acid



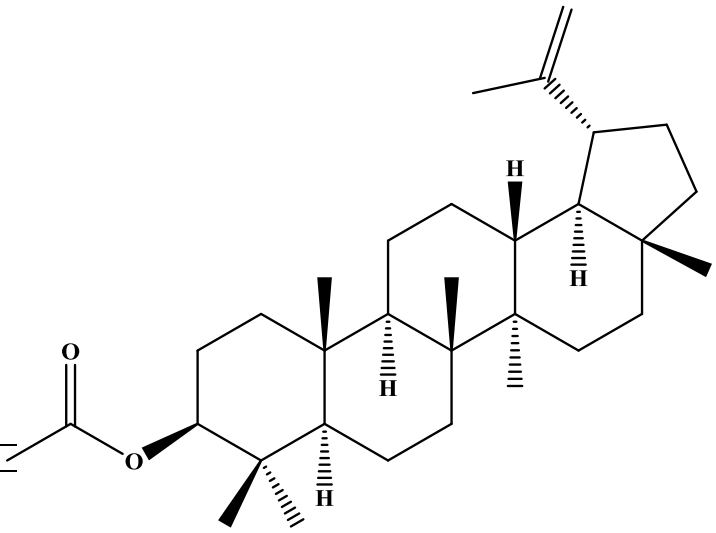
Hexadecenoic acid, methyl ester



Stigmasterol



Phytol



Lup-20(29)-en-3-ol, acetate, (3.beta)

Figure 3: Some of the major compounds identified in whole plant 50 and 100 % ethanolic extract of *A. mannii*

3. Discussion

Other Nigerian researchers had earlier reported on the biological evaluation of *A. mannii* leaf extracts (Aliyu *et al*., 2010; Musa *et al*., 2015). Traditional herbalists have reported using *A. mannii* whole plant extract to treat diabetes and certain diseases (Abdulrahman, 2021). This motivated us to look into the antioxidant, anti-inflammatory, anti-diabetic, and photochemical properties of the whole plant extract as a future

potential natural product. Water and ethanol as the diluent for the extract of the whole plant material since it was known to be safe for consumption (Abdulrahman, 2021; Ramli *et al*., 2017). The high yield obtained from ethanolic extracts could be due to the solvent's ability to extract more chemicals from the samples (Abdulrahman *et al*., 2019). Because ethanol has a slightly lower dipole and is dielectric, it is slightly polar and has superior dissolving capabilities than aqueous (Mahmoud Abdulrahman, 2021). Furthermore, only ethanol, ethyl acetate, and acetone are approved for use in the manufacture of food products (Ramli *et al*., 2017).

The phytochemical analysis of the extract confirmed the existence of compounds with medicinal and physiological properties. Similarly, flavonoids, saponins, tannins triterpenes, and alkaloids are reported to be present in the n-butanol, ethyl acetate and methanol fractions of *A.mannii* (Aliyu *et al*., 2010). Plants create natural antioxidants such as flavonoids, phenolic acids, and tocopherols in the form of phenolic compounds (Djeridane *et al*., 2007; Yadav & Agarwala, 2011). The absence of the compounds in the other extract is a result of the disparity in the nature and the way the compounds interact with the solvents (Escriche & Juan-Borrás, 2018). Because the composition of the phytochemical elements found in plants varies so widely, no single solvent has been developed that can extract all of the compounds from the plant (Omoruyi *et al*., 2012). Polyphenols are significant plant chemicals that have been documented to have a variety of biological effects, including antioxidant activity (Omoruyi *et al*., 2012). Their antioxidant activity stems mostly from their redox characteristics, hydrogen donors, and singlet oxygen quenchers, which can help adsorb and neutralize free radicals (Omoruyi *et al*., 2012).

The relevance of antioxidant components in plant materials in maintaining health and preventing heart disease and cancer are given much attention (Dogara *et al*., 2021). Antioxidants have a critical role in avoiding oxidative food deterioration by scavenging, neutralizing, or eliminating damaging free radicals (Hussain *et al*., 2012). In vitro screening approaches are effective in providing the necessary early observations for selecting crude plant extracts with features that could be beneficial in chemical and pharmacological research (Shibula & Velavan, 2015).

Inflammation is caused by the release of chemical mediators from injured tissues and migratory cells in the majority of cases (Abdulkhaleq *et al*., 2018). Chemical mediators like histamine and serotonin, lipids like prostaglandins, and small peptides like kinins are all unique and dependent on the type of inflammatory response (Abdulkhaleq *et al*., 2018). The plants extract revealed to possess above qualities which enable it to show significant activity against the inflammation assay.

The used of α-amylase and α-glucosidase inhibition on the management of diabetes will be very useful in addressing the ailment (Stojkovic *et al*., 2019; Tundis *et al*., 2010). The results of the study revealed that 100% ethanolic extract of the whole plant has a high inhibitory potential, which may indicate the presence of bioactive chemicals in the plant extracts with inhibitory properties. The results is in agreement with the other in vitro studies carried out on medicinal plants with ethanolic solvents (Mahmoud Abdulrahman, 2021; Abdulrahman *et al*., 2019). Previously effectiveness of α-glucosidase inhibition was reported as compared to the α-amylase (Kazeem *et al*., 2013). Previous research on the study enzymes inhibitors extracted from plants with medicinal values suggests that numerous possible compounds belong to the class of flavonoid, which contains properties that inhibit the activities of α-amylase and α-glucosidase enzyme (Kazeem *et al*., 2013). Natural α-amylase and α-glucosidase inhibitors from 100 and 50 % ethanolic extracts have been shown to have strong activity on α-amylase and α-glucosidase, and thus the whole plant ethanolic extracts can be utilised as a potential therapy for management of hyperglycemia. Natural products are a valuable source of therapeutic compounds (Gavamukulya *et al*., 2015).

The discovery of new sources of economically viable phytocompounds for the synthesis of complex chemical substances, as well as identifying the genuine relevance of traditional medicinal knowledge, necessitates understanding of plant chemical contents (Sermakkani & Thangapandian, 2012).

Previously GCMS was adopted for the chemical contents profiling of plant extracts (Gavamukulya *et al*., 2015; Sermakkani &

Thangapandian, 2012). Hexadecenoic acid, Octadecadienoic acid and Phytol are reported to be the major constituents of the 100 % ethanolic extract. Hexadecanoic acid, ethyl ester (34%), oxirane, hexadecyl- (11%), and 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z) were the most common chemicals (9.6%) are the major compound in the hexane leaf extract of *A. mannii* (Musa *et al*., 2015). The following compounds previously reported to inhibit significant activity against the antidiabetic, antioxidant, anticancer and hyper-cholesterol (Sermakkani *et al*., 2012). The maximum peak of 9-Octadecenoic acid (28.48) could be a good reason for the best antidiabetic properties in the following study. The study therefore believed the chemicals discovered are thought to have a synergistic or individual activity. As a result, the whole plant extracts could be used to treat a variety of health problems linked to the metabolites studied. The increased extraction capability of ethanol, according to the findings, may have resulted in the extraction of a vast number of active components responsible for a wide variety of biological functions. More study is needed to elute unique active compounds from the whole plants extracts, so that they can be employed in the development of herbal medicines and modern drugs which could lead to the development of a new way to cure many incurable diseases.

4. CONCLUSIONS

There is a revived interest in natural-source pharmaceuticals these days simply because they are considered green medicine, which is always assumed to be safe. The more effective the medicinal plant is, the higher its demand will be, and the likelihood of non-availability will rise. To accommodate the increased demand, different parts of the plant must be explored. The study examines the whole plant extracts potentiality. It can be concluded that the whole plant extracts of *A. mannii* is an effective medicinal agent. The study proved the claim of the traditional herbalist about the utilisation of the whole plant extract to treat diabetes. It provides the possibility for different therapy regimens to be developed. ethanol extracts displayed moderate inhibitory potentials, and this might be due to synergistic effect of the phytochemical constituents present in it. However, further study is needed to isolate the bioactive compound in the whole plant extract.

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Conflict of Interest

There is no conflict of interest.

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