A PROJECT REPORT ON

CHEMICAL CHARACTERIZATION AND INHIBITORY EFFECTS OF ARISTOLOCHIA RINGENS ROOT EXTRACT ON DIGESTIVE ENZYMES

BY

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CERTIFICATION

This is to certify that this project is the original work carried out by ABDULWAHAB QUAMDEEN (HND/23/SLT/FT/0930) of the Department of Science Laboratory Technology, Biochemistry unit, Institute of Applied Science (IAS), Kwara state Polytechnic, ilorin and has been approved in partial fulfillment of the requirement for the award of Higher National Diploma (HND) in Science Laboratory Technology.

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DEDICATION

This project research dedicated to the Almighty Allah for his provision, mercy,protection, loving kindness and guidance throughout the period of my stay in the Polytechnic.

It is also dedicated to my loving mother, Hajia Rosheedah Abdulwahab, for her unwavering support, love, and her sacrifice,encouragement and prayers I'm forever indebted to her And once again thanks for the love and the sacrifice, May your gentle soul continue to rest in peace.

ACKNOWLEDGEMENT

First foremost, I give all thanks and adoration to the lord of the universe, for guiding me throughout this journey, grating me wisdom, strengthening and perseverance, your divine present in my life has been my source of inspiration and comfort.

To my beloved parents, [Mr and Mrs. Abdulwahab], I'm deeply grateful for your unwavering support, love, and sacrifice your love, guidance, encouragement and prayers have been my pillars of strength, and I'm forever indebted to you both And for the mum thanks for the love and the sacrifice, May your gentle soul continue to rest in peace.

To my lecturers, I'm grateful for your mentorship, guidance, and expertise. You passion for teaching and commitment to excellence have inspired me to strive for greatness. Thank you for shaping my academic journey and helping me throughout.

To my friends, your friendship has been a blessing. I'm thankful for the memories, laughter and the wonderful moments we had, your support made this journey enjoyable and unforgettable.

To my dear sister and brother, I'm so grateful for your love, support and encouragement throughout my academic journey. Your presence in my life has made a significant difference, and I'm thankful for the memories and adventures we shared.

Thank you for being my confidant, role model , your individual strength and talent have inspired me to strive for excellence, and I'm so proud to call you my siblings.

With Love and appreciation and gratitude I {Abdulwahab Quamdeen] say a very big

Thank you to you all.

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ABSTRACT:

Aristolochia ringens is a medicinal plant that has been used traditionally in the management of several diseases. This study focused on investigating the phytochemical contents, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of Aristolochia ringens (Vahl.) root.

The plant material was collected, dried, coarsely grounded, and extracted using methanol. The methanol extract was partitioned into n-hexane and ethyl acetate to obtain the respective extracts. The qualitative phytochemical screening of the extracts was carried out using standard methods. Selected elements were determined from the plant material using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The antioxidant assays were carried out using the reducing power and 2,2-Diphenyl-l-1-picrylhydrazyl assay methods. The alpha-amylase inhibitory activities were determined preliminarily using the starch-iodide assay.

The extraction gave the methanol extract (ArMe) which on partitioning gave the n-Hexane (ArnH), ethyl acetate (ArEa), and the residual methanol extracts (ArRMe). Qualitative phytochemical screening showed the presence of flavonoids, cardiac glycosides, terpenoids, steroids, phlobatannins, and phenols in all the extracts; alkaloids, tannins, and reducing sugar in ArMe, ArnH, and ArRMe, while saponins were found in ArMe, ArnH, and ArRMe. Elemental analysis showed a significant level of the selected elements Ca, Mg, K, Fe, Zn, Na, Cu, Co, and Se in ppm. Antioxidant assay results showed that the extracts exhibited dose-dependent reducing properties and an increased DPPH scavenging activity

These results confirmed some of the traditional uses of A. ringens in the management of high blood pressure, diabetes, and inflammatory conditions. Keywords:

Aristolochia ringens, phytocontents, elemental contents, antioxidant activities, alpha-amylase inhibitory

CHAPTER ONE

INTRODUCTION

1.1 Background to Study

Plants are known in ancient and modern civilizations for their healing properties. They remain the sole source of healing principle to man's ailments until the 19th century when development of chemistry, particularly organic compounds led to the experimental and clinical validation of their efficacy (Edeoga et al., 2005; Natarajan et al., 2011).

The medicinal properties of plants have been connected to their phytochemical contents Tapsell et al.2013, To understand fully and utilize the health benefits of phytochemicals, there is a need to study the several thousands of medicinal plants yet to be explored [2]. New drug development has its origin from the explored medicinal plants which are reported to constitute about 25% of conventional medications still in use today [Rao 2004; Sofowora et al., 2013. Some medicinal plants are used in their crude forms for the treatment of diseases (Sofowora et al., 2013)

Aristolochia ringens, a related local species, resembles over 500 species of Aristolochia (family Aristolochiaceae) that spread across tropical and sub-tropical regions of Asia and Africa, and used traditionally for cancerous sore, dysentery, lung inflammation, scorpion and snake bite (Kumar and Suryanarayana, 2008; Vermal et al., 2008; Abhijit and Jitendra, 2011; Thirumal et al., 2012). Aristolochia species are

herbaceous vine with woody stock and widely distributed throughout the southwestern Nigeria (Kanjilal et al., 2009; Kumar et al., 2011). The leaves are variably glabrous, obovate - oblong with sub-pandurate entire and inflated pale green perianth up to 4cm long (Das et al., 2010). Like other species, Aristolochiaringens has been studied to contain essential oil, aristolochia acid and other phytochemicals that are of biological importance (Ashokkumar et al.,2010; Sinha and Choudhury, 2010; Tajkarimi et al., 2010; Abhijit and Jitendra, 2011; Samy et al., 2011). According to an ethnomedicobotanist's oral interview conducted at Bode, a popular herbal market in Ibadan metropolis within Nigeria, A. ringens is used to treat various ailments such as wounds, dysentery, throat infections and skin problems of which are linked to microbial infestations. In Asian countries, especially India, over 2,500 plants have been studied to have provided alternative medicine and curative properties to the available synthetic drugs (Sarmiento et al., 2011; Thirumal et al., 2012). Extracts from Aristolochia sp, especially, phytochemicals and essential oil have been receiving earnest in-vitro investigations for their numerous activities.

Among the documented activities traced to such phytochemical properties of A. ringens are antimicrobial, anti-inflammatory, anti-venom, antipyretic, antiseptic, abortifacient, emmenagogues, storage stability (preservative), foaming (lather), curative, taste, flavours and aroma on one hand and potent nephrotoxic, anti fertility and antispermatogenic on the other (AshokKumar et al., 2010; Sinha and Choudhury, 2010; Tajkarimi et al., 2010; Abhijit and Jitendra, 2011; Kumar et al., 2011). In recent years, the traditional application of natural compounds of plant origin has been receiving a lot of attention as an alternative source of remedy for the treatment of diseases coupled with the belief of their better safety nature and of less or non toxicity. This has led to the increase in laboratory (in-vitro) research into herbal medicine to establish their acclaimed efficacy and their therapeutic applications. This study aimed at ascertaining the acclaimed antimicrobial property possessed by A. ringens as an ingredient in the production of herbal medicine that serves as effective therapeutic agent against pathogenic microorganisms and their associated infections.

The earth's crust is well endowed with heavy metals, which are highly beneficial to

Man but required in trace quantities because of the possibility of becoming toxic at high concentrations (Jackson et al., 2014) These metals gain entry into the body system through food, drinking water, and air as trace metals [Jackson et al., 2014 ; .Soetan et al., 2010]. Common examples are selenium, copper, and zinc which are useful for the maintenance of the metabolism of the human body. Magnesium, calcium, and potassium, are useful for muscular function and blood pressure control (Jackson et al., 2014 ; .Soetan et al., 2010). However, they are required in minute quantities as they may become toxic at high concentrations (Soetan et al., 2010). This work is reporting the phytochemical, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of Aristolochia ringens (Vahl.) root extracts

1.2 Aims:

This study focused on investigating the phytochemical contents, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of Aristolochia ringens (Vahl.) root.

1.3 Objectives:

The specific objectives of the study are

1. To determine the phytochemical constituents and mineral content of Aristolochia ringen root
2. To determent the Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate
3. To determining the free radical scavenging and alpha-amylase inhibitory activities of Aristolochia ringens root.

CHAPTER TWO

LITERATURE REVIEW

2.1. Phytochemical

The earth's crust is well endowed with heavy metals, which are highly beneficial to Man but required in trace quantities because of the possibility of becoming toxic at high concentrations (Akoro et al 2017) These metals gain entry into the body system through food, drinking water, and air as trace metals (Akoro et al 2017) Common examples are selenium, copper, and zinc which are useful for the maintenance of the metabolism of the human body. Magnesium, calcium, and potassium, are useful for

muscular function and blood pressure control. Jackson SL(2018)]. However, they are required in minute quantities as they may become toxic at high concentrations OlaiyaCO (2013). This work is reporting the phytochemical, mineral contents, free radical scavenging, and alpha-amylase inhibitory activities of Aristolochiaringens (Vahl.)SoetanKO(2016)

No single method is sufficient to study the bioactivity of phytochemicals from a given plant. An appropriate assay is required to first screen for the presence of the source material, to purify and subsequently identify the compounds therein. Assay methods vary depending on what bioactivity is targeted and these may include antimicrobial, anti-malarial, anticancer, seed germination, and mammalian toxicity activities. The assay method however should be as simple, specific, and rapid as possible. An in vitro test is more desirable than a bioassay using small laboratory animals, which, in turn, is more desirable than feeding large amounts of valuable and hard to obtain extract to larger domestic or laboratory animals. In addition, in vivo tests in mammals are often variable and are highly constrained by ethical considerations of animal welfare. Extraction from the plant is an empirical exercise in which different solvents are utilized under a variety of conditions such as time and temperature of extraction. The success or failure of the extraction process depends on the most appropriate assay.

2.2. Minerals

Minerals build bones, teeth, blood, and help the body use energy. Minerals combine in important ways in the body, like Calcium in our bones. Each mineral has a specific role in our body. They also regulate body processes. For example, the Iron in red blood cells transports oxygen. We can obtain all the minerals we need through a healthy

diet.

The two main minerals that are especially important for all individuals are Iron and Calcium.

Iron: iron is involved in transporting oxygen around the body in the red blood cells. With folate and B12, it prevents anemia.

The best source is red meat e.g. beef and lamb. Green leafy vegetables, whole meal breads and fortified breakfast cereals contain iron but it is not absorbed as well from these foods. Having a vitamin C source at the same meal will increase the amount of iron the body absorbs from these foods, e.g. having a fresh orange or orange juice with your breakfast cereal. Calcium: Bones are composed of several minerals, the most important being calcium. Vitamin D works with calcium. Calcium requirements are higher during teenage years, pregnancy, in breastfeeding women and post-menopausal women. And it can be found in milk, cheese and yogurt, (low fat varieties have the same amount). Smaller amounts are found in white bread, nuts, green leafy vegetables and tinned fish.

2.3. Aristolochia Ringens

Aristolochiaringens is a species of perennial plant in the family Aristolochiaceae. It is found from Panama through Bolivia, Colombia, and Venezuela.zygia (2019.). It is reported to be used traditionally as analgesics, anti-cancer agents, anti-inflammatory agents, sedatives muscle relaxants, antihistaminics, antiallergics, antimicrobials, and antimalarials Kubmarawa D, Ajoku GA(2019). AristolochiaringensVahl.is a perennial plant in the Aristolochiaceae family. In the south-western Nigeria (Yoruba), the plant is commonly known as 'Akogun'. It is an aromatic liane, scrambler, a climbing shrub or rhizome (Albizia, 2018). The plant contains alkaloids and aristolochic acids. The plant is used locally in the treatment of wounds, dysentery, throat infections and skin problems. The antimicrobial potential and phytochemical composition of A. ringens root and bark have been investigated. 26 In addition, the antidiabetic, antitrypanosomal

and anticancer activities of the plant have been reported.

A ringens has been reported for short-time management of disorders like worm infestation, gastrointestinal, oedema, and inflammatory disorders . Previous works also reported the antidiabetic properties of A. ringens. The aqueous root extract of A. ringens has been used to manage and treat inflammation Studies have shown that A. ringens displayed antioxidant activities as it has proven folkloric uses in the treating and managing of some skin ailments such as eczema, psoriasis, and heat rashes because it contains tannins and flavonoids which are examples of naturally occurring antioxidants in plants Formagio (2014)

2.4. Antioxidant

Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson & Cheng, 2016). Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin et al., 2008; Jayasri et al., 2019). Antioxidants exert their activity by scavenging the „freeoxygen radicals‟ thereby giving rise to a fairly „stable radical‟. The free radicals are metastablechemical species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may

damage crucial bio molecules like lipids, proteins including those present in all membranes, mitochondria and, the DNA resulting in abnormalities leading to disease conditions (Uddin et al. 2018). Thus, free radicals are involved in a number of diseases including: tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer‟s diseases), AIDS and even early senescence (Chen et al., 2006; Uddin et al., 2018). The human body produces insufficient amount of antioxidants which are essential for preventing oxidative stress. Free radicals generated in the body can be removed by the body‟s own natural antioxidant defences such as glutathione or catalases (Sen, 1995). Therefore this deficiency had to be compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, β-carotene and natural products in plants (Madsen &Bertelsen, 1995; Rice- Evans et al., 2017; Diplock et al., 2019).

Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids hat are rich in antioxidant activity (Madsen &Bertelsen, 1995; Cai& Sun, 2003). Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, caratenoids, flavanols and phenolics which possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases (Hertog&Feskens, 2019; Anderson &Teuber, 2001). Many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or C, and thus might contribute significantly to

protective effects in vivo (Jayasri et al., 2019).

Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Due to safety concerns of synthetic compounds, food industries have focused on finding natural antioxidants to replace synthetic compounds. In addition, there is growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants.

2.5. Anti-diabetic

Cinnamaldehyde, a phytoconstituent extracts have been reported to exhibit significantantihyperglycemic effect resulting in the lowering of both total cholesterol and triglyceride levels and, at the same time, increasing HDL-cholesterol in STZ-induced diabetic rats. This investigation reveals the potential of cinnamaldehyde for use as a natural oral agent, with both hypoglycaemic and hypolipidemic effects. Recent reports indicate that Cinnamon extract and polyphenols with procyanidin type-A polymers exhibit the potential to increase the amount of TTP (Thrombotic Thrombocytopenic Purpura), IR (Insulin Resistance), and GLUT4 (Glucose Transporter-4) in 3T3-L1 Adipocytes. It was suggested that the mechanism of Cinnamon‟s insulin-like activity may be in part due to increase in the amounts of TTP, IRβ, and GLUT4 and that Cinnamon polyphenols may have additional roles as anti-inflammatory and/or anti-angiogenesis agents (Jakhetia et al., 2010).

CHAPTER THREE

MATERIAL AND METHODS

3.1. General instrumentation and materials

All chemicals used in this work are of analytical grade. Ultraviolet Spectroscopy data were obtained using Spectrum Lab 752s. Elemental analysis was carried out using Agilent 5800 ICP-OES.

3.2. Collection and extraction of plant materials

The plant material, Aristolochia ringens root was purchased at the Mushin market, Lagos, Nigeria. It was then authenticated at the University of Ilorin . The plant material was air-dried and grounded into a coarse form using an electrical blender. The grounded plant materials (250.15 g) were extracted by macerating for 72 h in 80% methanol. The extract was filtered and concentrated using a rotary evaporator and further dried in an air oven at 40 $^{\circ}$C to obtain a completely dried sample (Ar Me, solid, 6.99 g).

3.3. Partitioning of the crude methanol extract

The crude methanol extract (ArMe, 6.99 g) was dissolved in methanol and water (3:1). The extract was partitioned in n-Hexane (50 mL × 3): The solvent, n-Hexane (50 mL) was added to the crude methanol extract in the separating funnel, and the mixture was homogenized and left to stand for a while to give a clear separation. The fraction was separated and dried to give a solid (ArnH, green solid, 0.44 %). The residual methanol extract was further partitioned with ethyl acetate (50 mL × 3) and the fraction was separated and dried to give a solid (ArEa, greenish-brown solid, 8.54 %) and the

residual methanol extract also a solid (ArRMe, greenish-brown solid, 29.11%). The extracts were kept in the refrigerator until when required for further use.

3.4. Qualitative phytochemical screening

The extract was screened to detect the presence of secondary metabolites: alkaloids, flavonoids, saponins, tannin, phlobatannins, cardiac glycoside, terpenoids, steroids, reducing sugar and phenol using the standard methods described by Sofowora et al (2014)

3.4.1. Test for alkaloid

3.4.1.1. Wagners Test

A few drops of Wagner's reagents were added to the plant extracts. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

3.4.1.2. Dangendroff Test

The Dragdendroff's reagent (a few drops) was added to I mL of the filtrate obtained by boiling 0.01 g of the extract which was dissolved in 1% aqueous hydrochloric acid. The presence of a reddish-brown colouration indicates the presence of alkaloids.

3.4.2. Test for flavonoids

To the plant extract (about 0.01 g) was added dilute ammonia solution (1.0M, 5 cm3), followed by the addition of 5 cm3 of concentrated hydrogen tetraoxosulphate (VI) acid. The formation of a yellow colouration which disappeared on standing shows the presence of flavonoids

3.4.3. Test for Saponins

Frothing Test: A small amount of distilled water was added to the plant extract (about 0.01 g) and shaken. The appearance of froth indicates the presence of saponins.

3.4.4. Test for Tannin

Ferric Chloride Test: To the plant extract (about 0.2 g) was added 10 mL of distilled water and shaken for some minutes before filtering. $FeCl_3$ (15%) was added to the filtrate. Tannins are indicated by the formation of deep blue colour.

3.4.5. Test for Phlobatannins

Each of the extracts was boiled with hydrochloric acid (1%, 5 cm3), and the

deposit of a red precipitate shows a positive test.

3.4.6. Test for Glycoside

3.4.6.1. Keller kelliani Test

About 0.01 g of the plant extract was treated with chloroform and evaporated to dryness. 0.4 mL of glacial acetic acid containing a trace amount of ferric chloride was added, followed by the careful addition of 0.5 mL of concentrated H2SO4. The presence of blue colour in the acetic layer indicates the presence of glycosides.

3.4.6.2. Legal Test

The extract was dissolved in pyridine and five drops of 2% sodium nitroprusside together with four drops of 20% of NaOH were added. A deep colour indicates the presence of glycoside.

3.4.7. Test for Terpenoids

To each of the plant extracts (0.01 g), a mixture of chloroform (2 cm3) and concentrated hydrogen tetraoxosulphate (VI) acid (3 cm3) was added to form a layer. The presence of a reddish-brown colouration at the interface shows a positive result for the presence of terpenoids.

3.4.8. Test for Steroid

Salkowskis test: 0.01 g of the extract was dissolved in 2 mL of chloroform. Concentrated hydrogen tetraoxosulphate (VI) acid was added carefully to form a lower layer. A reddish-brown colouration at the interface indicates the presence of a steroidal ring (aglycone portion of the cardiac glycoside).

3.4.9. Test for Reducing Sugar

To the extract solution (5 mL), an equal volume of Fehling A and B solutions was added and the mixture was warmed. The formation of a brick-red precipitate at the bottom of the test tube indicates reducing sugar.

3.4.10. Test for Phenols

The extract solution was treated with four drops of FeCl3 solution, the formation of bluish-black colour indicates the presence of phenols.

3.5. Ashing and trace elements analysis

Air-dried and coarsely grounded A. ringens (5 g) were weighed into the crucible and were ashed in a muffle furnace at 550ºC for 4h. The ashed sample was dissolved

with 10 mL of aqua regia. This was transferred into a 100 mL volumetric flask to make a 100 mL solution. The trace elements were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [15]. The experiment was carried out in duplicate [16].

3.6. Antioxidant assay

Antioxidant assays were carried out on each of the plant extracts using the reducing power and DPPH free radical scavenging assay methods.

3.6.1. Reducing power assay of plant extracts

The reducing power of each of the plant extracts was determined as described by Oyiazu [17]. The extracts were mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide (1%, 2.5 mL). The mixture was incubated at 50oC for 20 mins after which a portion (1 mL) of 10% trichloroacetic acid was added to stop the reaction. The mixture was centrifuged for 10 mins at 3000 rpm. The upper layer of the solution (1.5 mL) was mixed with distilled water (1.5 mL) and FeCl3 (0.1 mL, 0.1%), and the content was incubated for 10 mins and the absorbance was measured at 700 nm. An increase in reducing power was indicated by an increase in absorbance as the concentration of extracts in the reaction mixture increased.

3.6.2. DPPH Scavenging activity of plant extracts

The DPPH solution (5 mL) was added to I mL of the plant extract solution at different concentrations. The reaction mixture was allowed to stand after shaking at room temperature for 30 mins. The absorbance of the solution formed was measured spectrophotometrically at 517 nm. For this experiment, Ascorbic acid was used as standard with the control prepared without the plant extract at different concentrations. The scavenging activity was expressed as the percentage inhibition and calculated using the formula below:

$$\% \text{ Scavenging activity} = \frac{\text{Control Absorbance (AC)} - \text{Sample Absorbance(AS)}}{\text{Control Absorbance (AC)}} \times 100\ \%$$

Where Ac = Absorbance of control at 517 nm and AS = Absorbance of the sample.

3.7. Alpha-amylase inhibitory assay

The alpha-amylase inhibitory assay was carried out using the starch iodide assay as described by Akoro et al.2017

3.7.1. Starch-Iodine Assay

To 250 µL of the plant extract in DMSO (concentration range 0, 1, 10, 100, 1000 µg/mL), was added to 250 µL enzyme solution (250 µg/mL pancreatic alpha-amylase enzyme) dissolved in 0.02M Sodium phosphate buffer with 0 0.006 M sodium chloride, the solution was incubated for 10 min at 37oC. Soluble starch (potato starch) (1%; 250 µL) was then added to all the test tubes and incubated again for 10 min 37oC. This was followed by the addition of 250 µL 1M HCl to terminate the enzymatic reaction and then the addition of 100 µL of iodine reagent. The colour changes were recorded.

3.8. Statistical analysis

Statistical analysis was carried out using MS Excel 2010. Results were expressed as mean ± standard deviation (SD). The student's t-test was used where appropriate to compare the significance in mean values at different concentrations at a probability level of < 0.05.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1. Extraction of plant material and phytochemical screening.

The plant material (219.15 g) was extracted with methanol by maceration to yield 6.32 g (2.88 %) of the methanol extract (ArMe). The methanol extract (6.32 g)was partitioned in n-hexane and ethyl acetate toyield the respective extracts (ArnH and ArEa) and the residual methanol extract (ArRMe). The results are summarised in Table 1.

4.2. Elemental analysis

The elemental analysis of the plant for some selected heavy metals and trace elements - Ca, Na, Mg, Zn, K, Fe, Cu, Se, and Co - (Table 3) was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

4.3. Antioxidant activities of the plant extracts

The antioxidant activities of each of the plant extracts were determined from the reducing power and DPPH radical scavenging assays (Figures 1 and 2).

Table 4.1. Yield, percentage yield, and colour of A. ringens extracts

Extracts	Mass of plants material (g)	Mass of extract obtained (g)	Percentage Yield	Colour
ArMe	219.15	6.32	2.88	Greenish brown
ArnH	6.32	0.66	10.44	Green
ArEa	6.32	0.54	8.54	Greenish brown
ArRMe	6.32	1.84	29.11	Greenish brown

Key: ArMe- crude methanol extract of A. ringens; ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe- residual methanol extract from the partitioning

Table 4.2. Phytochemical contents of the Aristolochia ringens

Phytochemicals	ArMe	ArnH	ArEa	ArRMe
Alkaloids	+	+	-	-
Flavonoid	+	+	+	+
Saponins	+	+	-	+
Tannins	+	+	-	-
Phlobatannins	+	+	+	+
Cardiac glycoside	+	+	+	+
Terpenoids	+	+	+	+

Steroid	+	+	+	+
Reducing sugar	+	+	-	-
Phenol	+	+	+	+

Key: + = Detected; - = Not Detected; ArMe- crude methanol extract of A. ringens; ArnH- n-hexane extract; ArEa- ethyl acetate extract; ArRMe-residual methanol extract from the partitioning.

Table 4.3. Mean percentage ash and mean amount of some selected elements detected in A. ringens in ppm

Mean % Ash	Element	Amount (ppm)
10.91±0.23	Ca	64.38±17.77
	Mg	21.46±5.93
	K	13.41±3.70
	Na	0.65±0.18
	Fe	3.25±0.90
	Zn	0.92±0.25
	Cu	0.43±0.12
	Se	0.12±0.03
	Co	0.18±0.05

Fig. 4.1. Graph of concentration against absorbance indicating reducing property

Fig. 4.2. DPPH scavenging activity of each extract

4.4. People Alpha-amylase assay of A. ringens Extracts

The colour changes observed in the preliminary alpha-amylase inhibitory assay using the starch iodide method are shown in Table 4.4. Preliminary phytochemical screening of the A. ringens extracts indicated the presence of flavonoids, cardiac glycosides, terpenoids, steroids, phlobatannins, and phenols in all the extracts; alkaloids, tannins, and reducing sugar were detected in only the crude methanol extract and n-hexane extract while saponins were detected in all the extracts except the ethyl acetate extract. (Table 2). The presence of these secondary metabolites may explain the various medicinal properties attributed to this plant

The elemental analysis of the plant for some selected heavy metals and trace elements - Ca, Na, Mg, Zn, K, Fe, Cu, Se, and Co - (Table 3) was carried out using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), which is used for elemental analysis in samples at trace level [Akoro et al., 2018]. ICP-OES is an elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. Samples are introduced into the plasma in a process that desolvates, ionises, and excites them. The results showed the following trend in the amount of elements: Ca > Mg > K > Fe > Zn > Na > Cu > Co > Se (Table 3). A. ringens in this work is known to give good support to the body framework (Soetan et al., 2010); magnesium and potassium are useful in the control of blood glucose and pressure (Akoro et al., 2017). Potassium is also useful in maintaining normal fluid levels in the body cells while sodium maintains the fluid level outside the body cells]. Cobalt is a major component of vitamin B12 (Osman et al., 2021) while iron is useful in the transportation of oxygen by the blood (Pittman 2011), manganese and zinc act as enzymes and they are useful elements that help in the proper functioning of the liver (Mohammad et al., 2012).

The antioxidant activities of each extract were determined from the reducing power and DPPH radical scavenging assays (Figures 1 and 2). The results of the

reducing power assay indicate activity in the n-hexane and ethyl acetate extracts. For the n-hexane and ethyl acetate extract, the reducing activity was not significantly different ($p<0.05$) at the concentrations studied (1, 10, 100, 1000 µg/mL). Also, in the residual methanol extract, the reducing activity was not significantly different ($p<0.05$) from those observed in the n-hexane and ethyl acetate at the lower concentrations indicating a lower reducing power than the n-hexane and ethyl acetate extracts. The results of the DPPH scavenging assay indicated activity in all extracts studied especially in the residual methanol extract (ArRMe). For the n-hexane and ethyl acetate extract,

Table 4.4. Alpha-amylase Inhibitory assay

Concentration (µg/mL)	Colour Observed		
ArnH	ArEa	ArRMe	
0	Orange	Orange	Orange
1	Orange	Orange	Blue-black
10	Deep orange	Blue-black	Blue-black
100	Deep orange with a trace of blue-black	Blue-black	Blue-black
1000	Deep orange with a trace of blue-	Blue-black	Blue-black

	black		
Conclusion	Dose-dependent Partial Inhibition	Dose-dependent inhibition	Inhibition at all the concentration

the DPPH scavenging activity was not significantly different ($p<0.05$) at the lower concentrations (1-100 µg/mL) but was pronounced and significant at 1000 µg/mL. However, the residual methanol extract showed higher reducing activity than all other extracts. However, the DPPH scavenging activity was not significantly different ($p<0.05$) at all the concentrations studied. In the two antioxidant assay methods used, the ascorbic acid standard showed relatively higher activity than all the extracts. These results can be concluded to indicate a moderate antioxidant activity in support of the folkloric use of A. ringens to mope up free radicals. (Kubmarawa et al., 2007; Yu et al., 2007)

The preliminary alpha-amylase inhibitory activities of each of the extracts were determined using the starch iodide assay. A dose-dependent inhibitory activity was observed in the n-hexane (ArnH) and ethyl acetate (ArEa) extracts based on the trend in the colour change from 10 µg /mL to 1000 µg/mL, with partial inhibition in ArnH and a move from partial inhibition to complete inhibition in ArEa (Table 4). However, in the methanol extract (ArRMe), there was complete inhibition at all concentrations (Table 4). The ability of the extract to inhibit alpha-amylase enzyme activity was indicated by the

presence of starch (blue-black colour in iodine); partial inhibition was indicated by orange or brownish blue colouration while yellow colour indicated complete digestion of the starch (Houston et al., 2008). The measurement of post-prandial blood glucose is one of the parameters monitored in the management of type-2 Diabetes mellitus [Kubmarawa et al 2007., , Yu et al., 2007].

The inhibition of the activities of digestive enzymes like alpha-amylase, and alpha-glucosidase plays a major role in the The antioxidant activities of each extract were determined from the reducing power and DPPH radical scavenging assays (Figures 1 and 2). The results of the reducing power assay indicate activity in the n-hexane and ethyl acetate extracts. For the n-hexane and ethyl acetate extract, the reducing activity was not significantly different ($p<0.05$) at the concentrations studied (1, 10, 100, 1000 µg/mL). Also, in the residual methanol extract, the reducing activity was not significantly different ($p<0.05$) from those observed in the n-hexane and ethyl acetate at the lower concentrations indicating a lower reducing power than the n-hexane and ethyl acetate extracts. The results of the DPPH scavenging assay indicated activity in all extracts studied especially in the residual methanol extract (ArRMe). For the n-hexane and ethyl acetate extract, the DPPH scavenging activity was not significantly different ($p<0.05$) at the lower concentrations (1-100 µg/mL) but was pronounced and significant at 1000 µg/mL. However, the residual methanol extract showed higher reducing activity than all other extracts. However, the DPPH scavenging activity was not significantly different ($p<0.05$) at all the concentrations studied. In the two antioxidant assay methods used, the ascorbic acid standard showed relatively higher activity than all the extracts. These results can be concluded to indicate a moderate antioxidant activity in support of the folkloric use of A. ringens to mope up free radicals. (Kubmarawa et al 2007., , Yu et al., 2007)

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CHAPTER FIVE

CONCLUSION

The results of this study support and further explain the folkloric uses of A. ringens in the management of several diseases including high blood pressure, diabetes, and inflammatory conditions associated with free radical accumulation

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