



Characterization Of Herbal Mixture Solution And In Vivo Modulation of Metabolites Associated with Risk Factors of Erectile Dysfunction

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ABSTRACT

Background: Erectile dysfunction is well known to be associated with hypertension and diabetes. Homeostasis of blood glucose and lipid profile could help lower some risk factors of erectile dysfunction. This study examined the effect of *Syzygium guineense*, *Allium sativum*, potash and *Syzygium aromaticum* mixture (herbal mixture solution and potash locally used as an aphrodisiac) on fasting blood glucose level and lipid profile. **Method:** The plant solution was also subjected to preliminary characterization using Fourier Transform Infrared (FTIR) spectroscopy as well as acute toxicity study. Thirty (30) male and six female *Wistar* rats were used. Varying concentrations (100, 200, 400 mg/kg) of the extract were administered to the test groups while 5 mg/kg of Sildenafil citrate was administered to the positive control group. The normal control received distilled water. The control and test groups contained six rats per group each. Animals were treated for 2 weeks. The female rats were used for the acute toxicity study (Up and Down procedure). **Results:** The LD₅₀ was above 5000 mg/kg. FTIR characterization revealed 4 spectra. Each spectrum is a reflection of the functional groups present in the mixture. Fasting blood glucose (6.60 ± 0.30^a mmol/L) and lipid profile (TG: 71.20 ± 3.28^a ; HDL-C: 27.00 ± 2.35^a mg/dl) were not altered significantly by the plant solution and Sildenafil citrate. **Conclusion:** These findings indicate that the plant solution was able to maintain homeostasis of blood glucose and lipid profile, hence would not vary the levels of these metabolites that are risk factors to erectile dysfunction.

Keywords: Acute toxicity, aphrodisiac, LD₅₀, Sildenafil citrate, *Syzygium guineense*.

1.0 INTRODUCTION

The safety and therapeutic potential of herbal mixture solution to a large extent depends on the source and quality of constituent material used for its formulation. Herbal medicine is increasingly gaining attraction worldwide in recent times and is gradually being incorporated into the core healthcare system (Tahir *et al.* 2022). Exploring the therapeutic potential of a single herbal product is what is mostly and widely reported with little reports on safety and efficacy of combination of mixture of herbal products. Most times, available reports on efficacy or toxicity of herbal products (singly or combined) are silent about the Constituents that are responsible for their action and this poses a major challenge to the understanding of the mechanism of action of herbal products. Just as the pathogenesis of most diseases have been reported to be multi-factorial (Wagner & Ulrich-Merzenich, 2009; Efferth & Koch, 2011), combination of phyto constituents are more potent against diseases than the use of a single compound (Ananta, 2022) possibly because of the synergistic or antagonistic action of the constituents within and/or between herbal mixture solution (Lindsay & Nadja, 2019). This could also be the reason for the untoward outcome that may occur from combination of herbal drug. *Syzygium guineense*, *Allium sativum*, potash and *Syzygium aromaticum* combination is a herbal mixture

solution locally used as an aphrodisiac. Each of these components may possess beneficial or hazardous effect when used singly or combined. *Syzygium guineense* is an evergreen tree that belongs to the family of Myrtaceae. Its health benefits have been widely reported, including being used for the treatment of diabetes (Mira *et al.*, 2022), hypertension (Melese *et al.*, 2021), sleep disorders and anemia (Nguyen *et al.*, 2016). *Allium sativum* (Garlic) is one of the most researched spices used as medicinal herb. Its curative potentials have been attributed to combinations of variety of active chemicals that work in synergy. Allinase, an enzyme, alliin (S-allylcysteine sulfoxide)- a sulfur-containing compound, and allicin (a product of enzyme action on alliin) are some of the active ingredients in garlic (Sunanta *et al.*, 2023). Its therapeutic potentials on many diseases which include; hypercholesterolemia, diabetes, hypertension, rheumatoid arthritis etc had been reported (Sunanta *et al.*, 2023). Potash known in Igbo Language as "Akanwa" or "Kanwa" in Hausa and "Kaun" in Yoruba Language is a dry, hydrated lake salt commonly used in Nigeria. Theophilus *et al.* (2019) reported its usage as a tenderizing agent in addition to many other health-promoting potential. *Syzygium aromaticum* commonly known as clove is a spice that is used locally for preservation of food and also known to contain many phytochemicals that possess pharmacological potential

(Gaber *et al.*, 2020). Erectile dysfunction (ED) is the inability to have or sustained erection during sexual intercourse. Disorders of the endocrine system such as diabetes mellitus, hypogonadism and cardiovascular disorders (ischemic heart disease, peripheral vascular disease and hypertension) are part of the key factors of ED. Nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) has been revealed to be the mediators of penile erection. NO activates guanylyl cyclase that lead to increased cGMP synthesis. The NO/cGMP signaling pathway regulates myosin light-chain kinase/phosphatase/intra-cellular calcium which enhances penile erection (smooth muscle relaxation) (Doaa *et al.*, 2023). Increased production of reactive oxygen species induced by hyperglycemia has been noted to contribute to impairment in endothelium-dependent vascular relaxation by the inactivation of nitric oxide (Zahra *et al.*, 2023). It is expected that proper maintenance of blood glucose and lipid profile could help lower some risk factors of erectile dysfunction since hyperglycemia and hyperlipidemia are predisposing factors to the risk factors of ED. This study therefore examined the effect of *Syzygium guineense*, *Allium sativum*, potash and *Syzygium aromaticum* mixture on fasting blood glucose level and lipid profile.

2.0 Materials and Methods

2.1 Collection and Identification of Plant Sample

The roots of *Syzygium guineense* were collected in Dogonruwa Village in Katsina State. Fresh spices (garlic and cloves) and potash were purchased from Dutsin-Ma Central Market, Katsina State, all in the month of October, 2021. The plant samples were identified at the herbarium unit in the Department of Biological Sciences, Federal University Dutsin-Ma, Katsina, Nigeria.

2.2 Preparation of Plant Sample

The root of *Syzygium guineense* was cleaned, shade dried and ground into powder form using mortar and pestle. Garlic and clove were cut to smaller size, dried under shade and ground to powder using mortar and pestle. The powdered plants samples (*Syzygium guineense*, garlic, and clove) and the potash were weighed (in the ratio of 1:1:1:0.1 respectively) for extraction by decoction. The weighed samples were boiled at 100°C for 1 hour with hot plate. The mixture was filtered using cheese cloth and filter paper. The solution was concentrated with water bath at 50 -55°C. The slurry obtained was further dried in a desiccator. Characterization of the plant extract was done using Fourier Transform infrared (FTIR) spectroscopy machine (Agilent-Cary 630 FTIR).

2.3 Experimental Animals

Wistar rats of either sex (male (30) and female (6)) weighing 160 – 250 g were purchased from a breeder in Zaria, Kaduna State, Nigeria. The animals were kept in

the animal house, at a temperature between 25-30°C with a 12-hour natural light and dark cycle. The animals were domiciled in a well-ventilated woody cage and had free access to standard commercial diet and water. The animals were marked for identification. All experiments were performed according to the principles for laboratory animal use and care as contained in the European Convention on Animal Care Guidelines and Protocol. This work was approved by the Departmental Ethics and Animal Welfare Committee of our institution, in a letter with reference no: BCHEAWC-12/10/2021.

2.4 Experimental Design

Thirty (30) *Wistar* albino male rats were assigned into five (5) groups of six rats per group. The plant extract was administered orally with the aid of a gavage for 2 weeks as follows:

Group 1: Normal control (NC), fed with normal feed and water.

Group 2: Positive control (PC), was given standard drug-Sildenafil Citrate (5mg/kg).

Group3: Test group, administered 100 mg/kg of plant extract

Group 4: Test group, administered 200 mg/kg of plant extract

Group 5: Test group, administered 400 mg/kg of plant extract

2.5 LD₅₀ Determination

The female rats (n-6) were used for the determination of LD₅₀ using the Up and down procedure method (OECD, 2022). In this procedure, 2 rats in each group were sequentially administered 1000, 2000, and 5000 mg/kg. Each group was monitored for 48hrs for mortality before the administration of the next dose.

2.6 Body weight

The body weights of the animals were measured on weekly basis after basal value determination using weighing balance.

2.7 Fasting Blood glucose

The fasting blood glucose of the animals were measured on weekly basis after basal value determination using glucometer as described previously (Osibemhe *et al.*, 2020)

2.8 Measurement of Biochemical Parameters

2.8.1 Assay of Lipid Profile

Serum levels of lipid profile (Total cholesterol (TCHOL), Triglyceride (TG), High density lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C)) were determined by routine laboratory methods(kits) using asemi-auto analyzer.

2.9 Statistical Analysis

Data were presented as means \pm SEM(n=6). Statistical significant differences among groups were analyzed by one-way ANOVA followed by Duncan's multiple range tests. Statistical significant difference was considered at $p < 0.05$.

3.0 RESULTS

3.1 FTIR Characterization

The results of the FTIR characterization of the mixture of the plant extract solution revealed 4 spectra in the fingerprint and functional group regions. Each spectrum is a reflection of the functional groups present in the mixture as shown in Fig. 1 and Table 1.

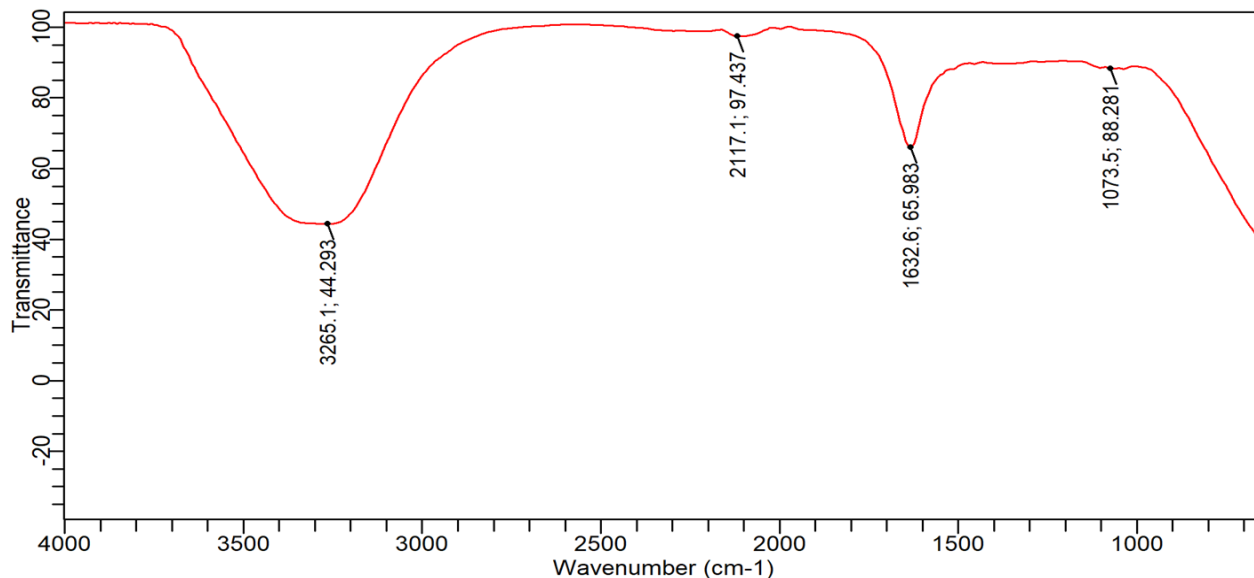


Fig. 1: FTIR Spectra of mixture of plant extract

Table 1: FTIR bands in the spectra of the examined mixture of plant extract

| Peak Number | Wavenumber (cm ⁻¹) | Intensity |
|-------------|--------------------------------|-----------|
| 1 | 1073.47330 | 88.28078 |
| 2 | 1632.57397 | 65.98296 |
| 3 | 2117.12789 | 97.43665 |
| 4 | 3265.14795 | 44.29299 |

3.2 Acute Toxicity Study

The results of the acute toxicity assay are shown in Table 2. No animal died from the administered dose of extract within the 48hrs of observation period. However, some of the notable signs of toxicity observed include sedation, decreased locomotion and feed intake.

Table 2: Acute toxicity using up and down procedure

| Mean Body Weight (g) | Number rats per group | Dose (mg/kg) | Ratio of dead rats |
|----------------------|-----------------------|--------------|--------------------|
| 173 | 2 | 1000 | 0/2* |
| 170 | 2 | 2000 | 0/2 |
| 169 | 2 | 5000 | 0/2 |

*Number of dead animals/ number of animals used. LD₅₀ was above 5000 mg/kg.

3.3 Body weight

Table 3 shows the body weight results. Except 400 mg/kg that had a reduction in body weight in the second week, no significant change was observed in all other groups.

Table 3: Body weight (g) of animals administered mixture of plant extract solution

| Group | Basal | Week 1 | Week 2 |
|-------|-------------------------------|-------------------------------|-------------------------------|
| NC | 205.2 \pm 4.31 ^a | 200.0 \pm 6.02 ^a | 202.6 \pm 3.91 ^a |
| PC | 237.8 \pm 5.15 ^a | 235.4 \pm 7.53 ^a | 235.4 \pm 4.30 ^a |

| | | | |
|-----------|---------------------------|---------------------------|---------------------------|
| 100 mg/kg | 252.0 ± 0.95 ^a | 251.0 ± 1.07 ^a | 241.0 ± 2.73 ^a |
| 200 mg/kg | 232.2 ± 2.84 ^a | 235.0 ± 4.75 ^a | 229.6 ± 3.76 ^a |
| 400 mg/kg | 258.0 ± 2.06 ^a | 256.0 ± 2.12 ^a | 240.6 ± 4.51 ^b |

Data were presented as means ± SEM (n=6). Values in the same row with same superscript alphabet represent no significant difference (p>0.05). NC= Normal control, PC= Positive control.

3.4 Fasting blood glucose

As shown in Table 4, no significant change was observed in the fasting blood glucose results.

Table 4: Fasting blood glucose (mmol/L) of animals administered mixture of plant extract solution

| Group | Basal | Week 1 | Week 2 |
|-----------|--------------------------|--------------------------|--------------------------|
| NC | 6.54 ± 0.25 ^a | 6.94 ± 0.34 ^a | 6.34 ± 0.16 ^a |
| PC | 6.44 ± 0.16 ^a | 6.78 ± 0.30 ^a | 6.28 ± 0.11 ^a |
| 100 mg/kg | 6.60 ± 0.30 ^a | 6.38 ± 0.23 ^a | 6.44 ± 0.28 ^a |
| 200 mg/kg | 6.22 ± 0.13 ^a | 6.04 ± 0.18 ^a | 6.06 ± 0.15 ^a |
| 400 mg/kg | 6.84 ± 0.26 ^a | 6.64 ± 0.25 ^a | 6.38 ± 0.09 ^a |

Data were presented as means ± SEM (n=6). Values in the same row with same superscript alphabet represent no significant difference (p>0.05). NC= Normal control, PC= Positive control.

3.5 Lipid profile

Lipid profile levels were not altered significantly by administration of the extract and the standard drug (Table 5).

Table 5: Lipid Profile (mg/dl) of animals administered mixture of plant extract solution

| Group | TCHOL-C | TG | HDL-C | LDL-C |
|-----------|---------------------------|---------------------------|---------------------------|---------------------------|
| NC | 97.60 ± 2.54 ^a | 75.8 ± 3.17 ^a | 25.20 ± 1.39 ^a | 77.40 ± 1.72 ^a |
| PC | 95.20 ± 3.01 ^a | 63.60 ± 1.17 ^a | 24.40 ± 0.51 ^a | 77.60 ± 2.80 ^a |
| 100 mg/kg | 93.40 ± 6.23 ^a | 71.20 ± 3.28 ^a | 27.00 ± 2.35 ^a | 73.60 ± 4.08 ^a |
| 200 mg/kg | 92.40 ± 6.37 ^a | 62.40 ± 3.92 ^a | 23.20 ± 3.18 ^a | 75.00 ± 4.89 ^a |
| 400 mg/kg | 93.20 ± 3.07 ^a | 70.00 ± 4.34 ^a | 24.00 ± 1.18 ^a | 74.40 ± 2.02 ^a |

Data were presented as means ± SEM (n=6). Values in the same column with same superscript alphabet represent no significant difference (p>0.05). NC= Normal control, PC= Positive control.

4.0 DISCUSSION

Hyperglycemia-induced oxidative stress is believed to be the major cause of endothelial dysfunction. hyperlipidemia is also noted to cause oxidative stress via a mechanism similar to hyperglycemia. This is because the acetyl CoA derived from free fatty acid (FFA) oxidation as well as that from β-oxidation of fatty acids, generates the same electron donors (NADH and FADH₂) via tricarboxylic acid cycle (TCA) (Brownlee, 2005). Transfer of electrons from these electron donors in the electron transport chain under abnormal blood metabolites (glucose and FFA) causes a block at complex III and subsequent backup of the electron at coenzyme Q from where they are donated one at a time to molecular oxygen leading to the production of superoxide (Brownlee, 2005). Endothelial dysfunctions imply means impairment in the endothelium-dependent vasodilatations caused by an imbalance between vasodilating and vasoconstricting substances produced by the endothelium or acts on the endothelium (Sun, *et al.*, 2020). Nitric Oxide is an example of a substance produced in the endothelium and also plays a significant role in it. Hyperglycemia-induced oxidative stress disrupts endothelial function by reducing the activity of the enzyme; endothelial nitric oxide synthase (eNOS)

(Brownlee, 2005). Endothelial dysfunction is associated with erectile dysfunction and vascular diseases. Penile erection is described as the relaxation of smooth muscle in the corpus cavernosum. Nitric oxide (NO) is noted to be the main mediator of smooth muscle relaxation which acts by increasing the cellular level of cGMP (Wareham & Calkins, 2020). Drugs with therapeutic effects on endothelial dysfunction may contribute to the effective management of erectile dysfunction (ED) (Shindel *et al.*, 2008). The potency of herbal drugs is inherent in the active compounds contained in them. These compounds, mostly act as antioxidants and some of them could be dangerous especially if consumed in excess. FTIR spectroscopy is a device that can be used to characterize active ingredients present in plants based on functional groups revealed in the spectra. In this study, the observed 4 spectra in the mixture of plant solution indicate that the solution contains simple organic/ inorganic compounds or small molecular weight compounds (Nandiyanto, *et al.*, 2019). The broadband noted at 32651 cm⁻¹ indicates the presence of OH-stretching which portend possible presence of polyphenol (Nandiyanto, *et al.*, 2019; Bello, *et al.*, 2022). Still in the functional group region, the bands: 2117 cm⁻¹ and 16326 cm⁻¹ may be attributed respectively to triple bond (C≡C) and double bond or carbonyl bond (C=O) presence. These bonds may

indicate presence of aromatic ring deformation that could be linked to the presence of polyphenols. The band 1073cm^{-1} present at the fingerprint region may connote C-O stretching which may mean the presence of aromatic ring vibration (Nandiyanto, *et al.*, 2019; Bello, *et al.*, 2022). Inadequate data on the safety and dosage of herbal preparations is one of the factors contributing to their low patronage most especially by the elites. The above 5000 mg/kg LD₅₀ observed in this study indicates that the herbal mixture is practically non-toxic (Edwige, *et al.*, 2020). Pang-Kuei *et al.* (2021) have reported LD₅₀ above 5000 mg/kg in rats in their examined plant extract. Female rats are believed to be generally sensitive to the toxic effect of chemicals or drugs and their usage in determining toxicity of drugs suffices as against using both sexes of animals (OECD, 2022). Hence, the use of female rats in this study for LD₅₀ determination is justifiable. Body weight changes can be used as an index of assessment of the general health status of animals (El Hilaly *et al.*, 2004). The nonsignificant change in body weight at lower doses may imply that the extract did not influence the feed utilization; however, 400 mg/kg at week 2 may have influenced the feed utilization of the animals which must have contributed to the increased weight observed. Hyperglycemia and hyperlipidemia are predisposing factors of erectile dysfunction. This is so because, the superoxide derived from the oxidation of these abnormal metabolites could initiate processes that may lead to endothelial dysfunction. The nonsignificant change in fasting blood glucose and assayed lipid profile indicate that the herbal mixture was able to maintain homeostasis of these metabolites. This effect may not be unconnected with the presence of polyphenolic compounds in the herbal mixture. Oksana & Chin-Kun (2022) have reported the potential of polyphenols to maintain glucose homeostasis through diverse mechanisms in the liver, muscle, adipocytes, and pancreatic β -cells.

5.0 CONCLUSION

The findings from this study have revealed the presence of phenolic and polyphenolic compounds in the herbal mixture. The data also showed that the synergistic actions of the constituent of the herbal mixture are not deleterious and will not produce abnormal metabolites believed to be predisposing factors to erectile dysfunction. Hence, the local usage of the herbal mixture as an aphrodisiac may be considered for scientific validation.

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CONFLICT OF INTEREST:

The authors have no conflict of interest regarding the manuscript.

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