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In-vitro Antidiabetic Assessment of Bioactive Phytochemicals in Locally used Mango and Soursop Leaves.

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Abstract
Diabetes mellitus is a chronic metabolic disorder that is characterized by prolonged hyperglycemia. Some plant-based herbal formulations have been reported to lower blood sugar levels and control diabetic complications. Harnessing the inherent potentials in plants phytochemicals for specific health therapy remains a challenge. In this study, and using cold maceration and gravimetric methods for extraction, we isolated, identified and characterized some bioactive phytochemicals in Mangifera indica (mango leaf) and Annona muricata (soursop leaf). The phytochemicals were further screened and analysed using qualitative and quantitative techniques. The results of the both samples revealed the presence of alkaloids, flavonoids, tannins, phenols, terpenoids and saponins with 15.62% flavonoids and 20.50% saponin yield respectively for mango leaf (MGL) and soursop leaves (SSL); followed by phenol and tannin then alkaloids and terpenoids that gave the least yield. The antidiabetic potentials of six (6) selected phytochemicals were also investigated via alpha amylase inhibitory assay and glucose uptake capacity of yeast cells. Our data revealed impressive alpha amylase inhibitory activity and glucose uptake across the plasma membrane of the yeast cells, with Flavonoids, saponin and terpenoids fractions of the samples possessing the highest alpha amylase inhibitory activity and glucose uptake capacity. It is our conclusion, therefore, that Mangifera indica (mango leaf) and Annona muricata (soursop leaf) ethanolic extract posses anti diabetic potentials by inhibiting alpha amylase and increasing glucose uptake within the cell.

Key words: *Mango leaf, Soursop leaf, Phytochemical, Antidiabetic, Extracts and Spectra.*

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I. Introduction

Diabetes has become a healthcare emergency; it is a chronic metabolic disorder that is characterized by prolonged hyperglycemia (Samuel C. Iwuji, Sixtus A. Okafor and Chioma C. Okey-Mbata 2020), due to the pancreas not producing sufficient insulin or the body cells not being able to respond adequately to the insulin secreted or both, resulting in defective carbohydrate, protein and fat metabolism (Muruli 2020). It is associated with hyperglycemic emergencies (Sixtus et al., 2022), retinopathies, nephropathies, cardiopathies, stroke as well as amputations of the lower limb. Almost one in ten persons has been projected to be living with diabetes by 2050. Diabetes complications have been implicated as the leading cause of diabetic mortality and morbidity (Samuel C. Iwuji, Sixtus A. Okafor and Chioma C. Okey-Mbata 2020).

Herbal decoction, maceration, infusion and juice extracts of the stem, leaves, bark and seeds of plants have been recommended for the management of diseases (Samuel Iwuji et al., 2021 and Murul, 2020). Mango and soursop leaves extracts possess phytochemicals which include Alkaloids, Flavonoids, Glycosides, Polysaccharides, Sterols, Peptidoglycans, Amino acids and their derivatives, Saponins, Terpenoids, Carotenoids (Ardalani et al., 2021 and Tran et al., 2020 and Ogbonna et al., 2023), The use of most of these bioactive molecules to manage diseases including diabetes mellitus and as anti microbial (Okafor et al., 2021) have been reported. Some have been implicated in the reduction of blood sugar levels as they are thought to promote glycemic control, induce weight loss, and glucose uptake (Kumar et al., 2021).

The use of herbal mixtures for the management of diabetes and its complication is on the increase (Vasilyev 2007, Ismail et al., 2020, Abdel-karim et al., 2021 and Lattibeaudiere 2022). This is thought to be as a result of the high cost of conventional diabetes medication, cheap, availability and fewer side effects of the herbal therapy. (Verma et al., 2018). Plants with antimicrobial and antidiabetic properties are known to thrive (Okafor et al., 2022) and are well distributed across geographical locations, are not impeded by the various climatic conditions (Mahammed et al., 2015) and are usually consumed as food, spices or food supplements.

Therefore, to reduce the disease burden and its associated complications, achieve better glycemic control, reduce cardiovascular and oxidative stress, and enhance therapeutic efficacy: there is the need to standardize locally available phyto-compounds in herbal plants formulation for the management of diabetes and its complications.

II. MATERIALS AND METHODS

2.0 Materials and Methods

2.1 Plant Specimen

The plant materials used are: Mangifera indica (Mango leaf) and Annona muricata (Soursop leaf),

2.2 Reagents

Reagent used include Alpha amylase (Molychem), Ethanol (Emsure), Methanol (Sigma-Alorich), Sodium Chloride (JHD), Petroleum Ether (JHD), Ammonium Hydroxide (JHD), n-butanol, n-hexane, Folin-Denis reagent, standard tannic acid, DPPH, Potassium ferrocyanide, H2SO4, Sodium phosphate, Ammonium molybdate, Starch azure, Sodium acetate buffer, Cacl2 and Trichloroacetic acid (TCA), Acetone, Ammonium sulphate (NH4)2SO4 all were sourced from ECCL England, Activated charcoal and Acetic acid.

2.3 Plant Specimen Preparation

We collected 200g each leaf samples from the Botanical garden, Crop Science Department Federal University of Technology Owerri. They were identified by a Botanist, washed under tap water and air-dried at normal room temperature for 4 weeks, then pulverized into powder using an electric blender and stored in airtight ziplock samples bags to avoid moisture and contamination.

2.4 Extraction of Phytochemicals

Using 95% ethanol in a sample to solvent ratio of 1:6 (w/v), we extracted the phytochemicals via crude ethanolic extraction and the extract stored in a well labeled air-tight specimen bottle. The powder was soaked at room temperature and allowed to stand for 3days, with occasional agitation to ensure complete extraction, followed by filtration using muslin cloth folded into two. The extract was concentrated under vacuum in a rotary evaporator with the heating bath set at 45^0C.

2.5 Phytochemical Analysis

Phytochemical analysis and screening of the ethanolic extracts were carried out according to (Roghini & Vijayalakshmi 2018), Alkaloids and Saponin content were determined according to (Roghini & Vijayalakshmi 2018), Terpenoid content determined according to (Azeemi 2017), Flavonoid determined by the method reported by Boham and Kocipai in (Ezeonu & Ejikeme 2016), Total phenolic contents determined according to the method described by (Phuyal et al., 2020) and Tannin content determined as described by Pearson (Bukola, Catherine & Gabriel 2018).'

2.6 Antioxidant Assay

Antioxidant assay of the crude ethanolic extracts was carried out using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, Ferric Reducing Antioxidant Power (FRAP) Assay and Total Antioxidant Capacity Assay (TAC) as described by (Rahman et al., 2015).

2.7 Antidiabetic Assay (Alpha Amylase Inhibitory Assay)

Antidiabetic assay of some selected phytochemicals was conducted using alpha amylase inhibitory assay according to the method of Hirasawa (De Morais & Takaki 1998 and Lehoczki et al., 2018)

2.8 Glucose Uptake Assay

Glucose uptake capacity of yeast cells was carried out according to the method described by Cirillo (Rehman et al., 2018).

III. RESULTS

The results of the phytochemical screening analysis of the crude ethanolic extracts of Annona muricata (Soursop leaf) labeled as SSL and Mangifera indica (Mango leaf) labeled as MGL, revealed abundance of alkaloids, flavonoids, tannins, phenols, terpenoids, and saponins.

Table 1: Qualitative analysis of ethanolic extract of Mango leaf (MGL) and soursop leaf (SSL). Tannins, Carbohydrates, Alkaloids, Saponins, Flavonoids, Quinones, Glycosides, Phenols, Terpenoids, Cardiac glycosides, Ninhydrins, Coumarins, Anthraquinones and Steroids were screened.

S/N	Extract	Tan	Cab	Alk	Sap	Fla	Qui	Gly	Phe	Ter	Cd	Nin	Cou	Anq	Ste
1	SS	+++	+++	++	++	++	-	+	+++	+++	-	-	+++	-	++
2	MG	+++	+++	+++	+++	+++	-	+	+++	++	-	-	++	-	++

Ethanolic extract of powdered leaf samples of MGL (Mango leaf) and SSL (Soursop leaf) by cold maceration yielded 15.62% and 20.50% respectively, which shows that soursop leaves produced a higher yield than Mango leaves.

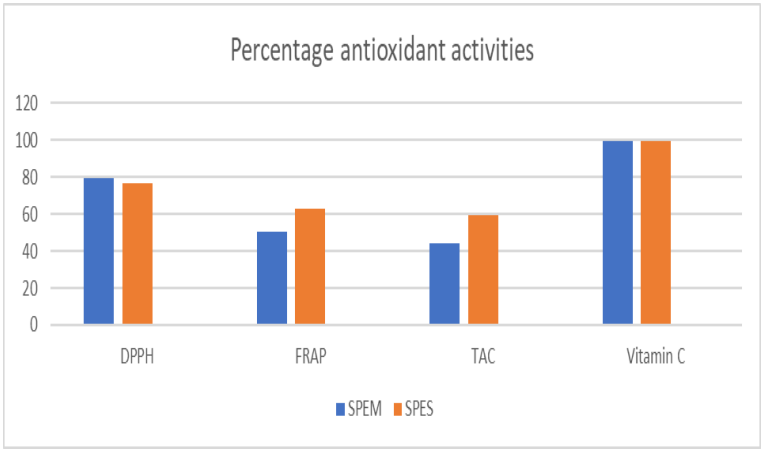

Figure 1: Percentage Antioxidant Activities (DPPH, FRAP and TAC assay results) revealed; for DPPH 79.66% / 76.95%, for FRAP 50.40% / 63.10%, TAC 44.40% / 59.10% and standard sample vitamin C 99.5% / 99.6% for MGL /SSL respectively.

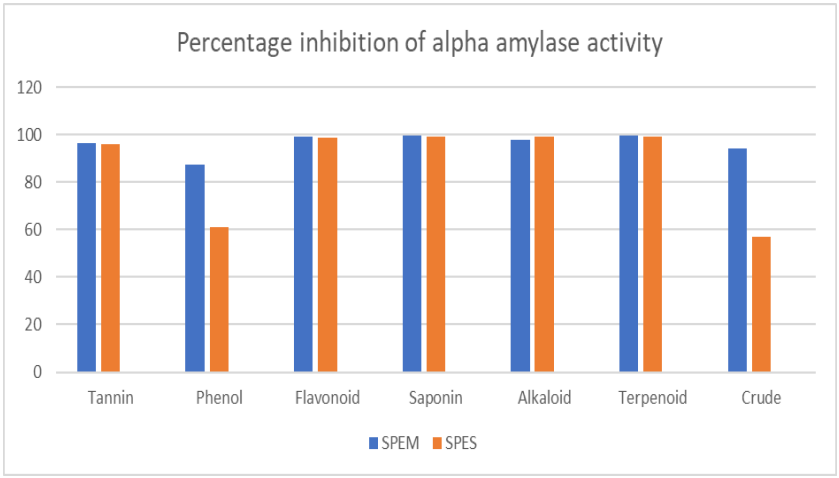

Figure 2: Percentage Inhibition Alpha Amylase Activities of crude ethanolic extracts of MGL/ SSL as well as the selected antidiabetic phytochemicals (tannin, phenol, flavonoid, saponin, alkaloid and terpenoid).

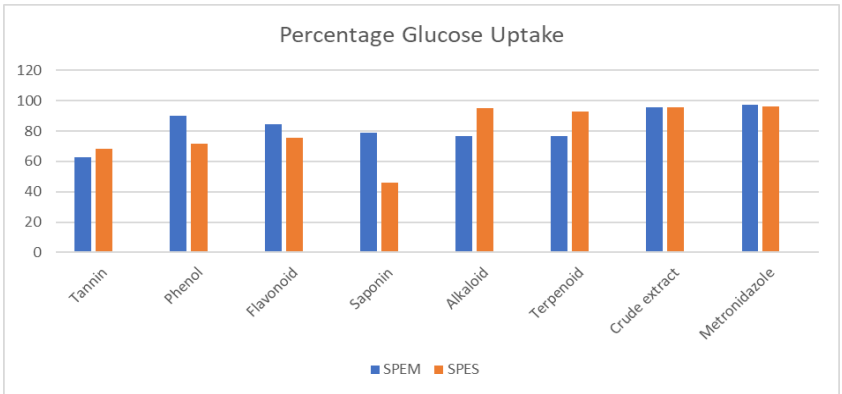

Figure 3: Percentage Glucose Uptake capacity of yeast cells on crude ethanolic extracts of MGL/ SSL as well as the selected antidiabetic phytochemicals (tannin, phenol, flavonoid, saponin, alkaloid and terpenoid) and standard drug metronidazole

IV. DISCUSSION

In this study, the ethanolic extracts of MGL, SSL were screened to identify the phytochemicals present in the extract and based on the colour intensities of each phytochemical components screened, six phytochemicals; alkaloids, flavonoids, tannins, phenols, terpenoids, and saponins were selected for further study. The result collaborated the findings of (Usunobun et al., 2014, Agu & Okolie 2017 and Uniyal & Rahal 2022) but at slight variance with (Nwaehujor et al., 2019) for Annona muricata (Soursop leaf) [See Table 1].

Also, the quantitative analysis results of MGL and SSL samples collaborated the findings of (Chitra 2021 and Kazi et al., 2019) for Mangifera indica sample and (Nguyen, 2020; Nwaehujor et al., 2019) for Annona muricata sample, showing high flavonoids, saponins, tannins and phenol contents. But the result of the crude ethanolic yield was similar to the observations of (Aquisman et al., 2021) for mango leaves using 75% ethanol but slightly higher than that obtained by (Suhendar 2019) for soursop in microwave assisted extraction (MAE) using 70% ethanol [See Table 1].

However, antioxidant assay which was carried out to establish the free radicals scavenging ability and hydrogen atom donating ability of the samples provided an insight into their in-vitro response to oxidative stress. DPPH, FRAP and TAC assay results for MGL and SSL revealed high percentage antioxidant activities [See Figure 1], collaborating (Das et al., 2014, Orak et al., 2019, Qorina et al., 2019 and Udem et al., 2018), though not comparable to the standard sample vitamin C. They were lower in comparison to (Hasmila et al., 2019 and Ibrahim et al., 2020) on Mangifera indica and higher for Annona muricata.

The antidiabetic activity guided search for potent phytochemicals were carried out on the six (6) selected phytochemicals through alpha amylase inhibitory assay and glucose uptake capacity of yeast cells. The crude extracts of the selected phytochemicals promoted glucose uptake across the plasma membrane of the yeast cell with more pronounced effect on the crude extracts of both MGL and SSL samples, followed by alkaloid and terpenoid of SSL (though, they both had very low yield) and phenol and flavonoid of MGL. Moreso, saponin of SSL gave the lowest glucose uptake followed by tannin of both MGL and SSL samples [See Figure 3]. However, the glucose uptake across the yeast cells were observed not to increase according to dose i.e. not dose dependent and this was consistent with (Pitchaipillai & Ponniah 2017) but in contrast with (Pulivarthi et al., 2020).

Our data, however, showed that our samples have a potent alpha amylase inhibitory activity [See Figure 2], which was high than that reported by (Kulkarni & Rathod 2018 and Francis & Amos 2021). The flavonoid and saponin fractions of MGL gave high alpha amylase inhibitory activities of 99.06% (for flavonoid) and 99.68% (for saponin). Moreso, the flavonoid and saponin fractions of SSL revealed an alpha amylase inhibitory activity of 98.75% and 99.16% respectively.

V. Conclusion

We conclude that, mango and soursop leave extract should be incorporated in herbal formulations for the management of diabetes mellitus and its complications.

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