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Antioxidant and Antidiabetic Activities of Ethanolic Leaf Extract of *Melochia corchorifolia*

[1]Ya'u, A., [2]Ayo R. G. and [1]Bello Oluwasesan M.

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1. Department of Chemistry, Federal University Dutsin-Ma, P.M.B 5001, Katsina State,
2. Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

**Corresponding Author:*
Yau Abubakar
yabubakar@fudutsinma.edu.ng

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Abstract

Polyphenolic extract of Melochia corchorifolia leaf has demonstrated high antioxidative activity and very promising anti-diabetic activities in vitro. The antioxidant and antidiabetic inhibition properties of the polyphenolic-rich extracts were also studied in this study and it revealed that the plant extract showed better activities than the conventional antidiabetic drug acarbose. The biological activity of Melochia corchorifolia leaf extracts was investigated. The maximum inhibition for the DPPH inhibition assay of Melochia corchorifolia was recorded at 10 µg/ml, where the scavenging activity of the control drug (Ascorbic acid) was found to be better than that of the plant extract at all dosages except for 20 µg/ml and 50 µg/ml, where Melochia corchorifolia showed better activity than the control. The ABTS inhibition assay result showed that the activity of the extract and the control is dose-dependent. The ethanolic leaf extract of Melochia corchorifolia demonstrated significant antioxidant (DPPH IC_{50}: 66.63 µg/mL; ABTS: 87.37 mM TE/g) and antidiabetic (α-amylase inhibition: 68.62 %) activities, surpassing acarbose in α-glucosidase inhibition ($p < 0.05$). The activity increases with an increase in the dosage. The best activity was recorded at the lowest dosage i.e., 10 µg/ml, where the plant extract inhibition activity was (6.474) at the same dosage against (27.394) of the positive control. This plant may hold the potential against diabetes.

Keywords: *Melochia corchorifolia*; Ascorbic acid; anti-diabetic and antioxidant

Introduction

The occurrence of bioactive plant components, mainly called phytochemicals, has been considered of fundamental nutritional importance in the prevention of many diseases such as cancer, cardiovascular disease, and diabetes (Aruoma, 2003). It has been discovered that regular utilization of fruits, vegetables, herbs, and spices has been associated with health benefits for humans and animals. But not until recently, these valuable compounds (phytochemistry and biological activity) were discovered to possess a wide range of biological activity (Sheetal and Jamuna, 2009). These biological activities of plants help in health benefits beyond basic nutrition (Oomah and Mazza, 2008).

Hence, biological activities studies of medicinal plants that are common in northern Nigeria will go a long way in providing a cheaper way of treating diseases such as jaundice, intestinal worm infection, wounds, malaria, venereal diseases, epilepsy, diarrhea, hemorrhoid, cancer, asthma and fever afflicting substantial number of communities in the region. Natural products are chemical organic substances that are produced by living organisms found in nature through the pathways of secondary and primary metabolisms (Woldeyes *et al.*, 2012).

Natural products have pharmacological activities that can be useful in treating various diseases, and may act as active substances for both modern medicines and traditional medicine (Raafat, 2013). Medicinal plants are plants that have at least one of their parts roots, stems, barks, or leaves, used for therapeutic purposes (*Chinyere et al.,* 2011).

MATERIALS AND METHODS

Chemicals / Reagent

All the chemicals and reagents used in the studies were of analytical grade and obtained from the Federal University Dutsin-Ma, Katsina State, in the Department of Applied Chemistry. Dichloromethane, n-Hexane, ethyl acetate, ethanol, concentrated sulfuric acid, chloroform, 5% acetone, hydrochloric acid, acetic anhydride, distilled water, disinfectant, amongst others were used in the work.

Equipment

Rotatory evaporator (Bibby Scientific Limited, Stone Staffordshire, ST15 OSA.UK), Vacuum pump, water bath, electric shaker. Analytical balance, Top loading balance, Hot water bath and ultraviolet light (254nm & 366nm).

Methods

Collection of Plant Material

During the rainy season in September 2022, the leaves of *Melochia corchorifolia* were collected from Wakaji village in the Dutsin-Ma local government of Katsina state, Nigeria. It was discovered at the Federal University of Dutsin-Ma Katsina, Department of Plant Science. The leaf was cleaned with water and air-dried for two weeks in the laboratory. Pestle and mortar were used to grind the leaf. The powdered samples were kept in clean, airtight containers at room temperature until they were needed.

Extraction of Plant Materials

The powdered sample (1.2 kg) was packed in a speculator and was extracted (Maceration) successively using ethanol (maceration). At the end of the extraction, the extracts obtained were concentrated at 40 ^{o}C using a rotatory evaporator, dried to a constant weight in a pre-weighed petri-dish and then kept in a refrigerator before subsequent analysis.

Antioxidant Activity

1-1-dipheny l–2 picrylhydrazyl free radical scavenging activity (DPPH):

The free radical scavenging ability of the extracts against DPPH (1, 1- diphenyl–2 picrylhydrazyl) free radical was evaluated using a modified method as described by Tuba and Gulcin (2008). Briefly, a 0.3 mm solution of DPPH was prepared in methanol and 500 µL of the DPPH solution was added to 1 mL of the extracts at various concentrations (15–240 µg/mL). These solutions were mixed and incubated in the dark for 30 minutes at room temperature. The absorbance was read at 517 nm against blank samples lacking a scavenger.

Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity of ABTS scavenging activity:

The ABTS scavenging activity of the plant extract was determined using different

methods of Analysis were carried out in triplicate.

Alpha amylase inhibitory activity

The α-amylase inhibitory activity was measured according to (Shai *et al*., 2010) with slight modifications. A volume of 250 µL of each extract or acarbose at different concentrations (100-500 µg/mL) was incubated with 500 µL of porcine pancreatic amylase (2µ/mL) in phosphate buffer (100 mM, pH 6.8) at 37 °C for 20 minutes. Then, 250 µL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) was further added to the reaction mixture and then incubated at 37 °C for 1 hour. Then, dinitro salicylate color reagent (1 mL) was added then left to boil for 10 minutes. The mixture resulted in an absorbance that read at 540nm, and the inhibitory activity was expressed without the inhibitors as a percentage of the control.

Alpha-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was determined by the method described by (Ademiluyi and Oboh, 2013) with slight modifications. Briefly, 250 µL of each extract or acarbose at different concentrations (30-240 µg/mL) was incubated with 500 µL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (100mM, pH 6.8) at 37 °C for 15 minutes. Then, 250 µL of pNPG solution (5 mM) in phosphate buffer (100 mM, pH 6.8) was added and the reaction mixture was further incubated at 37 °C for 20 minutes. The absorbance of the released *p*-nitrophenol was measured at 405 nm and the inhibitory activity was expressed as a percentage of the control without the inhibition. All assays were carried out in triplicate. The inhibitory activities of the extracts on the α-glucosidase and α-amylase were calculated by using the following formula:

Inhibitory activity % = (1− as/Ac) x 100

Where As: is the absorbance in the presence of the sample and

Ac: is the absorbance of the control.

The concentration resulting in inhibition of 50% enzyme activity and scavenging activities (IC50) weas obtained by calculating from the plot of percentage inhibition against log (concentration of the samples).

RESULTS AND DISCUSSION

EXTRACTION OF *Melochia corchorifolia*

Cold maceration extraction was used to extract 1000g of the *M. corchorifolia* using bottles. Hexane was initially used, followed by dichloromethane, and ethanol, respectively. Each of the crude extracts was concentrated by using a rotary evaporator, and the resulting crude extracts were air-dried until constant weights were obtained. The yield of crude methanol extract (0.56%) is the highest among the four samples, whereas the yield of crude dichloromethane extract (0.34 %) is the lowest compared to the other samples.

Yield % = weight of dry extract/weight of dry plant × 100%

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Table 1: Extraction Yield

SOLVENTS	Weight of extract (g)	PERCENTAGE YIELD (%)
n-hexane	4.00	0.4
Dichloromethane	3.60	0.34
Ethanol	6.00	0.56

Biological Activity of *M. corchorifolia*

Table 2: DPPH inhibition assay of Ethanol fraction

Dosage	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml
PFMC	31.67	40.03	46.99	62.12	66.63
ASC	30.07	41.82	51.97	60.81	65.41

Table 2 shows that the activity of the plant extract in DPPH solution is dose-dependent. The positive control used is ascorbic acid, which is the conventional drug against oxidative stress. From the table, maximum inhibition was recorded at 10 µg/ml where the scavenging activity of the control drug (Ascorbic acid) was found to be better than that of the plant extracts at all dosages. *M. corchorifolia* showed better activity than the control. In general, the scavenging activity of both the control and the plant extract increases with a corresponding increase in dosage. The ABTS inhibition assay result showed that the activity of the extract and the control is dose-dependent. The activity increases with a decrease in the concentration. The best activity was recorded at the highest dosage i.e., 150 µg/ml. *M. corchorifolia* has the best activity at (6.474), whose inhibition activity was at the same dosage against (27.39) of the positive control. The plant extracts showed better activity than the control at all concentrations.

Table 3: ABTS Inhibition Assay of Ethanol Fraction

Dosage	*10 µg/ml*	*20 µg/ml*	*50 µg/ml*	*100 µg/ml*	*150 µg/ml*
PFMC	6.47	31.14	56.82	87.37	87.37
ASC	27.39	55.80	78.2 2	86.35	90.10

Table 4: H_2O_2 Inhibition Assay of Ethanol Fraction

Dosage	10 µg/ml	*20 µg/ml*	*50* µg/ml	100 µg/ml	150 µg/ml
PFMC	13.89	24.44	34.28	47.32	52.92

ASC	11.87	26.70	39.47	63.28	74.61

Table 5: α-amylase Inhibition Assay of Ethanol Fraction

Dosage	20 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µf/ml
PFMC	**29.90**	40.52	29.41	21.07	17.15
ASC	**42.48**	58.00	63.88	67.15	68.62

Table 6: α-glucosidase Inhibition Assay of Ethanol Fraction

Dosage	20µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml
PFMC	**22.77**	24.20	24.74	26.52	28.74
ASC	**90.33**	93.27	93.700	93.72	94.03

Table 7: Advanced Glycation End-product assay

Dosage	*M. corchorifolia*
1mg/ml	24.84726

Results from the H_2O_2 inhibition assay showed that the activity of the plant extracts is dose-dependent. This result also follows a similar trend to other antioxidant assays studied in the research. Maximum activity was recorded at the lowest dosage, 150 µg/ml, where the scavenging activity of ascorbic acid was the best. The antidiabetic potential of the leaf extract of *M. corchorifolia was* studied. The result showed that the activity of the plant extracts is dose-dependent. The activity increases with increasing dosage of the extracts. The best activity was recorded at a dosage of 500 µg/ml. Table 6 shows the α-glucosidase activity of *M. corchorifolia.* The table shows that the inhibition activity of *M. corchorifolia* leaves extract is dose-dependent. There is an increase in the activity of both polyphenolic-rich extracts with a corresponding increase in dosage, with optimum activity achieved at a dosage of 500 µg/ml. In general, the activity of M. *Melochia* was the best at the optimum dosage. Table 7 shows the advanced glycation end-products inhibition of *M. corchorifolia,* the plant extract showed activity on the AGE inhibition assay with an activity of 75.1% in *Melochia corchorifolia*.

Antioxidant activity

Oxidative stress and viral disease have long been regarded as major causes of morbidity and mortality in humans. In order to achieve stability, free radicals destroy other molecules by removing electrons from them. The adverse effects of routinely used conventional antioxidants have compelled the quest for natural antioxidants from plant extracts (Tsado *et al.,* 2016). The DPPH radicals were frequently utilized to study the scavenging activity of various natural substances. In a DPPH radical scavenging

assay, ethanol leaf extract of *M. corchorifolia* was found to have a substantial antioxidant effect, with a maximal inhibition of 34.1429 at 10 ug/ml. The decrease in DPPH absorbance generated by *M. Corchorifolia* polyphenolic-rich fraction is due to the reaction between antioxidant molecules and radicals, which results in radical scavenging via hydrogen donation. Natural products contain numerous antioxidant components. Flavonoids are phenolic chemicals that play essential roles in scavenging free radicals and consequently play critical functions in the prevention of oxidative stress-related disorders (Nahak and Sahu, 2010).

This strong scavenging property was related to the presence of hydroxyl groups in the chemical structure of phenolic compounds, which can offer the required components as a radical scavenger. These findings back up previous observations that plant metabolites such as phenol, tannins, and flavonoids have antioxidant and antibacterial activities. The DPPH antioxidant result obtained in this study goes hand in hand with the result obtained by Purushotham *et al.* (2019), where the control (Ascorbic acid) has slightly better activity than that of the plant extract. In another study conducted by Rao *et al.* (2012). *M. corchorifolia* showed good antioxidant activity in DPPH, Peroxide and superoxide assay with IC_{50} values of 179 µg/ml, 240 µg/ml and 127µg/ml, respectively.

The antioxidant activity of plant extracts is measured using the ABTS radical. The radical absorbs at 743nm to produce a bluish green color, and an electron is lost by the nitrogen atom of ABTS, resulting in the production of the radical (Abdulrahman *et al.,* 2021). Both plants employed in this study showed superior activity against the ABTS solution than the control (Ascorbic acid), this may be due to both of them being leaves and having high volume of polyphenol in them and there is a link between polyphenolic content and antioxidant activity, this is in tandem with the finding of Adegbola *et al.,* 2020 where the antioxidant activity of *A. hybridus* was related to polyphenolic chemicals detected in significant concentrations in the plant's methanol leaf extract.

Antidiabetic activity

Diabetes is a chronic condition of carbohydrate, protein, and lipid metabolism caused by an absolute or relative lack of insulin secretion with/without variable degrees of insulin resistance (Barar, 2000). It can also be characterized as a disease in which the body either produces little or no insulin, or grows increasingly resistant to its activity (Ranjan and Ramanujam, 2002). It is now an epidemic, with a global frequency of 5% in the general population. The global adult diabetes population will increase from 135 million in 1995 to 300 million by 2025 (Torben, 2002). Diabetes mellitus is probably the fastest-growing metabolic disease in the world and as knowledge of the heterogeneous nature of the disease increases, so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes, but only a few have been scientifically evaluated (Kumar *et al.,* 2012). In the alpha-amylase assay, *M. Corchorifolia* exhibited excellent inhibition activity, where the activity of the extract increased with increasing

concentration or dosage. This is supported by the findings of research carried out (Vinoth *et al.,* 2021), where *Melochia corchorifolia* inhibition is highly demonstrated in the enzyme at all concentrations. Plant phenolic and flavonoid compounds such as quercetin, ferulic acid, anthocyanins, catechin, and resveratrol were shown in epidemiological studies to regulate glycemia via increased glucose uptake, insulin secretion, and inhibition of lipid peroxidation, alpha-glucosidase, and alpha-amylase (Lin *et al.,* 2016). Studies have demonstrated that flavonols and flavones' enzyme inhibitory capacity depends on hydrogen bonds between the hydroxyl groups of the polyphenol ligands and the catalytic residues of the binding site and formation of a conjugated α-system that stabilizes the interaction with the active site (Xiao *et al.,* 2015). The recent findings show the structure-activity relationship of polyphenols inhibiting α-amylase have concluded that the hydroxylation and galloylation of flavonoids, including catechins, improved the inhibitory effects against α-amylase (Wong *et al.,* 2015). In the α-glucosidase and α-amylase antidabetic assays carried out in this study, the two plant samples compete favourably when compared to that observed for the control drug acarbose employed in this study. To further buttress this claim, Yang *et al.,* (2020) reported that the water extracts of A. gangeticus and A. inamoenus showed high α-glucosidase inhibitory activity (80-50%) at a concentration of 1 mg. Another study by (Mondal *et al.,* 2015), the methanol extract of *Melochia corchorifolia* showed significant α-glucosidase inhibitory activity (IC50 8.49 µM/ml) and that acarbose provides inhibition at IC_{50} values of 15.25 µM/ml.

Conclusion

In conclusion, the polyphenolic extract of *M. corchorifolia* leaves has demonstrated high antioxidative activity and very promising anti-diabetic activities *in vitro* with better activity. The antioxidant and antidiabetic inhibition properties of the polyphenolic-rich extracts were also studied, and it revealed that the plant extract showed better activities than the conventional antidiabetic drug acarbose.

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