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In Vitro Antidiabetic Characterisation of the Combination of *Leptadenia Hastata* (Decne) Pers and *Momordica Balsamina* Linn Leaf Extracts

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Abstract: Diabetes is a multi-factorial disease that requires a multiple therapeutic approach to adequately control the multiple metabolic abnormalities of the disease. The present study evaluated the *in vitro* antioxidant and antidiabetic potentials of the combination (2:1) of the aqueous leaf extracts of *Leptadenia hastata* (LH) and *Momordica balsamina* (MB). The *in vitro* inhibitory effects of the combination on antioxidant indices and key enzymes of carbohydrate hydrolysis and polyol pathway were evaluated and the test extract's potencies were expressed in terms of IC_{50} values. The combination exhibited potent *in vitro* ferric reducing power (IC_{50} 29.28±0.29 µg/ml), scavenging activity (IC_{50} 90.05±25.89 µg/ml), alpha amylase (IC_{50} 15.86±1.03 µg/ml), alpha glucosidase (IC_{50} 330.93±26.47 µg/ml) and aldose reductase (IC_{50} 37.30±2.27 µg/ml) inhibitory potentials when compared to the standard drugs. Findings of the present study demonstrated the anti-diabetic potential of the combination as evidenced by their observed *in vitro* biochemical activities. This provides a background for further studying the antidiabetic and antioxidant potentials of the polyherbal combination in animal models of diabetes.

Keywords: Diabetes mellitus, Antihyperglycemia, Antioxidant, *Leptadenia hastata*, *Momordica balsamina*

INTRODUCTION

Free radicals (FR) play central roles in the pathogenesis of metabolic diseases like diabetes mellitus and its complications (like peripheral neuropathy). An imbalance between the free radical generation system and antioxidant defense pathways causes oxidative damage to DNA, lipids and proteins, leading to altered cellular functions and antioxidants level [1,2]. Oxidative stress has been shown to contribute significantly to the onset and pathogenesis of diabetes as well as in the development of macrovascular, microvascular and neurologic complications of the disease [3, 4]. Scavengers of FR may have an effect on reducing the increased serum glucose level in diabetes and may alleviate diabetes as well as reduce its secondary complications [5]. Several lines of evidence have supported the association of oxidative stress markers with diabetes [6,7]. This has prompted a resurgent interest in traditional plant treatments for diabetes in the recent decades. Plants often contain substantial amounts of antioxidants including α-tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids and tannins and it has been suggested that antioxidant action may be an important property of plant medicines used in diabetes [8].

Leptadenia hastata (Pers.) Decne which belongs to the family Asclepiadaceae is a widely distributed African herb in which all its parts are used for dietary and medicinal purposes [9]. It is popularly known as "Yadiya" (Hausa) in the north western parts of Nigeria and Niger. The leaves of *L. hastata* are more abundant and fresh during rainy season and have been reported to contain phenolic glycosides, tannins, flavonoids, proanthocyanidins, alkaloids and saponins [10-12]. Bello et al. [11] reported the total phenolic, total flavonoid

and proanthocyanidin contents of *L. hastata* leaves to be in the ranges of 17-38, 10-16 and 4-10 mg/g respectively depending on the extraction solvent. Ethnobotanic survey with the traditional healers revealed that the consumption of the leaf stems and leaf extracts of *L. hastata* could be used in the management of diabetes mellitus and treatment of stomach upset [10]. The aqueous and methanolic extracts (300mg/kg body weight) of *L. hastata* were reported to possess hypoglycemic, hypolipidemic and alpha glucosidase inhibitiory effects in normal, alloxan-induced diabetic rats and in *In vitro* studies [11].

M. balsamina Linn, popularly known as "Balsam apple" (English) and "Garaffini" (Hausa) is a tendril-bearing wild climber, well-known for its bitter taste due to the presence of phytochemical alkaloid and possess diverse biological and pharmacological functions. It has been used as a traditional folk medicine in many countries especially in Asia, Latin America and Africa. *M. balsamina* contains several cucurbitane-type triterpenoids which were isolated from different parts including balsaminapentaol, balsaminol A, balsaminol B, cucurbalsaminol A, cucurbalsaminol B [13]. Bhardwaj *et al.* [14] have reported the leaves, fruits, seeds, and bark of the plant to contain resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside, saponins, each having various medicinal importance. Zhang *et al.* [15] identified the leaves of momordica species as an important source of triterpenoids. Traditionally, in Northern Nigeria postnatal mothers eat the leaves of *M. balsamina* mixed with porridge to stimulate milk production and sometimes with ground groundnut cake.

The activity based review of *M. balsamina* indicated that it possesses activities like antimicrobial, antispasmodic, anti-inflammatory, analgesic, anti-HIV, anti-diahorrial, hepatoprotective, anti-malarial, antioxidant, anticancer and wound healing properties [16,17]. The alpha glucosidase inhibitory effect and antioxidant effect *M. balsamina* alone and as an ingredient in a polyherbal formulation have been demonstrated by many studies [18,19]. The potential antioxidant activity of *M. balsamina* has been reported and its antioxidant components were identified as polyphenols, inhibiting 5-lipo-oxygenase enzyme that is responsible for inflammation [20]. *In vitro* studies on the antidiabetic potentials of *L. hastata* and *M. balsamina* alone and in combination is scarce. The present study therefore evaluated the *in vitro* antioxidant activity and anti-hyperglycemic potentials of the combination of the aqueous leaf extracts of *L. hastata* and *M. balsamina* using some selected antidiabetic biomarkers.

Methods

Preparation of aqueous leaf extract and Biochemical analysis

The fresh leaves of *L. hastata* and *M. balsamina* were collected in March, 2013 from Kumbotso Local Government Area of Kano State. Voucher specimens *L. hastata* (No.900220) and *M. balsamina* (No.1139) were submitted at the Herbarium unit of Biological Science Department, Ahmadu Bello University, Zaria. The leaves were dried under the shade and powdered using mortar and pestle. The powdered leaves (500 g) were extracted with water using maceration method. The resulting aqueous extracts of *L. hastata* (LH) and *M. balsamina* (MB) were stored at 4˚C till required.

The ability of the combination of the aqueous leaf extracts of *L. hastata* and *M. balsamina* to scavenge free radicals was assayed using the synthetic free radical compound 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in accordance to the method described by Chen *et al.* [38]. The reducing potential of the combination of *L. hastata* and *M. balsamina* was determined according to the method described by Kumar and Hemalatha [21] where substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Alpha Amylase inhibitory activity of the combination of *L. hastata* and *M. balsamina* was determined as described by McCue and Shetty [22] wherein the reducing groups released from starch are measured by the reduction of 3,5-dinitrosalicylic acid under the specified conditions. Crude alpha amylase from rat pancreas prepared as described by Chougale *et al.* [23] was used as the crude enzyme source. Alpha glucosidase activity of the combination of *L. hastata* and *M. balsamina* was determined in accordance to the method described by Kim *et al.* [24] using p-nitrophenyl-α-d gluco pyranoside as substrate under specified assay conditions. Crude alpha glucosidase enzyme prepared from rat small intestine was prepared using the method described by Kim *et al.* [25]. Aldose Reductase activity was assayed spectrophotometrically by measuring the decrease in the absorbance of NADPH at 340 nm over a 4-minute period in accordance to the method of Hayman and Kinoshita [26] with some modifications [39,40] and crude rat lens aldose reductase (rAR) was used as enzyme source.

Statistical Analysis

The potency of the combination of LH and MB was expressed in terms of IC_{50} values. Paired T test compares the level of significance using the GraphPad Instat3 statistical software for windows 2006. Values were considered significant when $P < 0.05$.

Results and Discussion

The combination of the aqueous leaf extracts of LH and MB and ascorbic acid showed scavenging activity against DPPH free radical in a concentration dependent manner with the maximum inhibitory activities observed at the highest extract/drug concentration (Figure 1). Based on their IC_{50} values (Table 1), ascorbic acid (IC_{50} 96.50±4.93 µg/ml) depicted a more significant ($p<0.05$) radical scavenging activity than the combination of LH and MB (IC_{50} 90.05±25.89 µg/ml). Figure 2 demonstrates an increase in ferric reducing power of the combination (2:1) of the aqueous leaf extracts of LH and MB and ascorbic acid with an increase in extract/drug concentration. The combination of LH and MB (IC_{50} 29.28±0.29 µg/ml) exhibited a more significant ($p<0.05$) reducing ability than ascorbic acid (IC_{50} 482±7.13 µg/ml) (Table 1). The different concentrations of the aqueous leaf extracts of the combination of LH and MB (2:1) and acarbose depicted a concentration dependent increase in alpha amylase inhibitory activities with the maximum inhibitory activities observed at the highest extract concentration as shown in Figure 3. The combination of LH and MB (IC50 15.86±1.03 µg/ml) displayed a more significant ($p<0.05$) alpha amylase inhibitory effect than acarbose (29.82±2.55 µg/ml) (Table 1).

Figure 4 presents the inhibitory activities of the combination of the aqueous leaf extracts of LH and MB (2:1) and acarbose against rat intestine alpha glucosidase enzyme. While acarbose displayed a dose dependent alpha glycosidase inhibitory activity, the alpha glucosidase inhibitory activity of the combination of LH and MB decreased with increase of extract concentration. The standard drug; acarbose (IC_{50} 37.95±0.22 µg/ml) exhibited a more potent ($p<0.05$) alpha glucosidase inhibitory activity than the combination of the aqueous leaf extracts of LH and MB (2:1) (IC_{50} 330.93±26.47 µg/ml). The inhibitory potentials of the aqueous leaf extracts of LH, MB and their combination (2:1) and Quercetin against rat lens aldose reductase are presented in Figure 5. The combination of LH and MB (2:1) and Quercetin exhibited a dose related increase in aldose reductase inhibitory activity. Maximum inhibition of the combination of LH and MB (2:1) and quercetin was observed at 100µg/ml respectively. Quercetin (IC_{50} 28.03±0.22 µg/ml) was more potent ($p<0.05$) in inhibiting rat lens aldose reductase enzyme than the combination of LH and MB (IC_{50} 37.30±2.27 µg/ml) based on an IC_{50} value (Table 1).

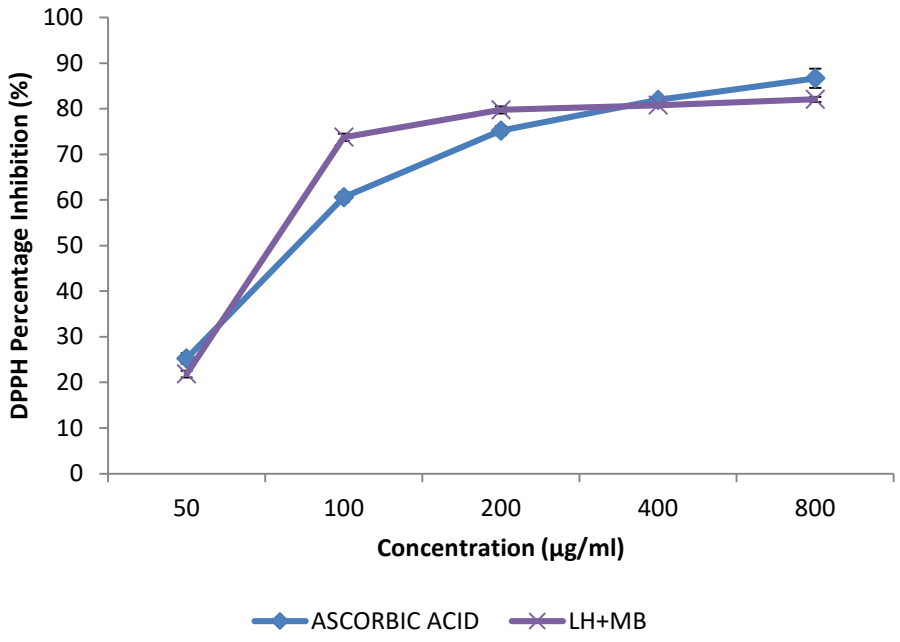

Figure 1: Radical Scavenging Activities of Aqueous Leaf Extracts of the Combination of LH and MB and Ascorbic acid (DPPH assay). Values expressed are Mean ± SD of 3 replicates.

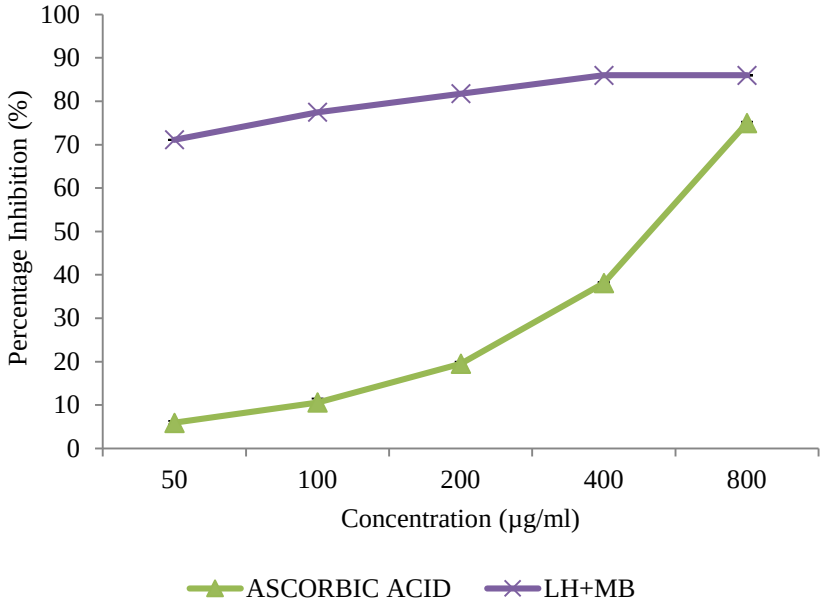

Figure 2: Ferric Reducing Power of the Aqueous Leaf Extracts of the Combination of LH and MB (2:1) and Ascorbic acid. Values expressed are Mean ± SD of 3 replicates.

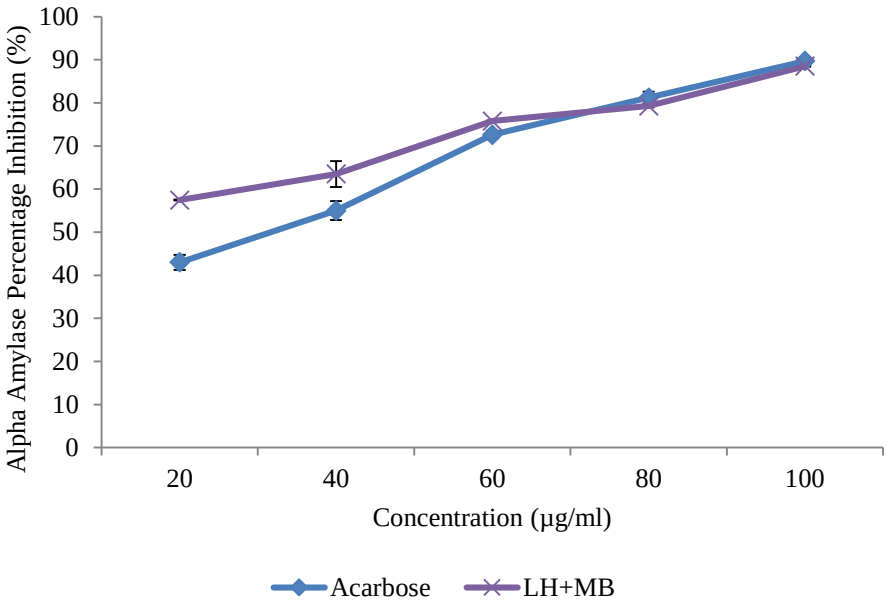

Figure 3: Alpha Amylase Inhibitory Activities of the Combination of LH and MB (2:1) and Acarbose. Values expressed are Mean ± SD of 3 Replicates.

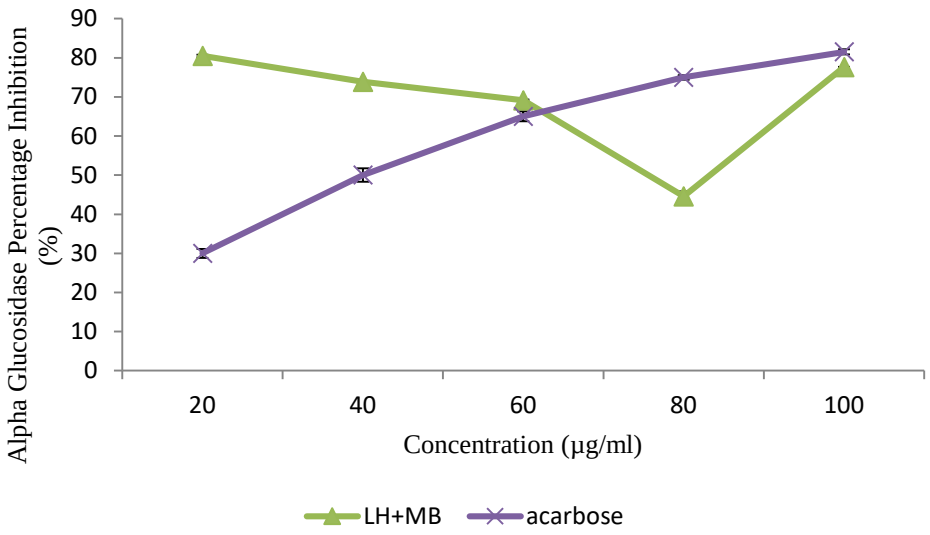

Figure 4: Alpha Glucosidase Inhibitory Activities of the Combination of LH and MB (2:1) and Acarbose. Values expressed are Mean ± SD of 3 Replicates.

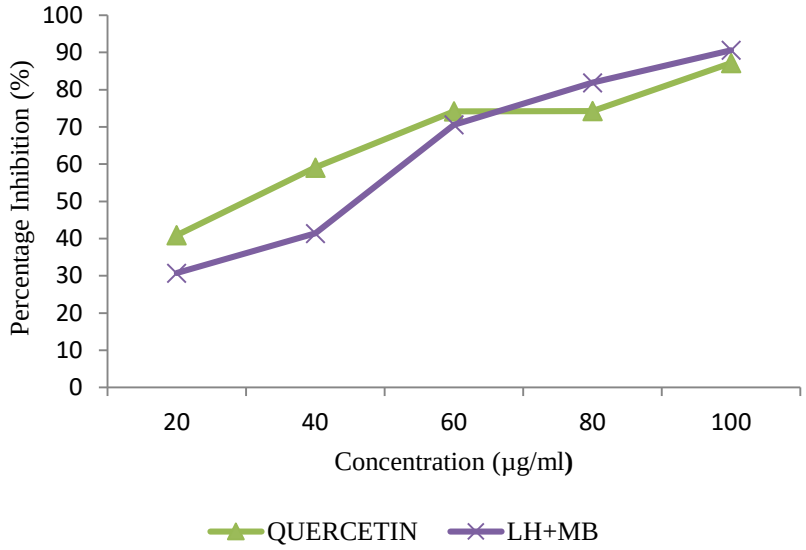

Figure 5: Aldose Reductase Inhibitory Activities of the Combination of LH and MB (2:1) and Quercetin. Values expressed are Mean ± SD of 3 replicates.

Table 1: IC_{50} values of Various *In vitro* Activities of the combination of the Aqueous Leaf Extracts of *L. hastata* and *M. balsamina*, Ascorbic acid, Acarbose and Quercetin

Extract/Drug	Ferric Reducing Power (µg/ml)	DPPH Radical Scavenging Activity (µg/ml)	Alpha Amylase Inhibition (µg/ml)	Alpha Glucosidase Inhibition (µg/ml)	Aldose Reductase Inhibition (µg/ml)
LH+MB	29.28±0.29[a]	90.05±25.89[a]	15.86±1.03[b]	330.93±26.47[a]	37.30±2.27[a]

1% Ascorbic acid	482.00±7.13[b]	96.51±4.93[a]	0.00	0.00	0.00
Acarbose	0.00	0.00	29.82±2.55[b]	37.95±0.22[b]	0.00
Quercetin	0.00	0.00	0.00	0.00	28.03±0.22[a]

Values expressed are mean±standard deviation of 3 replicates; values with different superscripts along a column are statistically significant (P<0.05).

Diabetes is a group of chronic diseases characterized by hyperglycemia. Persistent hyperglycemia is usually accompanied with an abnormally high level of reactive oxygen species which react with major macromolecules such as DNA, lipids and proteins leading to tissue damage [27]. Despite its high prevalence, diabetes requires lifelong pharmacological and non-pharmacological interventions to prevent the development and progression of both microvascular and macrovascular complications. Traditional medicine is accessible to more than three thirds of the world population. Most traditional herbal healers use polyherbal formulations with the knowledge of which plant parts might have hypoglycemic effects. The selection of the aqueous extracts of *Momordica balsamina* and *Leptadenia hastata* for this study is in consonance with the folkoloric use of aqueous solutions/concoctions of the two plant leaves by traditional herbal healers. The vast therapeutic properties of many medicinal plants originate from plants in the form of secondary metabolites.

Scavenging activity of free radicals by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing activity has been widely used for the *in vitro* evaluation of the antioxidant potentials of natural products from plant and microbial sources [28]. Akula and Odhav [20] have reported the use *M. balsamina* for combating the deleterious effects of oxidative stress. Souri *et al.* [29] attributed the antioxidant and DPPH radical scavenging activities of many medicinal plants LH and MB inclusive; to a variety of constituents such as phenols, flavonoids, tannins, ascorbate, carotenoids and terpenoides. Phenolic compounds are suggested to be antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers [30]. Tepe *et al.* [31] documented a positive correlation between total phenolic content and the antioxidant activity of many plant extracts. The phenolic content of *L. hastata* and *M. balsamina* could have therefore acted synergistically to exert potent antioxidant activity in the combination dose. The *In vitro* antioxidant activities observed in the polyherbal combination of *L. hastata* and *M. balsamina* can serve as a background for further studying its *in vitro* antioxidant effects on animal model of diabetes.

One of the therapeutic approaches for the treatment and management of diabetes is the control of postprandial hyperglycemia [32]. This can be achieved through the inhibition of carbohydrate hydrolyzing enzymes; alpha amylase present in saliva and pancreatic juice and alpha glucosidase present at the intestinal brush border. These enzymes act by breaking down polysaccharides contained in a carbohydrate meal in preparation of their absorption. Postporandial hyperglycemia have been shown to be a prominent and early defect in diabetes and regulating postporandial blood glucose elevation may attenuate progression towards diabetes associated secondary complications [33]. Sathish et al. [18] reported a decrease in the alpha glucosidase inhibitory activity of a poly herbal formulation containing *momordica charatia* with an increased extract concentration. Findings of the present study is in agreement with that of Sathish *et al.* [18] where they demonstrated a higher alpha glucosidase inhibitory activity at lower inhibitor dose of 31%. The observed decline with increase in extract concentration observed could be attributed to interference of certain substances at high concentration which generates an inhibitory reaction [34] or due to hormesis. Hormesis is a dose response phenomenon characterized by low dose stimulation and a high dose inhibition [35]. The alpha-amylase and alpha glucosidase inhibitory

activity of most antidiabetic plants have been attributed to the presence of phenolic compounds in the extracts that interact with and/or inhibit protein enzyme. Bello *et al.* [11] reported the alpha amylase and alpha glucosidase inhibitory activity of *L. hastata* in a dose response manner which correlates with the findings of our study.

One of the consequences of hyperglycemia in human diabetes mellitus is increased flux of glucose through the polyol pathway, which results in accumulation of sorbitol in cells. [36]. Increased sorbitol concentrations have been reported to alter redox potential, increase cellular osmolality, generate ROS and likely leads to other cellular dysfunctions. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. According to Bhatnagar and Srivastava, [37], inhibition of the conversion of glucose to sorbitol during diabetes, catalyzed by aldose reductase in the presence of NADPH may protect against oxidative stress and to abnormalities in nitric oxide action. Findings of the present study therefore demonstrate the potential of the combination (2:1) of LH and MB to confer protection against oxidative stress thereby reducing the risk of microvascular damage especially retinopathy.

Conclusion

The combination (2:1) of the aqueous leaf extracts of LH and MB demonstrated antidiabetic potentials exerted through the inhibition of hydrolysis of glucose via inhibition of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase), polyol pathway and antioxidant activity. This study provides the basis for studying the antidiabetic potentials of the combination of *L. hastata* and *M. balsamina* in animal models of diabetes and could be a candidate for natural antidiabetic drug.

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