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Research Article
Evaluation of Lens Aldose Reductase Inhibitory and Free Radical Scavenging Activity of Purified Fractions of *Hyptis suaveolens* Flower: Potential for Cataract Remediation

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Abstract

Background: Searching for effective and safe Aldose Reductase Inhibitor (ARI) agent is a major thrust area in the mainstream of anti-cataractogenic study. Plants constitute a rich source of bioactive chemicals that can be used for this purpose. **Objective:** To evaluate the ARI activity of fractions of *Hyptis suaveolens* for potential use in the development of a safe and effective anticataractogenic agent. **Materials and Methods:** Organic and aqueous extract of *Hyptis suaveolens* flower was screened for its phytochemical components. The ARI activities of the fractions and methanolic extract were studied *in vitro*. Partially purified aldose reductase extracted from homogenized goat lens was used in the study. The K_m and V_{max} of the enzyme in the presence and absence of the fractions was then estimated *in vitro* antioxidant capacities of the fractions were evaluated by determining the free radical scavenging activity against DPPH, NO, H_2O_2 and by evaluating the metal chelating and reducing power activity. **Results:** Phenol, flavonoid, tannin, alkaloid, terpenoid, quinones and phlobatannins were detected in both organic and aqueous extract but steroid was detected only in the aqueous extract. There was significant difference in the ARI activity of each fraction. It was found to be highest with aqueous fraction [IC_{50} (0.05 ± 0.01 mg mL^{-1})] followed by methanol extract [IC_{50} (0.07 ± 0.03 mg mL^{-1})] while it was least with chloroform fraction [IC_{50} ($>2.55 \pm 0.10$ mg mL^{-1})]. The hexane fraction showed non-competitive inhibition of AR while all other the fractions inhibited aldose reductase enzyme in an un-competitive manner. The lowest V_{max} was observed with methanol fraction (0.034 ± 0.002) while the highest V_{max} (0.111 ± 0.001) was observed with hexane fraction. All the fractions showed free radical scavenging activity and strong to moderate metal chelating and reducing power activity. **Conclusion:** All fractions of *Hyptis suaveolens* showed significant amount of ARI activity but this was highest with aqueous fraction. The fractions also showed free radical scavenging activity. These activities may be attributed to the phenolic components detected in this study. The study concluded that with further investigation *Hyptis suaveolens* may be used as base for development of anticataract agent.

Key words: Aldose reductase, antioxidant, cataract, *Hyptis suaveolens*, medicinal plant, phenolic compounds

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of medicinal plants, plant extracts or plant-derived pure chemicals to treat human ailments is an important alternative therapeutic approach. Presently, there is renewed interest in medicinal plant study, which possess good source of new bioactive drugs and now contributing 90% of newly discovered pharmaceuticals[1]. The drug discovery from natural products has therefore remained a significant hope in the 'Target rich lead poor' scenario of pharmaceutical study[2]. In the past decade an area of study interest in finding pharmaceutical agents against cataract [regarded as the main cause of blindness worldwide and accounting for 51% of world blindness[3,4] is the search for aldose reductase inhibitors.

Aldose reductase (ALR2, AR, EC 1.1.1.21) is a key enzyme in the polyol pathway that controls the conversion of glucose to sorbitol. It is found in almost all mammalian cells but richer in organs such as the lens, retina and sciatic nerves, which are affected by diabetic complications. Increased polyol pathway flux causes accumulation of sorbitol in the lens fiber, which in turn, causes an increased influx of water and the generation of osmotic stress, thereby leading to cataract formation[5]. Thus, reduction of the hyperglycemia- induced polyol pathway flux by AR inhibitors could be a potential therapeutic opening in the treatment and prevention of diabetic complications such as cataract formation. In normal tissue, aldose reductase has low substrate affinity for glucose. However, in diabetes mellitus, the increased availability of glucose in insulin-insensitive tissues such as the lens, nerve and retina leads to the increased formation of sorbitol through the polyol pathway[5,6].

In the prevention of diabetic complication, the elevated flux of blood and sorbitol through the polyol pathway in the target tissue is normalize by inhibition of aldose reductase. Aldose reductase enzyme and especially its inhibition by Aldose Reductase Inhibitors (ARIs) has therefore been gaining attention over the last years from the pharmaceutical community as it appears to be a promising pharmacotherapeutic target. Several authors have studied and reported on a number of structurally diverse naturally occurring and synthetic AR inhibitors that have proven to be effective for the prevention of diabetic complications in experimental animals, as well as in clinical trials[7,8]. Although, some synthetic ARIs have been developed as drug candidates, however, virtually all have not been successful in clinical trials due to adverse pharmacokinetic properties, inadequate efficacy and toxic side effects.

Cataract formation has also been attributed to oxidative stress triggered by Reactive Oxygen Species (ROS), which include superoxide anion (O^{2-}), nitric oxide (NO), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). The O^{2-} in itself is not highly toxic but it may react with other molecules, such as NO, yielding more reactive compounds[9]. An excess of NO, produced by Inducible Nitric Oxide Synthases (iNOS) upon stimulation is thought to cause cell injury by nitrosative stress and this may occur in certain diseases. In the eye, NO contributes to allergic conjunctivitis[10], glaucoma[11], diabetic retinopathy[12] and also cataract1[3-14]. The $OH^{\bullet}$ is another highly reactive free radical known to contribute to lens crystalline modification[15]. As far as free radical mediated damage in the eye lens is concerned, studies have shown that even in the earliest stages of cataract, lens proteins contained otyrosine, m-tyrosine, dityrosine, dihydroxyphenylalanine, valine hydroxide and leucine hydroxide and the levels of oxidized amino acids increased with the severity of cataract[14]. A compound therefore with antioxidant and free radical scavenging properties may be further justifiably evaluated as a potential approach to the management of cataract.

Hyptis suaveolens commonly called bush mint, bush tea, pignut or chan is known in Nigeria as daddoya-ta-daji (Hausa); efiri/jogbo (Yoruba); nchuanwu (Ibo) and tanmotswangi-eba (Nupe). It is considered a weed worldwide[16] and a very common plant found along roadsides and farmsteads in different parts of the world mainly in the tropics and subtropics. Although originally native to tropical America in Africa, it is found in Benin, Kenya, Nigeria, Sudan and Cameroon.

The plant has played an important role as a source of food as well as in traditional medicine. Different parts of the plant have been used by traditional healers in the treatment of various ailments and disease conditions. In the Northern part of Nigeria a decoction of the leaves is used for treating boils, eczema and diabetes mellitus[17,18]. Crushed leaves are applied on the forehead to treat headaches. Infusion made from the leaves and the inflorescence is used as stimulant, carminative, diuretic, antipyretic and eyes related disorder[19]. A decoction of the whole plant is also used to alleviate diarrhoea and various kidney ailments. However, to date, there appears to have been no study published on ARI activity of *Hyptis suaveolens*. Therefore, the present study was aimed at evaluating the aldose reductase inhibitory potential of the flower part extract and fractions of the extract *in vitro*. The study in addition was set up to evaluate the phytochemicals present in the plant and to investigate the efficacy of the extract and its fractions to scavenge free radicals *in vitro.*

MATERIALS AND METHODS

Quercetin, 2,2-diphenyl-1-picryl hydrazine (DPPH), TCA, glacial acetic, NADPH, ferrozine, napthylenediamine, dihydrochloride, 1,10-phenanthroline, sulfanilic acid, rutin and 2-mercaptoethanol were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All other solvents and chemicals used were of analytical grade and were obtained from commercial sources.

Plant materials: Whole plant of *Hyptis suaveolens* (with flowers) were collected from a botanical garden in Gaa-Osibi, Ilorin, Kwara State, Nigeria in January, 2016. The plant was authenticated at the herbarium of University of Ilorin where a voucher specimen (UILH/001/610) was deposited. The flowers were then removed, rinsed with distilled water and then shade dried for fourteen days. They were then pulverized.

Preparation of extract and fractions: Exactly 100 g of the pulverized flower was macerated in 500 mL of methanol for 7 days. It was then sieved over a muslin cloth first and further with filter paper. The extract was further macerated in another 500 mL of methanol for another 7 days and then filtered as above. The filtrate was combined and concentrated using a rotary evaporator. The concentrated product was further dried over a water bath at 40°C. The yield was 15.9%. The dried extract was then fractionated by suspending it in distilled water. Hexane was added to the suspension in ratio 1:2, shook well and allowed to stand for about 15 min until 2 layers were formed. The hexane layer was removed and more hexane was added to the aqueous layer. The process was repeated once and then a colorless hexane layer was seen. The two hexane layers were combined and dried to obtain the hexane fraction. The procedure was repeated with the aqueous layer using chloroform and ethyl acetate, respectively. Each fraction obtained including the aqueous fraction was then collected and dried. The aqueous layer was dried by lyophilization. The weight of the dried fractions were calculated and the sample was then stored in a desiccator for further use.

Isolation and partial purification of goat eye lenses: Eye ball was removed from goat immediately after sacrifice and stored in ice-cold container. Lenses were removed by lateral incision of the eye, washed with ice-cold distilled water and kept cold. The lenses were homogenized in 10 volumes of 100 mM ice-cold potassium phosphate buffer, pH 6.2 and centrifuged at 15,000×g for 30 min at 4°C. The resulting supernatant was used as the source of aldose reductase[5,8]. Saturated ammonium sulphate (100%) was added to the supernatant from the homogenate to reach 40% saturation and then allowed to stand for 15 min with occasional stirring to ensure the completeness of precipitation. It was then centrifuged and the precipitate was discarded. The same procedure was repeated for the resulting supernatant using 50 and 75% ammonium sulphate saturations. The final supernatant was used as the partially purified aldose reductase.

Aldose reductase assay: Aldose Reductase (AR) activity was assayed according to the method described by Hayman and Kinoshita[20]. Enzyme specific activity was calculated as IU mg^{-1} protein and this was defined as activity of the enzyme that can produce 1 µmol $NADP^+$ from NADPH in 1 min[21].

Inhibition study: Various concentrations (0.4-2.8 mg mL^{-1}) of the methanolic extract and its fractions were prepared in triplicate. Exactly 100 µL of concentrations prepared was then added to the assay mixture and incubated for 5-10 min. The reaction was initiated with the addition of NADPH. The absorbance was then read at 340 nm at the beginning and at the end of 30 min. The percent inhibition ARI (%) of the extract was then calculated as:

$$\text{ARI (\%)} = \frac{\Delta \text{Absorbance (negative control)-}\Delta \text{absorbance (fraction)}}{\Delta \text{ absorbance (negative control)}} \times 100$$

The AR activity in the absence of inhibitor was considered as 100%. The concentration of each test sample that gives 50% inhibition (IC_{50}) was then estimated. A negative control was prepared using 5% DMSO in phosphate buffer (pH 6.2).

Determination of kinetic parameters: The kinetic studies of inhibitory activity against aldose reductase of different fractions were analyzed using the lineweaver-burk plot.

Determination of free radical scavenging activity

DPPH radical scavenging assay: The DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging assay was carried out according to the method of Hemalatha *et al.*[22]. The solvent extracts of the sample were taken in the following concentration range: 100, 200 and 300 µL in each test tube and the volume was made up to 1 mL with the solvent and 3 mL of 0.1 mM DPPH was added to all the test tubes. The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517 nm

using a UV-spectrophotometer. All the experiments were performed in duplicate and the mean was taken. Scavenging activity was calculated from control sample OD using the following equation:

$$\text{DPPH-scavenging effect (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Ascorbic acid was used as positive control. The DPPH (0.1 mM) was prepared in methanol and 1.0 mL of its solution was mixed with 1.0 mL of extract (1.0 mL) prepared in methanol at different concentrations (20, 40, 60, 80 and 100 μg mL^{-1}).

The ability to scavenge DPPH radical was calculated using the equation:

- IC_{50} values been estimated from the graph. Lower IC_{50} value indicated strong free radical scavenging activity

Metal chelating activity: Each extract (0.5 g) was mixed with $FeCl_3$ (2 mM) and ferrozine (0.2 mL) in a test tube and the total volume was diluted with methanol (2 mL). The mixture was vigorously shaken and left standing for 10 min at room temperature. The absorbance of the solution was measured spectrophotometrically at 562 nm after the mixture had reached equilibrium. The procedure was repeated for the hexane, ethyl acetate and chloroform fraction. The EDTA was used a positive control and the percent inhibition of ferrozine-Fe^{2+} complex was calculated using the equation[23]:

$$\text{Percent scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where:
Control = Absorbance of ferrozine- Fe^{2+} complex
Sample = Absorbance of test compound

Hydroxyl radical scavenging activity: This was carried out according to the method reported by Wenli *et al.*[24]. The reaction medium was made up of 60 μL of 1 mM, $Fecl_3$, 90 μL of 1 mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 μL of 0.17 M H_2O_2 and 1.5 mL of various concentration of each fractions and methanol extract. Reaction mixture was kept at room temperature for 5 min incubation and absorbance was then measured at 560 nm using spectrophotometer. The concentration of the individual sample required to neutralize 50% hydroxyl radicals were considered as IC_{50} values. The alpha tocopherol (IC_{50}, 0.107 mg mL^{-1}) was used a reference compound.

Nitric oxide radical scavenging activity: Previously described method[25] was used for estimating nitric oxide radical scavenging activity. The reaction mixture containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4) and 0.5 mL of plant extract/fraction was incubated at 25°C for 2 h 30 min. After incubation time, 0.5 mL of reaction mixture was mixed with 1 mL of sulfanilic acid reagent (33 in 20% glacial acetic acid) and incubated for 5 min. This was followed by addition of 1 mL napthylenediamine dihydrochloride (0.1% w/v). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 560 nm using UV-VIS spectrophotometer. The amount of sample required to scavenge 50% nitric oxide radicals generated in the control set were calculated as IC_{50}. Rutin hydrate (IC_{50}, 0.165 mg mL^{-1}) was used a reference compound.

Hydrogen peroxide scavenging activity: The ability of plant extracts/sample to scavenge hydrogen peroxide was determined according to the earlier described method[26]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined by measuring absorbance at 230 nm using spectrophotometer. The extract/fractions prepared in distilled water were mixed with 0.6 mL of hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing plant extract/fraction in phosphate buffer without hydrogen peroxide. The absorbance of hydrogen peroxide (40 mM) without plant extract was considered as control (100%). The concentration of plant extract/fraction required to scavenge 50% hydrogen peroxide was calculated as IC_{50}. The alpha tocopherol (IC_{50}, 0.412 mg mL^{-1}) was used a reference compound for comparative study.

Reducing power assay: This was determined by the previously described method[27]. The reaction mixture containing 0.75 mL of various concentrations of plant extract/fraction, 0.75 mL of phosphate buffer (0.2 N, pH 6.6) and 0.75 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1% w/v) was incubated at 50°C in water bath for 20 min. The reaction was stopped by the addition of 0.75 mL trichloroacetic acid (10%) and then centrifuged for 10 min at 800 rpm. The supernatant (1.5 mL) of the individual reaction mixture was collected in different clean tubes and was mixed with 1.5 mL of distilled water followed by addition of 0.1 mL ferric chloride (0.1% w/v) and kept for 10 min. The absorbance of reaction mixture was measured at 700 nm as the reducing power. The absorbance of control was considered as 100% of

Fe^{3+} ions and IC_{50} values were determined as the concentration of plant extract/fraction required to inhibit 50% reduction of Fe^{3+} ions. Ascorbic acid (IC_{50}, 0.120 mg mL^{-1}) was used as standard reducing agent for comparative study.

Phytochemical screening: The methanolic and aqueous extracts of the plant was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures[28-30].

Data analysis: Data are expressed as Mean±SEM of 3 replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test to determine significant differences in all the parameters. Values were considered significantly different at $p<0.05$.

RESULTS

The yield of the crude methanolic extract and its sub-fractions is given in Table 1 while Table 2 showed the result of the phytochemical constituents of the aqueous and methanolic extract. The highest yield of 13.9% was obtained with ethylacetate fraction while the lowest yield (5.2%) was obtained with chloroform fraction. The result of the preliminary phytochemical screening carried out on the methanolic and aqueous extracts revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, phenols, flavonoids, tannins and phlobatannins in both extracts. Anthocyanins was not detected in any of the extract and steroids was detected only the in the aqueous extract.

Figure 1 is the result of changes in percentage inhibition of AR with increasing concentration of the extract/fraction of *Hyptis suaveolens* flowers while the result of IC_{50} is shown in Table 3. The least IC_{50} of 0.05±0.01 mg mL^{-1} was obtained with aqueous fraction and this was not different significantly ($p>0.05$) from IC_{50} of 0.07±0.03 mg mL^{-1} obtained with methanol fraction. The highest IC_{50} was obtained with ethylacetate fraction (2.84±0.01 mg mL^{-1}) and this was not significantly ($p>0.05$) different from the IC_{50} value of 2.55±0.10 mg mL^{-1} obtained with chloroform fraction.

Shown in Fig. 2 is the line weaver-burk plot while Table 4 is the results of aldose reductase kinetics when the enzyme was incubated with different fractions of *Hyptis suaveolens* flowers. Data from this study indicates that hexane fraction showed non-competitive inhibition. The V_{max} of 0.111±0.001 µm NADPH oxidised/h/100 mg protein obtained with hexane fraction was significantly

Table 1: Yield of extract/ fraction/ fraction

Extract/fraction	Yield (%)
Methanol	7.75
Aqueous	11.20
Ethyl acetate	13.93
Hexane	3.50
Chloroform	5.21

Table 2: Phytochemicals of *Hyptis suaveolens* flowers in aqueous and organic extract

Phytochemical constituents	Aqueous extract	Organic extract
Phenols	+	+
Phlobatannins	+	+
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	+	-
Alkaloids	+	+
Anthocyanins	-	-
Cardiac glycosides	+	+
Terpenoids	+	+
Quinones	+	+

+: Denotes detected and denotes not detected

Table 3: IC_{50} (mg mL^{-1}) of fractions of *Hyptis suaveolens* flowers (n = 3±SEM)

Extract/fraction	IC_{50} (mg mL^{-1})
Chloroform	2.55 ± 0.10^{a}
Methanol	0.07 ± 0.03^{b}
Ethyl acetate	2.84 ± 0.01^{a}
Aqueous	0.05 ± 0.01^{b}
Hexane	1.14 ± 0.03^{c}

Values in the same column with the same superscripts are not significantly different from each other

Table 4: Kinetics parameters of aldose reductase enzyme in the presence of different fractions of *Hyptis suaveolens* flowers (n = 3±SEM)

Extract/fraction	Vmax (A° 340 nm)	$K_m \times 10^{-3}$ mM
DL-glyceraldehyde	0.830 ± 0.012^{a}	6.313 ± 0.002^{a}
DL-glyceraldehyde+methanol	0.050 ± 0.002^{b}	4.054 ± 0.061^{b}
DL-glyceraldehyde+chloroform	0.038 ± 0.002^{c}	1.017 ± 0.001^{c}
DL-glyceraldehyde+hexane	0.111 ± 0.001^{d}	0.490 ± 0.021^{d}
DL-glyceraldehyde+ethyl acetate	0.034 ± 0.002^{c}	1.215 ± 0.002^{c}
DL-glyceraldehyde+aqueous	0.066 ± 0.004^{b}	5.782 ± 0.053^{b}

Values in the same column with the same superscripts are not significantly different from each other

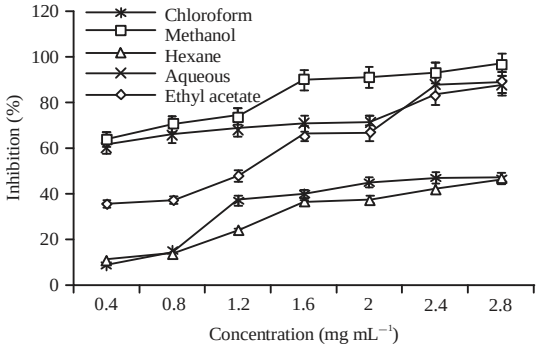

Fig. 1: Inhibitory effect of fractions of *Hyptis suaveolens* flowers on the specific aldose reductase activity

Table 5: Free radical scavenging activity of extract and fractions of *Hyptis suaveolens* flowers (n = 3±SEM)

Extract/fractions	DPPH IC_{50} (mg mL^{-1})	Metal chelating activity (%)	OH IC_{50} (mg mL^{-1})	H_2O_2 IC_{50} (mg mL^{-1})	Reducing power (mg mL^{-1})	NO IC_{50} (mg mL^{-1})
Methanol	0.21±0.02[a]	91	0.785±0.030[a]	0.516±0.001[a]	0.109±0.003[a]	0.916±0.001[a]
Aqueous	0.29±0.01[a]	82	0.609±0.021[b]	0.603±0.012[b]	0.121±0.001[a]	0.879±0.022[a]
Ethylacetate	0.35±0.01[b]	89	1.201±0.032[c]	0.805±0.001[c]	0.361±0.010[b]	0.988±0.014[b]
Chloroform	0.91±0.02[c]	45	0.916±0.035[d]	0.810±0.004[c]	0.402±0.002[c]	1.506±0.017[c]
Hexane	2.70±0.11[d]	22	1.521±0.012[e]	0.891±0.001[c]	0.608±0.003[d]	1.801±0.010[c]

Values in the same column with the same superscripts are not significantly different from each other

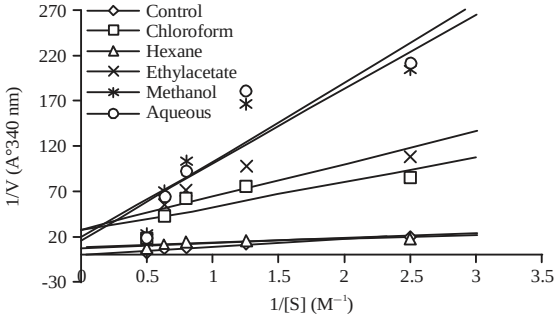

Fig. 2: Effect of different fractions of *Hyptis suaveolens* flowers on lineweaver-burk plot of aldose reductase activity

($p<0.05$) different from that obtained with glyceraldehyde (0.830±0.012 µm NADPH oxidised/h/100 mg protein). However, the K_m value of the enzyme when incubated with hexane fraction (0.490±0.021 mM) was not significantly ($p>0.05$) different from that observed with glyceraldehyde (0.313±0.002 mM). The result of aldose reductase kinetics when the enzyme was incubated with all other fractions viz. methanol, aqueous, ethylacetate and chloroform fractions indicates that the V_{max} and K_m of the enzyme in the presence of the fractions were significantly different from the value obtained with glyceraldehyde. The result suggests that these fractions showed uncompetitive inhibition of the aldose reductase enzyme.

Table 5 is the result of the free radical scavenging activities of the extract/different fractions of *Hyptis suaveolens* flowers. Table 5 indicates that methanol extract showed the highest DPPH scavenging activity (IC_{50}, 0.21±0.02 mg mL^{-1}) which was however not significantly ($p>0.05$) different from that of the aqueous fraction (IC_{50}, 0.29±0.01 mg mL^{-1}). The lowest DPPH scavenging activity was obtained with hexane fraction (IC_{50}, 2.70±0.11 mg mL^{-1}). All other fractions showed moderate activity in the DPPH IC_{50} activity. The highest H_2O_2 scavenging activity (IC_{50}, 0.516±0.001 mg mL^{-1}) was obtained with methanol fraction and this was significantly higher when compared with the H_2O_2 scavenging activity of the aqueous fraction (IC_{50}, 0.603±0.012 mg mL^{-1}). All other fractions showed ineffective H_2O_2 scavenging activities. The H_2O_2 IC_{50} obtained with diethyl ether, chloroform and ethyl acetate were not significantly different from each other but was significantly higher than that of the aqueous fraction. A similar pattern was seen in the variation in reducing power activity. However, the least reducing power IC_{50} obtained with methanol fraction (0.109±0.003 mg mL^{-1}) was not significantly ($p>0.05$) different from that of the aqueous fraction (0.121±0.001 mg mL^{-1}). Hexane fraction showed the least reducing power activity (IC_{50}, 0.608±0.003 mg mL^{-1}). Highest OH and NO scavenging activity were obtained with the aqueous fraction (IC_{50}, 0.609±0.021 and 0.879±0.022 mg mL^{-1}, respectively). The OH IC_{50} of 0.785±0.030 mg mL^{-1} obtained with methanol fraction was higher than that of the aqueous fraction, however, the NO IC_{50} of 0.916±0.001 mg mL^{-1} obtained with methanol extract was not significantly ($p>0.05$) different from that of the aqueous fraction. The weakest OH and NO scavenging activity were obtained with hexane fraction (IC_{50}, 1.521±0.012 and 1.801±0.010 mg mL^{-1}, respectively). Methanol extract also showed the highest metal chelating activity (91%) while hexane fraction showed the least metal chelating activity (22%).

DISCUSSION

This study identified phenol, flavonoids, tannins, alkaloids and phlobatannins to be present in both the aqueous and organic extracts of *Hyptis suaveolens* flowers thereby justifying its acclaimed medicinal properties. Reports have shown that medicinal plants contain some organic compounds which produce definite physiological action on the human body. These bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[31,32]. They are of great importance to the health of individuals and communities. Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Reports have implicated natural components especially phenolic compounds such as flavonoids as having

health beneficial properties. This medicinal property has been attributed to inhibition of certain enzymes particularly, aldose reductase and xanthine oxidase and also their antioxidant activity[33,34]. Phenolics have been known to possess a capacity to scavenge free radicals. This antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents and hydrogen donors. Studies have shown that they play an important preventive role in the development of cancer, heart diseases and ageing related diseases[35].

The present study also showed that both methanol and water were effective in extracting the bioactive principle of *Hyptis suaveolens* flowers. In a previous study, Boeing *et al.*[36] noted that among pure solvents, methanol was the most efficient solvent for extraction of antioxidant compounds, followed by water, ethanol and acetone. This observation is supported by another report by Zlotek *et al.*[33]. When the yield was compared after the methanolic extract was fractionated, the yield obtained with water and ethyl acetate was higher than that of chloroform and hexane. This report thus suggests that solvent polarity is important in the extraction of the antioxidant principle of *Hyptis suaveolens* flowers. This is in agreement with the report of Boeing *et al.*[36] and Urszula *et al.*[33]. This observation is also supported by the results obtained on aldose reductase activity and free radical scavenging activity where the methanol extract and aqueous fraction were noted to show better activities compared with the ethylacetate, chloroform and hexane fractions. This, we hypothesized, could have been due to the better solvation of antioxidant compounds present in the plant as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant molecules and the solvent[32]. Hexane gave the lowest activity because of their lower efficiency of solvation, since hexane molecules are only proton acceptors while methanol and water are also proton donors.

The inhibitory effects of plant phytochemicals, including polyphenols (which are currently regarded as natural antioxidants) against carbohydrate hydrolyzing enzymes and their antioxidant activities are important for human health[37,38]. In the present study it was observed that different fractions showed significant aldose reductase inhibitory activity. However, it was maximum in the case of aqueous fraction and followed by methanolic extract. All other fractions showed weak AR inhibitory activity. Aldose reductase is the key enzyme in the polyol pathway. The enzyme reduces glucose to sorbitol which is further metabolized to fructose by sorbitol dehydrogenase. Normally this pathway, accounts for less than 3% of glucose consumption. However in the presence of high glucose, the activity of this pathway is substantially increased and could represent up to 30% of total glucose consumption. Therefore, aldose reductase inhibition in the early onset of the secondary complication in the diabetic mellitus will be beneficial. Since in this study, both the aqueous fraction and methanolic extract showed good inhibitory activity against aldose reductase, the study suggests that the plant can be potentially used to treat cataract, a major diabetic complication and therefore may be a potential candidate for the development of anticataractogenic agent. Significant study efforts have been going on all over the world on the investigation of naturally-occurring biomarkers with potential ability to inhibit aldose reductase enzyme. In an attempt to develop potent, safe and new ARI agents from natural sources, many plant materials and isolated phytoconstituents have been tested for ARI activity in both *in vivo* and *in vitro* models. Some of the plants that has been investigated include root of *Salacia oblonga* and *Salviae multiorrhizae*[6], *Caesalpinia brevifolia and Anacardium occidentale*[7], *Hydrocotyl bonariensis*[39] and *Andrographis paniculata*[40].

The detection of phenols and flavonoids as reported in this study suggest that the aldose reductase inhibitory action of *Hyptis suaveolens* flowers may be due to these phytoconstituents. Flavonoids are commonly ingested from fruits and vegetables in the diet, although they have no nutritive value, they are capable of exerting various pharmacological activities, including antioxidative, superoxide-scavenging and aldose reductase inhibitory activity[6]. Several flavonoids, such as quercitrin, guaijaverin and desmanthin have been tested and proven for their inhibitory activity against aldose reductase[41]. Previous study has reported that phenolic compounds are one of the most widely occurring groups of phytochemicals and are of considerable physiological and morphological importance in plants[33,34]. There are many reports that this group of phytochemicals possesses biological activity. The antioxidant activity of polyphenols is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations[33,34,42]. Zhang *et al.*[43] reported that the presence of electron-donating and electron-withdrawing substituents in the ring structure of phenolics as well as the number and arrangement of the hydroxyl groups determines their antioxidant potential.

The kinetic study was performed for the entire fraction in order to elucidate the mode of inhibition of the aldose reductase enzyme by the fraction/extract. The result of the kinetic studies suggest that the aldose reductase inhibitory compounds present *Hyptis suaveolens* flowers

extract/fractions can interact and inhibit lens aldose reductase enzyme in an uncompetitive manner (except hexane fraction), appearing to interact with the enzyme at a site independent of either substrate or enzyme. An uncompetitive inhibitor binds exclusively to the enzyme-substrate complex, yielding an inactive enzyme-substrate-inhibitor complex. The effect of an uncompetitive inhibitor is to decrease both V_{max} and K_m. The K_m is a measure of substrate affinity for the enzyme. A lower K_m corresponds to a higher affinity. The presence of an uncompetitive inhibitor actually increases the affinity of the enzyme for the substrate. Since, the inhibitor binds the E-S complex, the inhibitor decreases the concentration of the E-S[44]. Despite their rarity in drug discovery programs, uncompetitive inhibitors could have dramatic physiological consequences. As the inhibitor decreases the enzyme activity, there is an increase in the local concentration of substrate. Without a mechanism to clear the buildup of substrate, the potency of the uncompetitive inhibitor will increase.

The role of free radicals in pathogenesis of diabetes and related disorders like cataract has been previously discussed. Pathophysiologically, one of the early signs of cataractogenesis is damage to the lens cell membrane[45], apparent by a decline in its ability to actively transport substances against electrochemical gradients, resulting in alterations in intraocular composition and metabolism. The plant's fractions investigated in the present study were found to possess significant free radical scavenging activity. A cursory look at the IC_{50} values presented in Table 5 clearly show that the aqueous fraction and the methanol extract are more efficacious than all other fractions in scavenging DPPH, $OH^{\bullet}$ and H_2O_2. The metal chelating activity of all the fractions as noted in Table 2 ranged from 22-91% whereas the reducing power showed IC_{50} activity ranging from 0.109-0.608 mg mL^{-1}. Again, the NO scavenging activity as indicated by the IC_{50} was also shown for all the fractions to range from 0.879-1.811.

CONCLUSION

The results of this study indicate that *Hyptis suaveolens* flowers can be considered as potential source for the identification of novel and effective AR inhibitors, anti-cataract and antioxidant agents. The methanol extract and the aqueous fraction were found to exhibit maximum aldose reductase inhibition and free radical scavenging activity. The plant may therefore serve as a base for the development of anti cataract agent. In order to fully explore the potentials of the plant, it is important to further isolate and identify the novel anti cataract agents. This is the thrust of the ongoing study in our laboratory.

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