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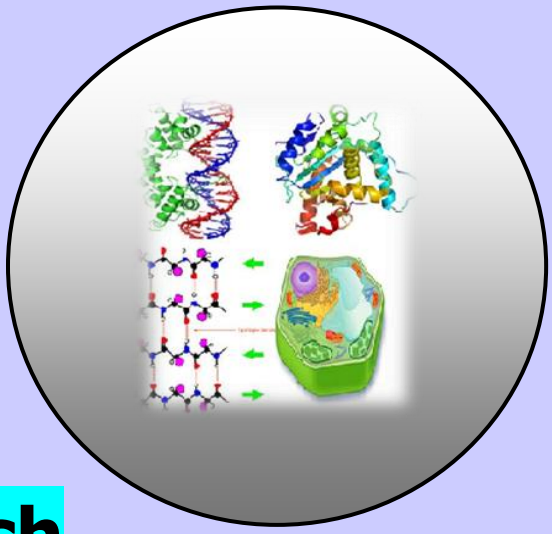
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66
Index Copernicus International Value
IC Value of Journal 47.86 Poland, Europe

J. Biol. Chem. Research
Volume 33 (2) 2016 Pages No. 865-880



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 33, No. 2: 865-880, 2016
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 33/2/140/2016

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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RESEARCH PAPER

Received: 29/10/2016 Revised: 08/11/2016 Accepted: 10/11/2016

In vitro Lens Aldose Reductase Inhibitory and Free Radical Scavenging Activity of Fractions of *Rauvolfia vomitoria* Leaves

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ABSTRACT

Searching for effective and safe aldose reductase inhibitor is a major thrust area in the mainstream of anti-cataractogenic research. The present study was set up to investigate the in vitro aldose reductase inhibitory (ARI) activity of fractions of methanolic extract of Rauvolfia vomitoria leaves on partially purified AR from goat lens. The phytoconstituents of the leaves were screened for in the aqueous and methanolic extract. The metal chelating capacity, reducing power, DPPH, superoxide, hydroxyl radical, H_2O_2 scavenging activity of the hexane, chloroform, ethylacetate and aqueous fractions of the methanolic extract of the leaves were evaluated in vitro. The percentage reduction in ARI and the kinetics of the aldose reductase when the enzyme was incubated with each fraction was also investigated and compared. Phenol, flavonoid, tannins, alkaloid, saponin and cardiac glycoside were detected in both extracts. All the fractions inhibited AR in an uncompetitive manner showing a reduced V_{max} and K_m when compared with glyceraldehyde. ARI activity was found to be highest with aqueous and chloroform fractions ($IC_{50,}$ 0.91 ± 0.01mg/ml and 0.90 ± 0.01mg/ml respectively). All other fractions showed mild AR inhibition capacity and this were found to be lowest with ethylacetate fraction ($IC_{50,}$ 3.25 ± 0.03mg/ml). All the fractions showed free radical scavenging activity and metal chelating activity but were highest with aqueous fraction. The study confirms the ARI and antioxidant capacity of Rauvolfia vomitoria which may be attributable to its phenolic constituents. This study suggests that R. vomitoria may serve as a base for the development of anticataract agent.

Kew words: Aldose Reductase, Cataract, Free Radical, Medicinal Plant and Phytochemical Constituents.

INTRODUCTION

As a result of decreasing efficacy of synthetic drugs, their non-affordability and the increasing contraindications of their usage, attention in recent years (particularly in Africa) is now focused on natural medicines (Biljana, 2012). Report indicated that about 80% of the world's population now relies on traditional medicinal system to augment and supplement the increasingly expensive orthodox medicine (WHO, 2010). One among such plants that is finding applications in this regard is *Rauvolfia vomitoria.*

R. vomitoria (Apocynaceae) is found mainly wild in Africa. It is a plant that is widely distributed in the humid tropical secondary and low land forests of Africa and Asia (Abayomi et al. 2015). The common names of the plant are Swizzle stick or Poison Devil's stick. In Nigeria, it is commonly known as 'Asofeyeje' (Yoruba), ira (Igbo) and "Wadda" (Hausa) (Ikewuchi and Ikewuchi, 2009). *It* is a common herb used traditionally for psychiatric management in Nigeria (Akpanabiatu et al. 2006). The extracts of the plant have been reported to have anti-inflammatory (Kato et al. 2009), antipyretic (Amole and Onabanjo, 2009), anti-diabetic and anti-cancer effect (Campbell et al. 2006). These effects have been attributed to the β-carboline alkaloid and alstonine (Bemis et al. 2006, Okon et al. 2003) content. *R. vomitoria* has been reported to be relatively safe with a LD_{50} of 17.5 g/kg (Amole et al. 2009). An earlier study by Jeanette et al. (2009) identified saponin, phenol, terpenoid and flavonoid as some of the phytoconstituents of the plant. The medicinal values of plants lie in their component phytochemicals which produce a definite physiological action on the human body. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research (Akinmoladun et al. 2007). In the past decade, an area of research interest in finding pharmaceutical agents against cataract is the search for aldose reductase inhibitors.

Cataract is opacity of the lens that interferes with vision, and is the most frequent cause of visual impairment worldwide, especially for the elderly. It is the leading cause of blindness and contributes to 50% of blindness worldwide (Haque and Gilani, 2005). The only present remedy for cataract is surgery. The opacity of lens occurs as a result of oxidation which is augmented by the free radical generation. The intensity of opacification of lens can therefore be reduced by the antioxidants which scavenge the generation of free radicals. Studies have confirmed that diet rich in vitamins, carotenoids and flavonoids may reduce the cataract intensity (Haque and Gilani, 2005, Rathnakumar et al. 2013). Aldose reductase, an enzyme of the polyol metabolic pathway has also been identified as a key enzyme in diabetic cataract. Reduction of glucose by the enzyme aldose reductase (AR) leads to the formation of sorbitol, which, in some tissues, is further oxidized to fructose upon sorbitol dehydrogenase-catalyzed oxidation (Suzen et al. 2006, Suzen et al. 2007). The importance of aldose reductase in the etiology long term diabetic complications lies in its role in sorbitol production, osmoregulation in the lens and kidney and regulation of sperm maturation (Garrat, 1964, Gui et al. 1995). This enzyme apart from its role in cataract formation is also involved in many pathological processes that have become major threats to human health. Such pathologies include a number of cardiac disorders, inflammation, mood disorders, renal insufficiency, and ovarian abnormalities (Alexiou et al. 2009, Oates, 2008).

Aldose reductase enzyme and especially its inhibition by aldose reductase inhibitors (ARIs), has 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 (Guzman and Guerrero, 2005, Patel et al. 2012a). Although, some synthetic aldose reductase inhibitors (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. The present study is therefore aimed at evaluating the aldose reductase inhibitory and the free radical scavenging activity of *R. vomitoria.*

MATERIAL 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 *R. vomitoria* was collected from a botanical garden in Moro Local government area of Kwara State in July 2015. The plant was identified at the herbarium of Plant Biology, Kwara State University, Malete, Nigeria where a voucher specimen (KS/PLH/BB01/0/001/981) was deposited. The leaves of the plant were then shade dried for two (2) weeks and then pulversised with a local kitchen blender.

Preparation of extract and fractions

Exactly 100 g of the pulverized leaves was macerated in 500 ml of methanol for 7days. It was then sieved over a muslin cloth first and further with filter paper. The residue was again re-extracted in another 500 ml of methanol for 7 more 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 40^0C. The yield was 6.7 %. 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 fifteen minute until two 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 aldose reductase from 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, pH6.2 and centrifuged at 15,000xg for 30 minutes at 4^0C. The resulting supernatant was used as the source of aldose reductase (Suryanarayana et al., 2004). Saturated ammonium sulphate (100%) was added to the supernatant from the homogenate

to reach 40% saturation and then allowed to stand for 15 minutes 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 (1965). Enzyme specific activity was calculated as IU/mg protein and this was defined as activity of the enzyme that can produce 1µmol $NADP^+$ from NADPH in 1min (Halder et al. 2003).

Inhibition study

Different concentrations (0.4-2.8 mg/ml) of the methanolic extract of the leaves 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 minutes. 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 minutes. The per cent inhibition (%ARI) of the extract was then calculated. 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 (pH6.2).

Determination of Kinetic Parameters

The kinetic studies of inhibitory activity against aldose reductase of different fractions were analyzed using the Lineweaver-Burk plot

Estimation of Lens Protein Concentration

The protein determination was carried out using the Stoschck (1990), modified method of Lowry *et al.* (1951).

Assay for free radical scavenging activity

DPPH Radical Scavenging Assay

The DPPH (1, 1- diphenyl-2-picryl hydrazyl) radical scavenging method was used to evaluate the antioxidant property of the plant. The antioxidant activity of each sample was expressed in terms of IC_{50}, and this was calculated from the graph after plotting inhibition percentage against extract concentration. The assay was carried out according to the method of Hemalatha *et al*., (2010). DPPH (0.1mM) was prepared in methanol and 1.0 ml of its solution was mixed with 1.0 ml of extract/fractions prepared in methanol at different concentrations (20, 40, 60, 80 and 100 µg/ml). The mixture was shaken well and incubated at room temperature for 30 minutes and absorbance was measured at 517 nm using a UV-spectrophotometer. All the experiments were performed in triplicate and the mean was taken. Scavenging activity was calculated from control sample absorbance using the following equation:

DPPH- Scavenging capacity (%) = $[(A^0\text{control} - A^0\text{sample}/A^0\text{control}) \times 100]$

IC_{50} values (concentration of extract/fraction required to reduce 50% of DPPH radical) were estimated from the graph and compared.

Metal chelating activity

The method described by Decker and Welch (1990) was used to estimate the metal chelating activity.

The extract/fraction (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 minutes at room temperature. The absorbance of the solution was measured spectrophotometrically at 562 nm after the mixture had reached equilibrium. EDTA was used as positive control and the percent inhibition of ferrozine-Fe^{2+} complex was calculated using the formula below.

- Percent Scavenging = $[(A^0control – A^0sample)/ A^0control] \times 100$
- Where: $A^0control$ = absorbance of ferrozine- Fe^{2+} complex

A^0 sample = absorbance of test compound

Hydroxyl radical scavenging activity

This was carried out according to the method described by Yu *et al*., (2004). 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/ 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.

Nitric oxide radical scavenging activity

Method previously described by Garrat (1964) 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 ^{0}C for 2 hours 30 minutes. 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 napthylenediaminedi hydrochloride (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}.

Hydrogen peroxide scavenging activity

The assay method described by Ruch*et al*., (1989) was used to determine the ability of plant extracts/fraction to scavenge hydrogen peroxide. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). 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}.

Reducing power assay

This was determined by the previously described method (Oyaizu, 1986). 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 ^{0}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.

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 (Harbone, 1973, Sofowora, 1993, Trease and Evans, 1989).

Statistics

The experimental results obtained were expressed as mean values of three replicates. Linear regression analysis was used to calculate the IC_{50} values. 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 $p<0.05$

RESULT

Result showed that the yield varied with the solvent fractions (Table 1). It was highest with aqueous fraction (9.17%) and lowest with ethylacetate fraction (3.33%). Result of phytochemical constituents (Table 2) also showed that a wide range of phytoconstituents were extracted by both the aqueous and organic solvents. Phenol, tannins, flavonoids, saponins, alkaloids and cardiac glycosides were detected in both extracts but quninone was detected only in the aqueous extract.

The profile of the percentage aldose reductase inhibition by the selected fractions is shown in Figure 1 while Table 3 showed the profile of the concentration of individual fraction required to achieve 50% AR inhibition. Figure 1 indicates that there were increases in percentage ARI with increased concentrations of the fractions until a peak was reached after which increasing the concentration of the fractions does not result in increase in percentage inhibition. All the fractions showed a mild to moderate inhibition of AR with the lowest IC_{50} observed with aqueous and chloroform fraction (0.90 ±0.01 and 0.91 ± 0.01 mg/ml respectively). The highest IC_{50} indicating lowest degree of inhibition was observed with ethtlacetate fraction (IC_{50} 3.25 ± 0.03 mg/ml). The Line weaver-Burk plot (Figure 2) indicates that all the fractions inhibited aldose reductase in an uncompetitive manner. The result of the aldose reductase kinetics indicates that both V_{max} and K_m obtained when the fractions were incubated with glyceraldehyde were significantly ($p<0.05$) lower (except with hexane fraction) than that obtained with glyceraldehyde (control) alone. The lowest V_{max} was obtained in the presence of aqueous fraction (0.017 ± 0.004 µm NADPH oxidized /hr/ 100mg protein). This was significantly lowered when compared with Vmax of 0.023 ± 0.010 µm NADPH oxidized /hr/100mg protein obtained with ethylacetate fraction. Highest V_{max} of 0.096 ± 0.026 µm NADPH oxidized /hr/ 100 mg protein was obtained with hexane fraction. Among all the fractions studied, highest Km of AR was obtained when incubated with aqueous fraction (1.922 ± 0.138 mM^{-1}) while the lowest K_m was obtained with hexane fraction (0.392 ± 0.024 mM^{-1}).

Shown in Table 5 is the result of the free radical scavenging activities of the fractions of *R. vomitoria*. Highest DPPH scavenging activity ($IC_{50,}$0.052 ± 0.012mg/ml), metal chelating activity (73.5%), hydroxyl radical scavenging activity ($IC_{50,}$ 0.712 ± 0.051mg/ml), H_2O_2 scavenging activity ($IC_{50,}$ 0.562 ± 0.016 mg/ml), NO scavenging activity ($IC_{50,}$0.610 ± 0.066 mg/ml) and reducing power activity (IC_{50} 0.216 ± 0.016 mg/ml)was shown by the aqueous fraction of *R. vomitoria.* All other fractions showed mild antioxidants activity with least activity shown by hexane fraction (DPPH scavenging activity, IC_{50} 0.106 ± 0.031 mg/ml), metal chelating activity (17.4%), hydroxyl radical scavenging activity (IC_{50}, 2.106 ± 0.061 mg/ml) and reducing power activity, (IC_{50}, 0.612 ± 0.016 mg/ml). Though the least H_2O_2 scavenging activity (IC_{50}, 0.927 ± 0.055 mg/ml) and NO scavenging (IC_{50}, 1.012 ± 0.042 mg/ml) were shown by chloroform fraction, the values obtained were not different from that of hexane fraction (IC_{50}, 0.916 ± 0.068 mg/ml and IC_{50}, 0.612 ± 0.016 mg/ml respectively).

DISCUSSION

Plants produce a very diverse group of secondary metabolites with antioxidant potential. Preliminary qualitative test according to Savithramma *et al*., (2011) is useful in the detection of these bioactive principles and subsequently may lead to drug discovery and development. Our study indicates that *Rauvolfia vomitoria* leaves contain tannins, phenols, flavonoids, saponin, steroids, alkaloids, cardiac glycosides and terpenoids. The phytochemicals detected in this study were the same with that which were earlier reported by Jeannette (Jeanette et al. 2009). These phytochemicals were detected in both aqueous and organic extracts indicating that both solvents were efficient in extracting the bioactive constituents. The presence of these phytochemicals may justify the inclusion of *Rauvolfia vomitoria* leaves in Traditional Medical Practices (Bemis et al. 2006, Campbell et al. 2006, Jun et al. 2013). Phenolic compounds are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Reports have implicated phenolic compounds as having health beneficial properties. This is because the compound is able to inhibit aldose reductase and xanthine oxidase enzymes. Furthermore, phenolic compounds because of their antioxidant activity (Balasundram et al. 2006, Devi et al. 2011, Urzula et al. 2015) have been known to possess a capacity to scavenge free radicals. This antioxidant property is principally due to their redox properties, which allow them to act as reducing agents and hydrogen donors. Reports in the literature indicates that the most widely used solvents for extracting phenolic compounds are water, ethanol, methanol, acetone, and their water mixtures (Bunea et al. 2012, Perez-Jimenez et al. 2008, Rabbah et al. 2010). The present study is in agreement with these studies. When the yields of the fractions were compared, result from the present study also indicates that aqueous fraction produced the highest yield whereas; ethylacetate fraction produced the lowest yield. This may suggest that solvent polarity may influence extraction of bioactive compounds. Data from this study suggest that all the fraction of *Rauvolfia vomitoria* leaves investigated showed significant aldose reductase inhibitory activity the ARI capacity was seen to be strongest with aqueous and chloroform fraction but weakest with hexane fraction. Ethylacetate and methanol fraction showed weak AR inhibitory activity. Significant research efforts have been going on all over the world on the investigation of naturally-occurring biomarkers responsible for inhibiting 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 (Ajani et al. 2009, Jung et al. 2008, Patel et al. 2012a, b). In one review that evaluated some indigenous plants reported to have potent ARI activities and their anticataract potentials against galactose-induced biochemical changes in rat lens organ culture, Patel *et al*., (2012a) reported that *Ocimum sanctum* with IC_{50} value of 20 µg/ml was the most potent AR inhibitor *in vitro*. Data from the present study indicates that the IC_{50} of fractions of *Rauvolfia vomitoria* ranged from 0.90 (chloroform fraction) to 3.25 mg/ml (ethylacetate fraction). The lowest AR inhibitory activity observed with hexane fraction support the fact that the solvent is least efficacious in extracting the antioxidant principles of the plant. This may due to low efficiency of solvation with the solvent. This is because hexane molecules are only proton acceptors while methanol and water are also proton donors.

The detection of phenols and flavonoids as reported in this study suggest that the aldose reductase inhibitory action of *Rauvolfia vomitoria* may be due to these phytoconstituents. Phenolic compounds are one of the most widely occurring groups of phytochemicals and are of considerable physiological and morphological importance in plants (Balasundram et al. 2006, Urzula et al. 2015). Studies have shown that many dietary polypenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamin E and C and thus might contribute significantly to protective effect *in vivo* (Catherine et al. 1997). The antioxidant activity of polyphenols has been attributed to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Amarowicz et al. 2004, Balasundram et al. 2006, Urzula et al. 2015). Zhang *et al*., (2003) 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. 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 (Patel et al. 2012b). Several flavonoids, such as quercitrin, guaijaverin and desmanthin have been tested and proven for their inhibitory activity against aldose reductase (Kato et al. 2009).

In this study, 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. Effect of different fractions on rat lens aldose reductase activity in Lineweaver-Burk plot using DL glyceraldehyde as a substrate was made between 1/velocity vs 1/DL-glyceraldehyde. The result of the kinetic studies suggest that the aldose reductase inhibitory compounds present in *Rauvolfia vomitoria* leaves extract/fractions can interact and inhibit lens aldose reductase enzyme in an uncompetitive manner thus 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. 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 (Copeland, 2005).

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. Data obtained in the present study about V_{max} and K_m indicates that aqueous fraction showed the maximum inhibitory potential whereas hexane fraction showed the least inhibition among the tested fraction.

Table 1. Yield of extract/ fraction (n=3± SEM).

Extract/ Fraction	Yield (%)
Methanol	7.75
Aqueous	9.17
Ethylacetate	3.33
Hexane	5.55
Chloroform	5.00

Table 2. Phytochemicals of *Rauvolfia vomitoria* Leaves.

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

Note: + denotes detected and – denotes not detected.

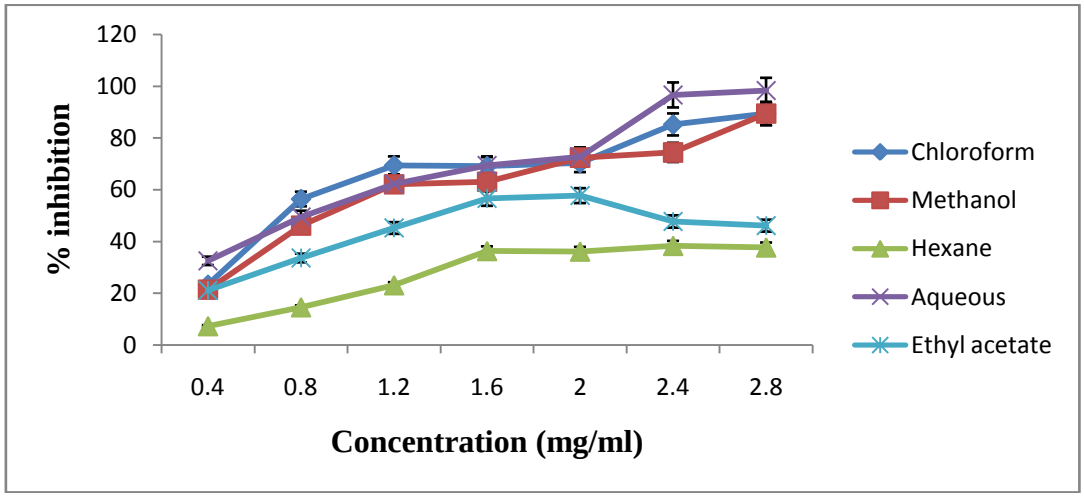

Fig. 1. Inhibitory effect of fractions of *Rauvolfia vomitoria* Leaves on the specific aldose reductase activity.

Table 3.IC_{50} of fractions of *Rauvolfia vomitoria* Leaves.

Fraction	IC_{50} (mg/ml)
Chloroform	0.90 ± 0.01^{a}
Methanol	1.13 ± 0.03^{b}
Ethyl acetate	3.25 ± 0.03^{c}
Aqueous	0.91 ± 0.01^{a}
Hexane	2.18 ± 0.31^{d}

♦ All values are expressed as mean± SD, n=3.

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

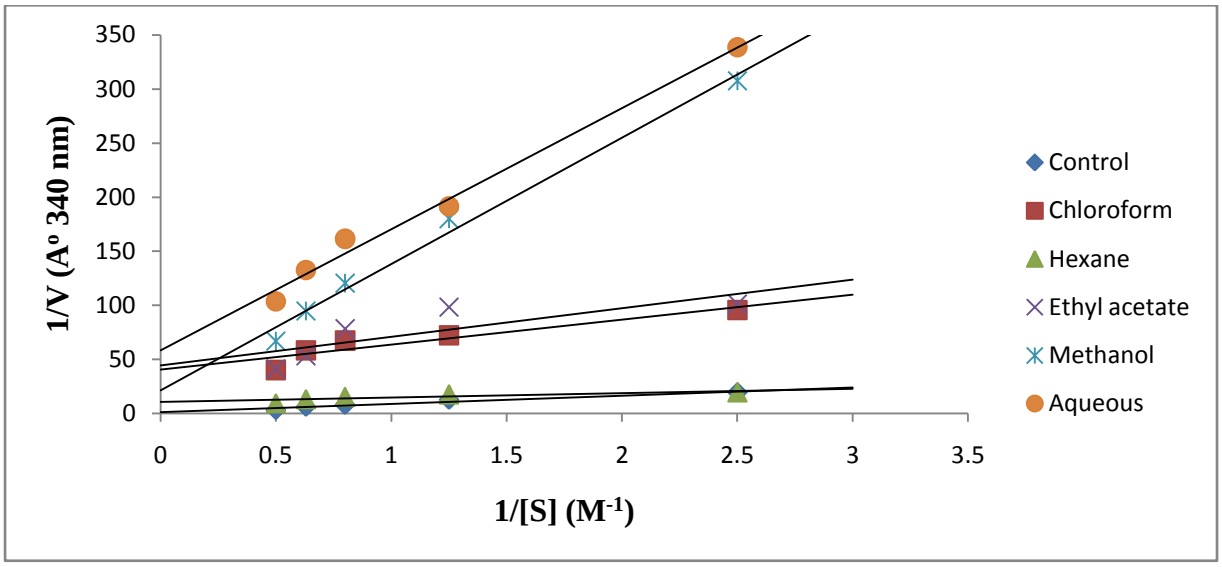

Figure 2. Effect of different fractions of *Rauvolfia vomitoria* Leaveson lineweaver-Burk plot of aldose reductase activity.

Table 4. Kinetics parameters of aldose reductase enzyme in the presence of different fractions of *Raufvolfia vomitoria* leaves.

Extract/fraction	**Vmax** (µm NADPH oxidised/hr/100mg protein)	**Km X 10^{-3}mM**
DL- glyceraldehyde	0.830 ± 0.031	6.313 ± 0.012
DL- glyceraldehyde + methanol	0.047 ± 0.011	5.502 ± 0.101
DL- glyceraldehyde + chloroform	0.025 ± 0.014	0.571 ± 0.068
DL- glyceraldehyde + hexane	0.096 ± 0.026	0.392 ± 0.024
DL- glyceraldehyde + ethylacetate	0.023 ± 0.010	0.595 ± 0.012
DL- glyceraldehyde + aqueous	0.017 ± 0.004	1.922 ± 0.138

♦ All values are expressed as mean ± SD, n=3.

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

Table 5. Free radical scavenging activity of extract and fractions of *Rauvolfia vomitoria* leaves.

Extract/Fractions	DPPH IC_{50} (mg/ml)	Metal chelating activity IC_{50} (mg/ml)	OH IC_{50} (mg/ml)	H_2O_2 IC_{50} (mg/ml)	Reducing power IC_{50} (%) (mg/ml)	NO IC_{50} (mg/ml)
Methanol	0.071 ± 0.011[a]	9.9	0.902 ± 0.131[a]	0.786 ± 0.011[a]	0.381 ± 0.046[a]	0.870 ± 0.036[a]
Aqueous	0.052 ± 0.012[a]	73.5	0.712 ± 0.051[b]	0.562 ± 0.012[a]	0.216 ± 0.016[b]	0.610 ± 0.066[a]
Ethylacetate	0.072 ± 0.026[a]	46.8	1.095 ± 0.087[a]	0.764 ± 0.051[a]	0.422 ± 0.016[a]	0.879 ± 0.052[a]
Chloroform	0.076 ± 0.022[a]	30.4	1.162 ± 0.031[a]	0.927 ± 0.055[b]	0.501 ± 0.042[a]	1.011± 0.042[a]
Hexane	0.106 ± 0.031[b]	17.4	2.106± 0.061[c]	0.916 ± 0.068[b]	0.612 ±0.016[c]	0.918 ± 0.024[a]

♦ All values are expressed as mean ± SD, n=3.

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

Reactive oxygen species (ROSs) are known to damage cellular membranes by inducing lipid peroxidation. They also can damage DNA, proteins, lipids and chlorophyll (Michalak, 2006). The most popular ROS are O_2^- (superoxide radical), H_2O_2 (hydrogen peroxide) and OH (hydroxyl radical) originating from one, two or three electron transfers to dioxygen (O_2). Under physiological conditions O_2^- is not very reactive against the biomolecules of the cell and in aqueous solutions at neutral or slightly acidic pH disproportionate to H_2O_2 and $O_2.H_2O_2$ is relatively stable and not very reactive, electrically neutral ROS, but is very dangerous because it can pass through cellular membranes and reaches cell compartments far from the site of its formation (Ramar et al. 2012). Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases. Cataract formation can be attributed to oxidative stress triggered by these reactive oxygen species (ROS). All the fractions investigated in this study were found to possess free radical scavenging activity. A cursory look at the IC_{50} values presented in Table 5 clearly showed that the aqueous fraction was more efficacious than all other fractions in scavenging DPPH, OH, H_2O_2, O_2^- and NO radicals. Data from the study thus suggest that the free radical scavenging capacities of the antioxidants present in the fractions of *Rauvolfia vomitoria* leaves reported here have strong relationships with the solvent employed in the fractionation. This also indicates that the antioxidant potential of compounds varies with the polarities of the solvents. This agrees with the observation of Moure *et al*., (2001). Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Anjali and Sheetal, 2013).

Result from this study indicates that the aqueous fraction of *Rauvolfia vomitoria* leaves when compared with other fractions investigated had a better reducing power and metal chelating capacity.

CONCLUSION

The present work confirmed that the leaves of *Rauvolfia vomitoria* are rich in phenolic compound. These compounds may be responsible for the strong free radical scavenging activity against DPPH, H_2O_2, NO, O_2^-, OH radicals exhibited by the leaves and also accounts for the reducing power and metal chelating activity reported in this study. The study also showed that the efficacy of *Rauvolfia vomitoria* extract in inhibiting aldose reductase enzyme is dependent on extractive solvents and that the aqueous fraction is more efficacious than other fractions in both inhibiting aldose reductase activity and in mopping up free radicals. Further investigation is ongoing in our laboratory to isolate and characterize the antioxidant constituents responsible for this action and to confirm these observed results *in vivo*.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Ajibade of Biochemistry Laboratory, Department of Biological Sciences, Landmark University, Omuaran for the kind assistance received and also to our research students who participated in the study.

REFERENCES

Abayomi, S. F., Adeleke, A., Bukunola, O. A., Olugbenga, O. O., Kuburat, T. O., and Emmanuel, O. A. (2015). Combined Administration of *Rauwolfia vomitoria* extract And Vitamin C Elicits Hypolipidemic and Antioxidative Potentials in High-Fat Fed Experimental Rats. *IOSR Journal of Pharmacy and Biological Sciences* (IOSR-JPBS) 10 (2): 52-60.

Ajani, E. O., Salako, A. A., Sharlie, P. D., Akinleye, W. A., Adeoye, A. O., Salau, B. A., and Adebawo. O. O. (2009). Chemopreventive and remediation effect of *Hydrocotylbonariensis Comm. Ex Lam* (Apiaceae) leave extract in galactose-induced cataract. Journal of Ethnopharmacol., 123:134-142

Akinmoladun, A. C., Ibukun, E. C., and Dan-Ologe, I. A. (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaenaodorata*. Sci. Res. Essays 2:191-194

Akpanabiatu, M. I., Umoh, I. B. and Eyong, E. U. (2006). Influence of *Rauwolfia vomitoria* root bark on cardiac enzymes o normal Wistar albino rats. Recent Prog Med Plants. 14:273–278.

Alexiou, P., Pegklidou, K., Chatzopoulou, M., Nicolaou, I.and Demopoulos, V. J. (2009). Aldose reductase enzyme and its implication to major health problems of the 21(st) century. Curr. Med. Chem., 16,734-752

Amarowicz, R., Pegg, R. P., Rahimi-Moghaddam, P., Barl, B. and Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 84:551-562.

Amole, O. O. and Onabanjo, A. O. (1999). Reserpine: The effect and uses of *Rauwolfia vomitoria*. *J Chemother*., 3: 45-47

Amole, O. O., Yemitan, O. K. and Oshikoya, K. A. (2009). Anticonvulsant activity of *Rauvolfia Vomitoria* (Afzel) *African Journal of Pharmacy and Pharmacology*. 3 (6): 319–322.

Angel, G. and Ricardo, O. G. (2005). Inhibition of aldose reductase by herbs extracts and naturalsubstances and their role in prevention of cataracts. *Rev. Cubana Plant Med*. 10:10-15.

Anjali, S. and Sheetal, P. (2013). Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. *Journal of Pharmacog*. and *Phytochemistry*., 2 (4):22-29

Balasundram, N., Sundram, K. and Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem*. 99:191-203.

Bemis, D. L., Capodice, J. L., Gorroocurn, P., Katz, A. E. and Buttyan, A. (2006). Anti-prostate cancer activity of -carboline alkaloid enriched extract from *Rauwolfiavomitoria*. Inter J Oncol.29:1065–1073.Biljana BP (2012), Historical review of medicinal plants' usage, *Pharma-cogn. Rev.* 6 (11): 1-5.

Biljana, B.P., (2012). Historical review of medicinal plants' usage, *Pharma-cogn. Rev*. 6(11): 1–5.

Bunea, C. I., Pop,N., Babeş, A. C., Matea, C., Dulf, F. V. and Bunea, A. (2012). Carotenoids, total polyphenols and antioxidant activity of grapes (Vitisvinifera) cultivated in organic andconventional systems. *Chem Central J.* 6:66-74.

Campbell, J. I. Mortensen, A. A. and Molgaard, P. (2006). Tissue lipid lowering-effect of a traditional Nigeian anti-diabetic infusion of *Rauwolfia vomitoria* foliage and *Citrus aurantium* fruit. *J Ethnopharmacol*. 10: 379-386

Catherine, R., Nicholas, M. and Geaoege, P. (1997). Antioxidant properties of phenolic compounds. *Trend in Plant Science* 2(4): 152-159

Copeland, R. A. (2005). Evaluation of Enzyme Inhibitors in Drug Discovery: *A Guide for Medicinal Chemists and Pharmacologists*. Wiley, New York. pp. 243-147.

Devi, S., Asha, J. and Ganjewala, D. (2011). Antioxidant Activities of Methanolic Extracts of Sweet- Flag (Acoruscalamus) Leaves and Rhizomes', *Journal of Herbs, Spices & Medicinal Plants.* 17: (1): 1 — 11

Decker, E. A. and Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *J Agricul. Food Chem*. 38:674-677.

Gabbay, K. H. (1975). Hyperglycemia, polyol metabolism, and complications of diabetes mellitus *Annu. Rev. Med*. 26**,** 521-536.

Garrat, D. C. (1964). The quantitative analysis of drugs, vol. 3. Chapman and Hall Ltd., Japan, pp.456-458.

Gui, T., Tanimoto, T., Kokai, Y. and Nishimura, C. (1995). Presence of a closely related subgroupin the aldo-keto reductase family of the mouse. *Eur. J. Biochem.* 227**,** 448-453.

Guzmán, A. and Guerrero, R. O. (2005). Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts *J. Rev Cubana Plant Med*. 10:3-4.

Halder, N., Joshi, S., and Gupta, S. K. (2003). Lens aldose reductase inhibiting potential of someindigenous plants. *J Ethnopharmacol*. 86:113-116.

Haque, S. E. and Gilani, K. M. (2005). Effect of Ambroxol, Spirulina and Vitamin E in naphthalene inducedcataract in female rats. *Ind J Physiol Pharmacol.* 49: 57–64

Harborne, J. B. (1973). Phytochemical Methods. Chapman and Hall td., London; 49-188Hayman Sand Kinoshita Jin H (1965). Isolation and Properties of Lens Aldose ReductaseJ. Biol. Chem.240:877-882.

Hayman, S. and Kinoshita, J. H. (1965). Isolation and Properties of Lens Aldose Reductase, *J. Biol. Chem*. 240: 877

Hemalatha, S., Lalitha, P. and Arulpriya, P. (2010). Antioxidant activities of the extracts of the aerial roots of *Pothosaurea* (Linden ex Andre). Der pharma chemical. 2(6):84-89

Ikewuchi, C. J. and Ikewuchi, C. C. (2009). Alteration of Plasma Lipid Profiles and Atherogenic Indices by *Stachytarpheta jamaicensis* L. (Vahl), Clinical Chemistry.18: 499-502.

Jeannete, Y. D. Gilles inès, F., Marie-claire, K., Josuéponé, W., Olivia, T. F., Jules-roger, K. and Blaise, M. M. (2009). *In vitro* antioxidant potential and phytochemical constituents of three Cameroonian medicinal plantsused to manage parasitic diseases. *Pharmacology online*, 1: 648-657

Jun, Y. U., Yan, M. and Jeanne Drisko, Q. C. (2013). Antitumor Activities of *Rauwolfia vomitoria* Extract and Potentiation of Carboplatin Effects against Ovarian Cancer *Current Therapeutic Research*. 75: 8–14.

Jung, H. A., Yoon, N. Y. and Kang, S. S. (2008). Inhibitory activities of prenylated flavonoids from *Sophora flavescens* against aldose reductase and generation of advanced glycationend products. *J Pharm. Pharmacol*. 60 (9): 1227-1236.

Kato, A., Yasuko, H. and Goto, H. (2009). Inhibitory effect of rhetsinine isolated from *Evodiarutae carpa* on aldose reductase activity. *J. Phytomedicine.*16 (2-3):258- 261.

Kweifio-Okai, G., Bird, D. and Field, B. (1995). Anti-inflammatory activity of a Ghanaianantiarthritic herbal preparation: III. *J Ethnopharmacol*. 46: 7-15.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, N. J. (1951). Protein measurement with folinphenol reagent. *J. Biol. Chem*. 193: 265-275.

Michalak, A. (2006). Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. *Polish J. of Environ. Stud.* 15 (4): 523-530.

Moure, A., Cruz, J. M., Franco, D. Domínguez, J. M., Sineiro, J., Domínguez, H., Núnez, M.J. and Parajó, C. (2001). Natural antioxidants from residual sources. *Food Chem.* 2001, 72: 145-171.

Oates, P. J. (2008). Aldose reductase, still a compelling target for diabetic neuropathy. Curr. Drug Targets 9: 14.

Okon, P. B. and Amalu, U. C. (2003). Using Weed to Fight Weed. Leisa Magazine. http://www.metafro.be/leisa/2003/194 21 pdf

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Jap. J. Nutri.* 44:307-315.

Patel, D. K., Kumar, R., Kumar, M., Sairam, K. and Hemalatha, S. (2012b). Evaluation of in vitro aldose reductase inhibitory potential of different fraction of *Hybanthusennea spermus* Linn F. Muell. *Asian Pacific Journal of Tropical Biomedicine*: 18:134-139.

Patel, D. K., Kumar, R., Sairam, K. and Hemalatha, S. (2012a). Pharmacologically tested aldose reductase inhibitors isolated from plant sources-A concise report Chinese *Journal of Natural Medicines* 10 (5): 0388-0392.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I. and Saura-Calixto, F. I. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Res Int.* 41: 274-285.

Rababah, T. M. Banat, F., Rababah, A., Ereifej, K.and Yang, W. (2010). Optimization of extraction conditions of total phenolics, antioxidant activities, and anthocyanin of oregano, thyme, terebinth, and pomegranate. *J Food Sci.* 75:626-632.

Ramar, M., Raman, T., Sivagnanam, B., Ganapasam, S. and Munuswamy, A. (2012). Curcumin prevents free radical-mediated cataractogenesis through modulations in lens calcium. *Free Radical Biology & Medicine* 48:483-492.

Rathnakumar, K., Jaikumar, S., Duraisami, R. and Sengottuvelu, S. (2013). Anticataract activity of *Ervatamiacoronaria*leaf extract on chemically induced cataractogenesis in rats. *Global J Res. Med. Plants & Indigen. Med*. 2 (4): 213-218.

Ruch, R. J., Cheng, S. J.and Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea*. J. Carcinogen.* 10:1003-1008.

Savithramma, N., Linga Rao, M. and Suhrulatha, D. (2011). Screening of Medicinal Plants for Secondary Metabolites Middle-East, *Journal of Scientific Research* 8 (3): 579-584.

Sofowara, A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria; 191-289.

Stosceck, C. M. (1990). Quantitation of protein. *Met. In. Enz.* 182: 50-69.

Suryanarayana, P., Anil Kumar, P., Megha, S, Mark, P. and Reddy, G. (2004). Inhibition of aldose reductase by tannoid principles of *Emblica officianalis*: Implications for prevention of sugarcataract*. Mol. Vis.* 10:148-154

Suzen, S., Bozkaya, P., Coban, T. and Nebioglu, D. (2006). Investigation of the in vitro antioxidant behavior of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparison with melatonin. *J. Enzyme Inhib. Med. Chem.* 21 (4**),** 405-411.

Suzen, S., Daş-Evcimen, N., Varol, P. and Sarikaya, M. (2007). Preliminary evaluation of rat kidney aldose reductase inhibitory activity of 2-phenylindole derivatives: affiliation to antioxidant activity. *Med. Chem. Res.* 16 (3), 112-118.

Trease, G. E. and Evans, W. C. (1989). Pharmacognosy. Bailliere Tindall London, Edn 11: 45-50.

Urszula, Z., Sylwia, M., Małgorzata, N. and Michaeł, S. (2015). The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi Journal of Biological Sciences,* in press.

WHO (2010). Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region 2001–2010. Document Reference AFR/RC50/Doc. 9/ R, World Health Organization, Harare, Zimbabwe.

Yu, W., Zhao, Y. and Shu, B. (2004). The radical scavenging activities of Radix puerariaeiso flavonoids: a chemilum inescence study. *Food Chem.* 86:525-529.

Zhang, H. Y., Sun, Y. M. and Wang, X. L. (2003). Substituent effects on O–H bond dissociation enthalpies and ionization potentials of catechols: a DFT study and its implications in the rational design of phenolic antioxidants and elucidation of structure–activity relationships for flavonoid antioxidants. *Chem. Eur. J*. 9 (2):502-508.

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