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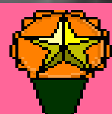
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Journal of Medicinal Plants Research

Full Length Research Paper

Chemical composition and evaluation of possible *alpha* glucosidase inhibitory activity of eight *Aloe* species

Abeer Mohamed El Sayed*, Shahira Mohamed Ezzat, Mohammed Nabil Khalil and Seham Salah El-Hawary

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Many of the health benefits associated with *Aloe* species have been attributed to the polysaccharides contained in the gel of the leaves. The aim of this study was to investigate the chemical composition as well as the biological evaluation of polysaccharides isolated from the leaves of eight different *Aloe* species, *A. vera* (A_1), *A. arborescens* (A_2), *A. eru* (A_3), *A. grandidentata* (A_4), *A. perfoliata* (A_5), *A. brevifolia* (A_6), *A. saponaria* (A_7) and *A. ferox* (A_8) grown in El Orman Botanical Garden, Giza, Egypt. Polysaccharides from the plants were isolated using hot extraction method and then hydrolyzed. The polysaccharide hydrolysates were identified using high performance liquid chromatographic technique. Maximum yield of total polysaccharides identified were obtained from A_7 (12.04%), A_1 (8.51%), A_8 (8.03%), A_2 (5.32%) and A_6 (2.18%) respectively. The isolated polysaccharides were tested for antihyperglycemic activity in alloxan-induced diabetic rats and *alpha* glucosidase inhibitory activity. Chromatographic investigation of the polysaccharides recorded the presence of 18 saccharides, glucuronic acid, stachyose, galacturonic acid, sucrose, glucose, xylose, galactose, rhamnose, mannose, arabinose, fructose polyol, mannitol and sorbitol in the eight *Aloe* species, but their quantitative composition differed among the species. Glucuronic acid, stachyose and galacturonic acid were the major detected saccharides. The results of the biological activities revealed significant antihyperglycemic activities with variable degrees. After four weeks of daily administration, polysaccharides isolated from *A. vera* (A_1) and *A. arborescens* (A_2) were the most active with 40 and 44% reduction in blood glucose level, respectively. All the tested polysaccharides showed significant *alpha* glucosidase inhibitory activity with IC50 (µg/ml) 11.70, 14.60 and 15.80 for A_7, A_6 and A_1 respectively. In conclusion, the tested polysaccharides contribute to the antidiabetic action of these *Aloe* species.

Key words: *Aloe*, *alpha* glucosidase inhibitors, antidiabetic, polysaccharide.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by high blood glucose levels (American Diabetes Association, 2009). Type-1 diabetes is an autoimmune disease characterized by T-cell mediated destruction of the

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pancreatic beta cells. In type-2 diabetes, there is a gradual development of insulin resistance and beta cell dysfunction, strongly associated with obesity and a sedentary lifestyle (Zimmet et al., 2001). Due to a higher incidence of the risk factors, the prevalence of diabetes is increasing worldwide, but more evidently in developing countries (Sherif and Sumpio, 2015). In the Middle East and North Africa Region, 1 in 10 adults have diabetes (Klautzer et al., 2014); the region has the highest prevalence of diabetes (10.9%). The International Diabetes Federation (IDF) estimated that there are 34.6 million people with diabetes in the Middle East and North Africa, a number that will almost double to 67.9 million by 2035 if concerted action is not taken to tackle the risk factors fuelling the epidemic of diabetes throughout the region (IDF, 2013). Egypt is now ranked eighth highest in the world in terms of the disease (IDF, 2013).

Diabetes mellitus is a chronic state of hyperglycemia which results in the development of important complications (Metelko and Brkljacic, 2013), where body cells cannot uptake and utilize glucose; therefore breakdown of fats increase with production of fatty acids and ultimately ketone bodies, this disorder is accompanied by decrease in protein synthesis (American Diabetes Association, 2009). Nevertheless, diabetes mellitus is an illness that is found among the non-transmissible chronic diseases and it is considered a health challenge due to the great number of existing cases, its growing contribution to general mortality, its identification as the most frequent cause of premature disability, its complexity, and the high cost of its treatment (Córdova et al., 2008).

In recent years, several synthetic drugs have been developed to combat against diabetes, but the situation has only marginally improved. Furthermore, these synthetic drugs are not able to combat with all the pathological complications and mostly palliative in their effect. Herbal medications have been used successfully by mankind from ancient time to counteract diabetes and its associated complications (Bailey and Day, 1989). Long-term complications of diabetes include retinopathy and loss of vision; nephropathy cause renal failure; peripheral neuropathy with risk of foot ulcers; and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular and sexual dysfunction. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (American Diabetes Association, 2009).

A number of reviews on medicinal plants used in the management of diabetes in different parts of the world, as well as those used specifically in certain regions, such as in West Africa, Central America and Asia exist (Ezuruike and Prieto, 2014). These include *Allium sativa, Gymnema sylvestre, Ocimum sanctum, Pterocarpus marsupium, Trigonella foenum graecum* and *Tinospora cordifolia*. Moreover, most of these plants were clinically tested; the results were recommendation that physicians can depend on herbs in alleviating diabetes and its complications (Ghorbani, 2013; Pandey et al., 2011). Therefore, these herbal plants could be an alternative therapy for diabetes and its complications (Khan et al., 2012).

Polysaccharide hydrocolloids including mucilages, gums and glucans are abundant in nature and are commonly found in many higher plants (Clifford et al., 2002). These polysaccharides constitute a structurally diverse class of biological macromolecules with a broad range of physicochemical properties which are widely used for applications in pharmacy and medicine (Franz, 1989).

The genus *Aloe* (family Xanthorrhoeaceae, subfamily Asphodeloideae) contains over 500 species of flowering succulent plants, among which the most widely known species is *A. vera* (L.) Burm. f. or true aloe, which is one of the most important pharmaceutical herbs (Grindlay and Reynolds, 1986; Boudreau and Beland, 2006). Different Aloe species have been used in the treatment of a variety of disorders including infections, dermatologic conditions and also used as a laxative since ancient times in the Greek Herbal of Dioscorides (ca 70 AD) the U.S. pharmacopoeia in 1820 (Davis, 1997; Park and Lee, 2006; Ulbricht et al., 2008). These plants have long meaty thick leaves with twisted sides which end in thorns (Grindlay and Reynold, 1986; Heggars et al., 1993). The substance inside the leaf called gel consists of 99% water with long chain polysaccharide, of acetylated glucomannan kind, and other carbohydrates. It was claimed that the polysaccharides in *Aloe vera* L. gel had therapeutic properties such as anti-inflammatory, wound healing, promotion of radiation damage repair, antidiabetic and anti-neoplastic activities (Chun-hui et al., 2007). The Aloe leaf structure is made up of three layers: (i) rind, the outer protective layer; (ii) sap, a layer of bitter fluid which helps protect the plant from animals; (iii) mucilage gel, the inner part of the leaf that is filleted out to make *A. vera* gel. The authors have cut the leaves open and take the inner content so it will include the mucilage and the sap.

During the past 20 years, reports have shown that *Aloe* preparations have beneficial therapeutic effects on diabetes. *Aloe* spp. is documented in ethnobotanical survey as one of the potential anti-diabetic plants (Gbolade, 2009). The dried sap, of the *Aloe* plant had been used for diabetes in the Arabian Peninsula (Ghannam et al., 1986). Its ability to lower the blood glucose was studied in five patients with noninsulin-dependent diabetes and in Swiss albino mice made diabetic using alloxan (Ghannam and Geissman, 1986). The hypoglycemic activity of *Aloe* species was first demonstrated by Agrawal (1985). Since then, the antidiabetic effects of *Aloe* preparations have been demonstrated in diabetic patients (Ghannam et al., 1986; Ajabnoor 1990; Bunyapraphatsara et al., 1996; Yongchaiyudha et al. 1996), and in alloxan- or streptozotocin-induced diabetic animal models (Beppu et

al., 1993; Rajasekaran et al., 2004, 2005, 2006; Beppu et al., 2006). *A. vera* gel was administered to STZ-induced diabetic rats decreased fasting blood glucose levels and improved the levels of the antioxidant enzyme (Nwanjo, 2006); moreover aqueous extract prevented the onset of hyperglycaemia in alloxan-induced diabetic rabbits (Akinmoladun and Akinloye, 2007); a polyphenol-rich *A. vera* extract (350 mg kg^{-1}) with known concentrations of aloin (181.7 mg g^{-1}) and aloe-emodin (3.6 mg g^{-1}) was administered to insulin resistant mice for 4 weeks improved insulin tolerance and fasting blood glucose levels (Perez et al.,2007).

The principle aim of this work was to evaluate the qualitative and quantitative components of the polysaccharide isolated from eight *Aloe* species growing in Egypt using HPLC analysis. In addition, the possible antidiabetic effect of the purified mucilage against alloxan-induced diabetes in rats and their *alpha*-glucosidase inhibitory activity were evaluated.

MATERIALS AND METHODS

Plant material

The leaves of *Aloe vera* L. Burm. f. (A_1), *Aloe arborescens* Mill. (A_2), *Aloe eru* A. Berger (A_3), *A. Aloe grandidentata* Salm-Dyck (A_4), *Aloe perfoliata* L.(A_5), *Aloe brevifolia* Mill. (A_6), *A. saponaria* L. (A_7) and *A. ferox* Mill. (A_8) were collected in April 2013 from El-Orman Botanical Garden, Giza, Egypt. The plants were kindly authenticated by Dr. Mohamed El-Gebaly, Botany Specialist. Voucher specimen 1842013 is kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Extractions of polysaccharides were conducted in Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt in 2014. HPLC analysis of polysaccharides were performed at Food Technology Research Institute, Agriculture Research center, Ministry of Agriculture and Land Reclamation, Giza, Egypt in 2015.

Extraction of polysaccharides

The polysaccharides were isolated from the fresh leaves of A_1, A_2, A_3, A_4, A_5, A_6, A_7 and A_8 (Figure 1), by cutting each one of them into small pieces with size range (1-1.5 cm long × 1-2 cm wide). 1 kg of each sample was extracted by hot extraction methods, with 10 L of boiling distilled water, filter while hot through a muslin cloth. The aqueous extract was concentrated to one third to make the aqueous solution saturated with polysaccharides and hence facilitate the polysaccharide precipitation and reduce volume of the used acetone. The polysaccharides were then precipitated in each case by addition of three times of acetone. The polysaccharide was collected by centrifugation at 20°C and 18,000 rpm for 1 h (Megafuge 1.0 R; Heraeus, Haneau, Germany). The mucilage was then vigorously stirred in absolute acetone, filtered and then dried in a vacuum desiccator over anhydrous calcium chloride. The percentage yields of polysaccharides were as follows 0.86, 0.49, 0.20, 0.15, 0.6, 0.25, 1.15 and 0.7% of A_1, A_2, A_3, A_4, A_5, A_6, A_7 and A_8, respectively.

Chemicals

Authentic sugars for high performance liquid chromatography (HPLC) (sucrose, D-glucose, D-sorbitol, mannitol, galacturonic acid, glucuronic acid, stachyose, xylose, galactose, rhamnose, mannose and arabinose, all with a purity exceeding 99.0% were purchased from Sigma Aldrich (Steinheim, Germany), and D-fructose was purchased from Merck (Darmstadt, Germany). Alloxan (Sigma Co., USA) freshly dissolved in saline was used for induction of diabetes. Biodiagnostic kit for assessment of blood glucose and glutathione levels (Epico, Egyptian Int. Pharmaceutical Industries Co.,A.R.E.). Carboxymethylcellulose sodium (CMC-Na) was purchased from Acros Organics (NJ, USA), Metformin (1,1-dimethylbiguanide hydrochloride) (Sigma Co., USA). α-Glucosidase enzyme from brewer's yeast (EC 3.2.1.20), the substrate; *p*-nitrophenyl α-D-glucopyranoside (p-NPG), and Phosphate buffer (pH 6.8) were purchased from Sigma Chemical Co., (St Louis, MO 63103 USA). The positive control, acarbose was purchased from Bayer Pharmaceuticals Pvt., Ltd (USA).

Acid hydrolysis

Ten milligram of each of the mucilage of each plant under investigation were separately heated in 2 ml of 0.5 M sulphuric acid in a sealed test tube for 20 h in a boiling water bath. At the end of the hydrolysis any precipitate was filtered off. The filtrate was freed of sulphate ions by precipitation with barium carbonate. The hydrolsates were separately concentrated under vacuum at a temperature not exceeding 40°C to a syrupy consistency. It was diluted with 10% isopropanol in water to about 10 ml. The individual authentic reference sugars were dissolved in distilled water. All samples were filtered through microfilter (0.45 µm) and stored in vials to be used in HPLC investigation (Gertz, 1990).

HPLC analysis of the polysaccharides

HPLC analysis was used to determine the free sugars in the isolated polysaccharide from the different plants under investigation qualitatively and quantitatively. HPLC analysis was performed on operating system model Shimadzu (SCL-10AVP) equipped with RI detector RID-10A and high pressure pump LC-10ADVP. Separation and determination were performed on column sugar SC1011 (Shodex SUGAR Series) (Particle size 6 µm; length 300 mm; diameter 8 mm) using deionized water as mobile phase with 1ml/min flow rate. Ten milligram of each residue of isolated polysaccharides, as well as of the aforementioned individual authentic sugars was separately dissolved in 1 ml of deionized water. 10 µl of each sample was injected into HPLC using an SGE syringe (Syringe Perfection, Australia). Quantitative determination was based on peak area measurement while qualitative identification was carried out by comparison of the retention times of the peaks with those of the authentic sugars (Figures 2A to D).

Evaluation of the anti-hyperglycemic activity

Animals

Adult male rats of Sprague-Dawley strain (130 to 150 g) were obtained from the laboratory animal facility of the National Research Center, Dokki, Giza. Animals were housed in steel cages under standard conditions, animals were housed in a temperature (20 to 23°C) and humidity (approximately 50%)-controlled colony room on a 12:12-h light: dark schedule. To facilitate measures of food intake, rats were housed conventionally in individual stainless steel hanging wire-mesh cages, and fed with standard pellets, commercial standard chow (18 % protein; Global 2018, Harlan Teklad, Madison, WI) and water *ad libitum*. All experimental procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care, and were

approved by the Ethics Committee (No. 9-031) in accordance with recommendations for the proper care and use of laboratory animals (NIH Publication No. 80-23; revised 1978).

Induction of diabetes mellitus in rats

Diabetes was induced intraperitoneally with a single dose of alloxan (150 mg/kg body weight). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of 140 mg/dl were included in the study. Treatment with polysaccharides samples was started 48 h after alloxan injection. Hyperglycemia was assessed after 72 h by measuring blood glucose and after 2 and 4 weeks intervals (Eliasson and Samet, 1969).

Assessment of anti-hyperglycemic effect

The eight polysaccharide samples (A_1-A_8), isolated from each of the eight tested species were tested for their anti-hyperglycemic activity over 28 days at a dose of 250 mgkg^{-1} body weight. The doses of the polysaccharides were determined as 250 mg kg^{-1} on the basis of a preliminary short-term pilot study with a range of variable doses. The control diabetic group in each experiment received a single daily dose of 1% Carboxymethylcellulose sodium (CMC-Na), as a vehicle for the tested sample. The vehicle, metformin and polysaccharides were given orally by gavage as single daily treatments for 4 weeks. At the end of each study period, blood samples were collected from the retro-orbital venous plexus through the eye canthus of anesthetized rats after an overnight fast. Serum was isolated by centrifugation, and the blood glucose level was measured by enzymatic colorimetric method at zero time, at days 14 and 28 from the treatment (Trinder, 1969).

Assay for alpha-glucosidase inhibitory activity

The assay was performed to measure the *alpha*-glucosidase inhibitory activity of the polysaccharide samples isolated from the eight *Aloe* species (A_1-A_8). The enzyme inhibition study was carried out spectrophotometrically in a 96-well microplate reader using a procedure reported by Li et al. (2005). Enzyme assay of 220 µl were prepared using phosphate buffer (50 mM, pH 7). It contained 50 µl *p*- nitrophenol-α-glucoside (α-NPG) (1.3 mM), 150 µl enzyme (0.026 U) and 20 µl of each polysaccharide sample. The inhibitor was replaced by water in case of incubations performed to determine 100% activity. Blank incubations were performed to cancel the color of the polysaccharide. Polysaccharide (10 mg) was dissolved in 600 µl hot water (90°C). The assays were incubated at 37°C for 7 min and color was measured at λ_{max}410 nm using a *Spectra Max 340* (Molecular Devices, USA) spectrometer. Controls contained the same reaction mixture except the same volume of phosphate buffer was added instead of the inhibitor solution. Acarbose (Bayer Pharmaceuticals Pvt., Ltd .USA) was dissolved in water and used as a positive control.

$$\text{Inhibition \%} = [(A_B - A_A)/A_B] \times 100\%$$

Where A_B is the absorbance of the control sample and A_A is the absorbance of test sample. The fifty percent inhibitory concentration (IC_{50} µg/ml) of the active samples against yeast glucosidase was calculated.

Statistical analysis

The statistical comparison of difference between the control group and the treated groups was carried out using two-way ANOVA followed by Duncan's multiple range test.

RESULTS

It was obvious from Table 1 and Figures 1 and 2A to D that all the investigated *Aloe* species were rich in polysaccharides; A_7, A_1 and A_8 had the highest polysaccharide contents (12.0, 8.5and 8.0%) respectively. While A_5 and A_4 had showed the lowest polysaccharide contents (0.5 and 1.2%) respectively. High concentration of glucuronic acid (2.1, 1.5 and 1.3%), was detected in A_7, A_8 and A_2 respectively. Moreover, Stachyose was major identified sugar as (5.4, 2.6 and 1.7%) in A_8, A_7 and A_1 respectively. Galacturonic acid was prevailed as major sugar (4.5, 1.45 and 0.3%) identified in A_1, A_7 and A_5 respectively. Whereas the lowest concentration of most of the sugars were found in A_2, A_3, A_4 and A_6. Chemical composition differs among the species of *Aloe*. For example, *A. barbadensis* Miller may contain 2.5 times the aloe-emodin of *A. ferox* Miller; the time of harvest may a factor into the composition variation. *Aloe ferox* is similar to *A. vera* but has many times more nutritional and medicinal value than *A. vera* (Bhaludra et al., 2013). However, discrepancies exist regarding the composition of polysaccharide species and an understanding of pulp structure in relation to its chemical composition has been lacking (Ni.et al., 2004).

HPLC analysis of the polysaccharides (Table 1) revealed that their composition is more or less similar qualitatively, except for the low detection of stachyose in A_1 and A_5. While, maltose greatly presented as (2.8 and 2.5%) only in A_2 and A_7 respectively. On the other hand, the eight samples differ greatly quantitatively in the ratios of the different sugars. Quantitative HPLC analysis revealed that *A. saponaria* (A_7) mucilage hydrolysate contained mainly glucuronic acid, stachyose, galacturonic acid, maltose and glucose in ratio 2.0, 2.4, 1.6, 2.5 and 0.5 % respectively (Figure 2D). *A. vera* (A_1) mucilage hydrolysate contained mainly glucuronic acid, stachyose, galactouronic acid, xylose and galactose in the ratio 1.0, 1.5, 4.4, 0.1 and 0.2% respectively. Glucuronic acid was the dominant sugar in all *Aloe* species investigated ranging from 486 to 20070 mg kg^{-1} other quantitatively important saccharides were galactouronic acid stachyose, sucrose, maltose, galactose and glucose.

As many species of *Aloe* such like *A.* v*era* was reported to cause hypoglycemic effects (Ghannam et al., 1986; Ajabnoor, 1990), it was felt that it would be interesting to study the influence of polysaccharides of different *Aloe* species *A. vera, A. arborescens, A. eru, A. grandidentata, A. perfoliata*, *A. brevifolia, A. saponaria* and *A. ferox* on diabetic rats model that was induced by intraperitoneal injection with alloxan. So, the aim of our study was to shed the light on the antidiabetic effect of the isolated polysaccharide of the eight species under investigation and evaluate their ability to inhibit *alpha*-glucosidae

Table 1. HPLC analysis of the isolated polysaccharides.

Identified sugar	R_t (min)	Concentration of saccharides (ppm)*							
		A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8
Glucuronic acid	5	10180±46.0	7781.5±311.5	5577.5±175.5	995.5±215.5	486±13.5	1055.5±261.5	20070±116.3	14959.5±274.5
Stachyose	5.42	15900.5±549.5	6185.5±167.5	6257.5±1236.5	1715±95	1171.5±126.5	1326±163	24772±1571	54868.5±414.5
Galacturonic acid	5.67	44420.5±569.5	6224±811	2339±322	884±75	3314±60	1329±364	16823±243	834.5±644.5
Sucrose	6.2	3300.5±730.5	10181±533	103±38	111±60	6±0.0	842±31.9	2997±23.2	145±80.0
Maltose	6.46	1298±1192	15029±492	299±132	221.5±32.5	12±0.0	1619±40.0	25364±551.0	521±0.0
Lactose	6.85	211±91.0	704±14.1	380.5±89.5	650.5±11.5	20±4.0	809±86.0	2163.5±611.5	198.5±70.5
D-glucose	7.75	499.5±139.5	143±43	531±195	867±61	112.5±2.5	140±78.0	3185.5±451.5	438.5±287.5
Glucose	7.78	674.5±105.5	198±16.1	398.5±93.5	795.5±338.5	12±3	168.5±72.5	6377±612	269.5±15.5
Xylose	8.7	1614.5±124.5	659±40.0	728±59	629.5±12.5	5.5±1.5	372.5±221.5	1497.5±265.5	411±84
Galactose	8.9	2826.5±316.5	500.5±14.5	1068±39	238.5±28.5	3.5±0.5	456±152	2437±512	616.5±61.5
Rhamnose	9.06	1990.5±369.5	477.5±181.5	1728.5±914.5	340±36	13.5±7.5	431.5±346.5	764±89	477.5±66.5
Mannose	9.18	650±20.0	1271.5±740.5	1001.5±66.5	1619.5±67.5	19±4	3938.5±699.5	1034.5±207.5	706.5±12.5
Raffinose	10.38	539.5±170.5	494.5±396.5	661±57	467.5±96.5	10.5±2.5	790.5±89.5	3965±1153	838±12.6
Arabinose	10.51	473±83.0	3201±90.0	277.5±32.5	1162±70	6±1.0	797±231.0	2417.5±251.5	434±2.0
Fructose	10.63	298.5±8.5	104.5±21.5	198.5±42.5	447±6	3.5±0.5	302.5±91.5	4531.5±157.5	400.5±126.5
Mannitol	14.02	201±39.0	15.5±2.5	66±19	133.5±52.5	6.5±4.5	576.5±57.5	573.5±4.5	1441±106
Sorbitol	18.1	35±25.0	8±3	40±21	76±11	1.5±0.5	106±20	234.5±153.5	1051±540
Ribose	19.51	39.5±0.5	23±5	235±126	679. ±433.5	6.5±4.5	280±64	1243.5±177.5	1771±1628
Total in ppm		85152.5	53201	21890	12033	5210	15340	120450.5	80382
Total in %		8.51525	5.3201	2.1890	1.2033	0.5210	1.5340	12.04505	8.0382

*Average of duplicate measurements; A_1 = *Aloe vera,* A2 = *Aloe arborences,* A_3 = *A. ero;* A_4 = *A. grandidentata,* A5 = *A. perfoliata,* A_6 = *A. brevifolia* A_7 = *A. saponaria* A_8 = *A. ferox*

activity. Although some of the investigated species were reported to have antidiabetic effects such as *A. vera* and *A. arborescens* the other species were not tested for this effect.

Oral administration of polysaccharide samples isolated form the eight studied *Aloe* species (250 mg kg^{-1} body weight) reduced the blood glucose levels starting from week 2 and continued to week 4 (Table 2). Moreover, the effect of *Aloe vera* and *A. barbadensis* was the most pronounced as they induced 40 and 44% reduction in glucose levels, respectively. This was in agreement with the reported literature (Ghannam et al., 1986; Ajabnoor, 1990). Standard samples, glucuronic acid, stachyose, galacturonic acid and mixture of these compounds at a dose of 50 mg kg^{-1} body weight were exhibited anti diabetic activity, reduced the blood glucose level by 22, 30, 23 and 24% respectively. From results presented (Table 2), synergistic effects of all sugar components of mucilage that isolated from different *Aloe* species as a hypoglycemic agents (to reduce the blood glucose level) was prevailed.

Our *in vitro* studies, had shown that the polysaccharide samples isolated form the eight studied *Aloe* species had moderate inhibitory activities on the *alpha* -glucosidase enzyme (Table 3), thus they can be used as useful functional foods according to their association with reduced risk of diabetes.

DISCUSSION

Type 2 diabetes mellitus is a complex disease (Leahy, 2005), which is characterized by

Table 2. Effect of mucilage samples isolated from the eight *Aloes* species (A1-A8) on blood glucose level in diabetic rats.

Dose mg/kg	Blood glucose level		
	Zero time	2 weeks	4 weeks
Diabetic non-treated	249.6±9.3	259.1±9.5	263.1±10.2
Diab. + A_1	256.7±9.6	203.1±8.1*	153.6±6.9*(40%) [a]
Diab. + A_2	253.6±8.7	216.2±8.3*	141.9±6.3*(44%) [a]
Diab. + A_3	258.3±9.2	209.3±7.9*	161.5±7.5*(37%) [a]
Diab. + A_4	263.7±9.6	221.4±8.2*	192.1±7.8*(27%) [a]
Diab. + A_5	257.2±8.9	198.2±7.6*	179.6±6.4*(30%) [a]
Diab. + A_6	255.8±9.4	210.7±10.3*	171.2±7.1*(33%) [a]
Diab. + A_7	262.4±9.8	223.6±9.2*	185.9±7.6*(29%) [a]
Diab. + A_8	248.9±8.6	206.3±9.8*	158.4±6.8*(36%) [a]
Diab. + Glucuronic acid	247.9±7.9	226.1±8.5*	193.6±8.2*(22%) [a]
Diab. + Stachyose	262.3±9.4	232.9±8.6*	182.4±8.1*(30%) [a]
Diab. + Galacturonic acid	248.7±8.2	221.8±9.7*	191.2±7.6*(23%) [a]
Diab. + Mixture	251.8±8.6	206.3±9.3*	168.9±7.4*(24%) [a]
Diab. + Metformin	263.7±9.4	181.2±7.2*	96.4±3.6*(63%) [a]

Diab., diabetic, the mucilage samples were injected at a dose of 250 mg kg^{-1} body weight; Glucuronic acid, Stachyose, Galacturonic acid and mixture (glucuronic, stachyose, galactouronic acid) were injected at a dose of 50 mg kg^{-1} body weight; metformin is injected at a dose 100 mg kg^{-1} body weight; (n=6). * Significantly different from zero time at $p<0.01$; a % of change, calculated as regards the effect at zero time A_1 = *A. vera,* A2 = *A. arborences,* A_3 = *A. ero* A_4 = *A. grandidentata,* A_5 = *A. perfoliata,* A_6 = *A. brevifolia* A_7 = *A. saponaria* A_8 = *A. ferox.*

Figure 1. Photos of the eight Aloe species.

abnormal hepatic glucose output, insulin resistance and impaired insulin production (Golay, 1988; Fujimoto, 2000). It may be assumed that in individuals with type 2 diabetes, many metabolic pathways are likely to be

Table 3. Inhibitory effects of mucilage samples isolated from the eight *Aloes* species (A_1-A_8) on *α*-glucosidase enzyme.

Tested sample	IC 50[a] (µg/ml)
A_1	15.80± 0.9
A_2	16.30± 1.3
A_3	25.00± 1.5
A_4	46.40± 3.1
A_5	18.10± 1.1
A_6	14.60± 1.4
A_7	11.70± 0.7
A_8	61.48± 3.2
Acarbose	2.6 ± 0.07

[a] average of duplicate measurements. A_1 = *A. vera,* A2 = *A. arborences,* A_3 = *A. ero* A_4 = *A. grandidentata,* A_5 = *A. perfoliata,* A_6 = *A. brevifolia* A_7 = *A. saponaria* A_8 = *A. ferox.*

affected and presumably play a role in their overall metabolic dysfunction. Thus, the identification of new biomarkers and pathways can improve the characterization of pathophysiological alterations associated with the disease condition (Bain et al., 2009). In non-treated mature-onset diabetics or most subjects with diabetic coma, basal glucuronic acid excretion is reduced (Fishman, 2014). Non treated diabetic patients have been demonstrated to have a reduced glucuronidation capacity after a menthol load, a finding related to the diminished glycogen content of the liver (Fishman, 2014). The glucuronidation capacity is actually reduced in diabetic patients (Muller-Oerlinghausen et al., 1967).

Glucuronidation is the addition of glucouronic acid to a xenobiotic such as drugs, pollutants, bilirubin, androgens, estrogens, mineralocorticoids and fatty acid derivatives. The human body uses glucuronidation to make xenobiotics and their metabolites more water-soluble, and, in this way, allow for their subsequent elimination from the body through urine or feces (*via* bile from the liver) (King et al., 2000). However, glucuronides may be hydrolyzed by β-glucouronidase present in intestinal microflora to the respective aglycone, which may be reabsorbed from the intestine and translocated back to the liver. On the other hand, glucuronic acid is a precursor of ascorbic acid as well as a powerful detoxifier this, binds the toxins entering the liver and eliminates them out of the body via kidneys.

Mucilages are classified as soluble fibers which imbibe water and swell to form gel or colloidal gel. This solution increases the viscosity of the intestinal content and reduces absorption and entraps sugars (Kaczmarczyk et al., 2012; Lattimer and Haub, 2010). Mucilage is not hydrolyzed in intestine to give their constituent sugar rather they pass the intestine intact, absorbs water and swell; the effect which adds bulk to the stool (Satija and Hu, 2012). Partial hydrolysis of plant polysaccharides by intestinal bacteria and subsequent release of glucuronic acid in large intestine may occur; however, kinetic studies show that plant polysaccharides could serve as potential glucuronic acid source. Benefits of plant polysaccharides for diabetic patients are enumerated in many publications. Previous studies reported that polysaccharides could reduce serum lipid in hyperglycemic rats through its antioxidant properties (Xu et al., 2010) and could have antioxidant and antihyperglycemic effects on diabetes mellitus induced by alloxan in rats (Long et al., 2012). In addition, polysaccharides could partially recover the secretory function of islet cells by antioxidant effects. Polysaccharides have antihyperglycemic effect in type 2 Diabetes mellitus and may partially recover the secretary function of islet cells, leading to elevated serum levels of insulin and amylin and improved glucose metabolism regulation (Li et al., 2012)

Postprandial hyperglycemia is the key problem in diabetes mellitus. Ingestion of carbohydrate rich diet causes elevation in blood glucose level by the rapid absorption of carbohydrates in the intestine aided by the action of glycoside hydrolysis which breaks complex carbohydrates into absorbable monosaccharides (Winchester and Flee, 1992). Thus, use of glycosidase inhibitor such as *alpha*-glucosidase inhibitors would be a prospective therapeutic agent for the effective management of diabetes. *Alpha*-glucosidase inhibitors inhibit the disaccharide digestion and impede the postprandial glucose excursion to enable overall smooth glucose profile (Casirola and Ferraris, 2006). Several *alpha*-glucosidase inhibitors have been isolated from medicinal plants to develop as an alternative drug with increased potency and lesser adverse effects than the existing drugs (Toeller, 1994). Our *in vitro* studies, had shown that the polysaccharide samples isolated form the eight studied *Aloe* species had some inhibitory activities on the *alpha*-glucosidase enzyme (Table 3). They could act by impeding the binding of the substrate to the enzyme. This will reduce hydrolysis of disaccharides and oligosaccharides present in food, which in turn will reduce the amount of free sugars absorbed, consequently, reduces postprandial increase in blood glucose.

Conclusion

Glucuronic acid, and galacturonic acid the major identified sugar acids in all polysaccharide samples of *Aloe* species could be attributed to the observed anti hyperglycemic effect. Polysaccharides constituents of eight *Aloe* species have synergistic *in vivo* anti hyperglycemic activity in alloxan-induced diabetic rats. Moreover, they have moderate *alpha*-glucosidase inhibitory activity. Therefore, these plant polysaccharides can be used as useful functional foods to control elevated blood glucose levels in diabetic patients. Traditional anti-diabetic plants might provide new antidiabetic

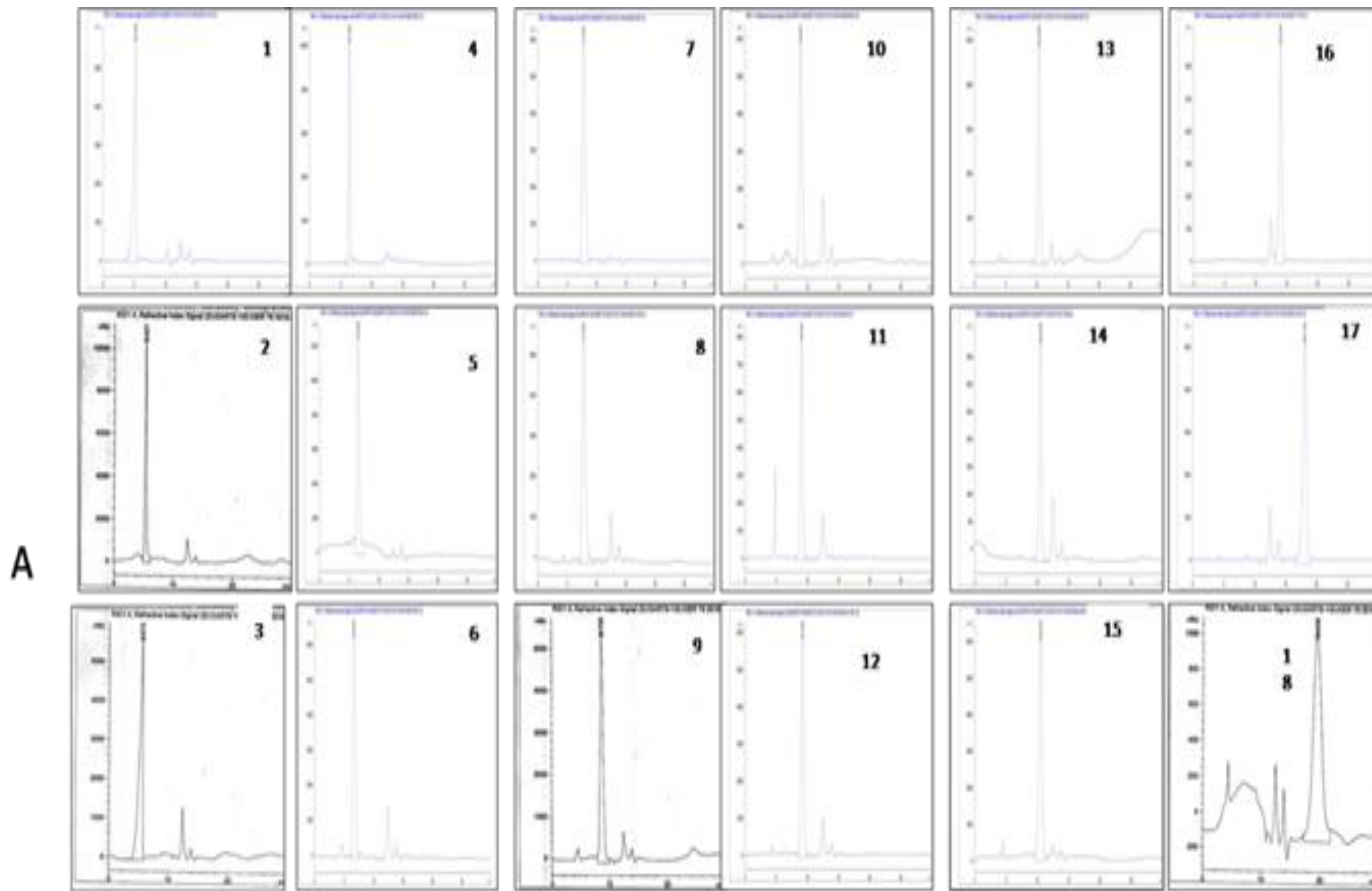

Figure 2A. Chromatograms of HPLC analysis of standard authentic sugars. 1= Glucuronic acid; 2=Stachyose; 3= Galacturonic acid;4=Sucrose; 5=Maltose;6=Lactose ; 7=D-glucose ;8=Glucose; 9=Xylose; 10=Galactose; 11= Rhamnose; 12=Mannose; 13=Raffinose ;14=Arabinose;15=Fructose; ;16=Mannitol;17=Sorbitol;18=ribose.

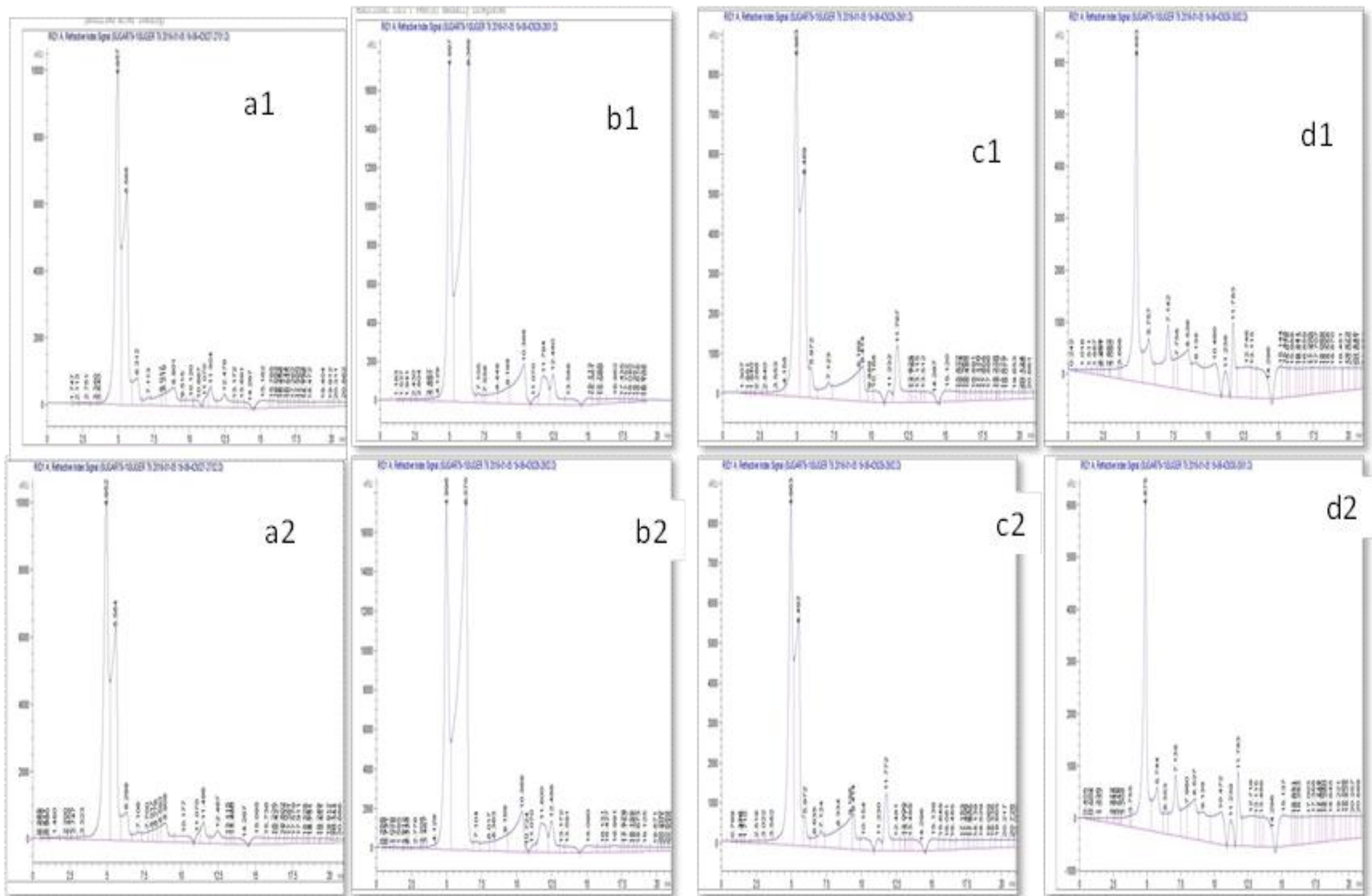

Figure 2B. Chromatograms of HPLC analysis of mucilage hydrolsate (duplicate run 1,2) for four samples of different *Aloe* species. a= *A. vera*; b= *A. arborences*, c = *A. eru* d= *A. grandidentata*.

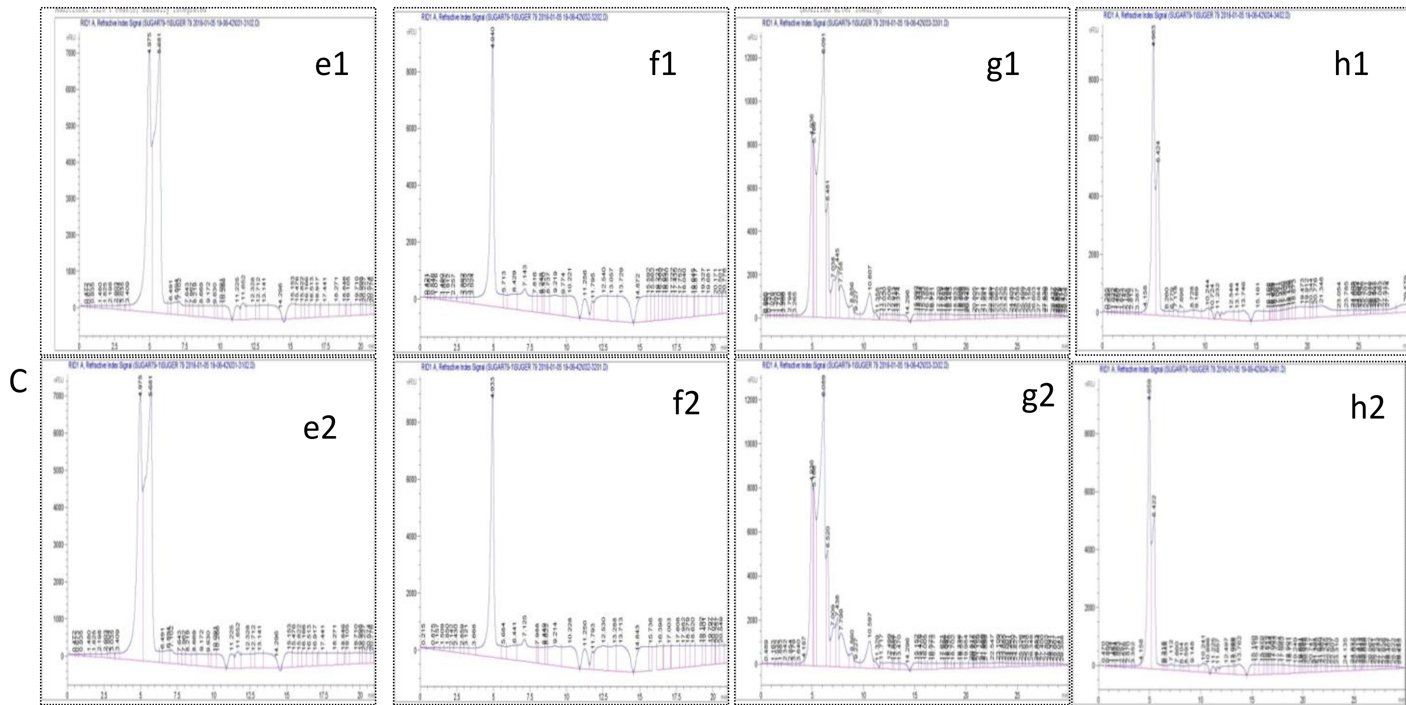

Figure 2C. Chromatograms of HPLC analysis of mucilage hydrolysate (duplicate run 1,2) for four samples of different *Aloe* species e= *A. perfoliata*, f= *A. brevifolia* g= *A. saponaria* h= *A. ferox.*

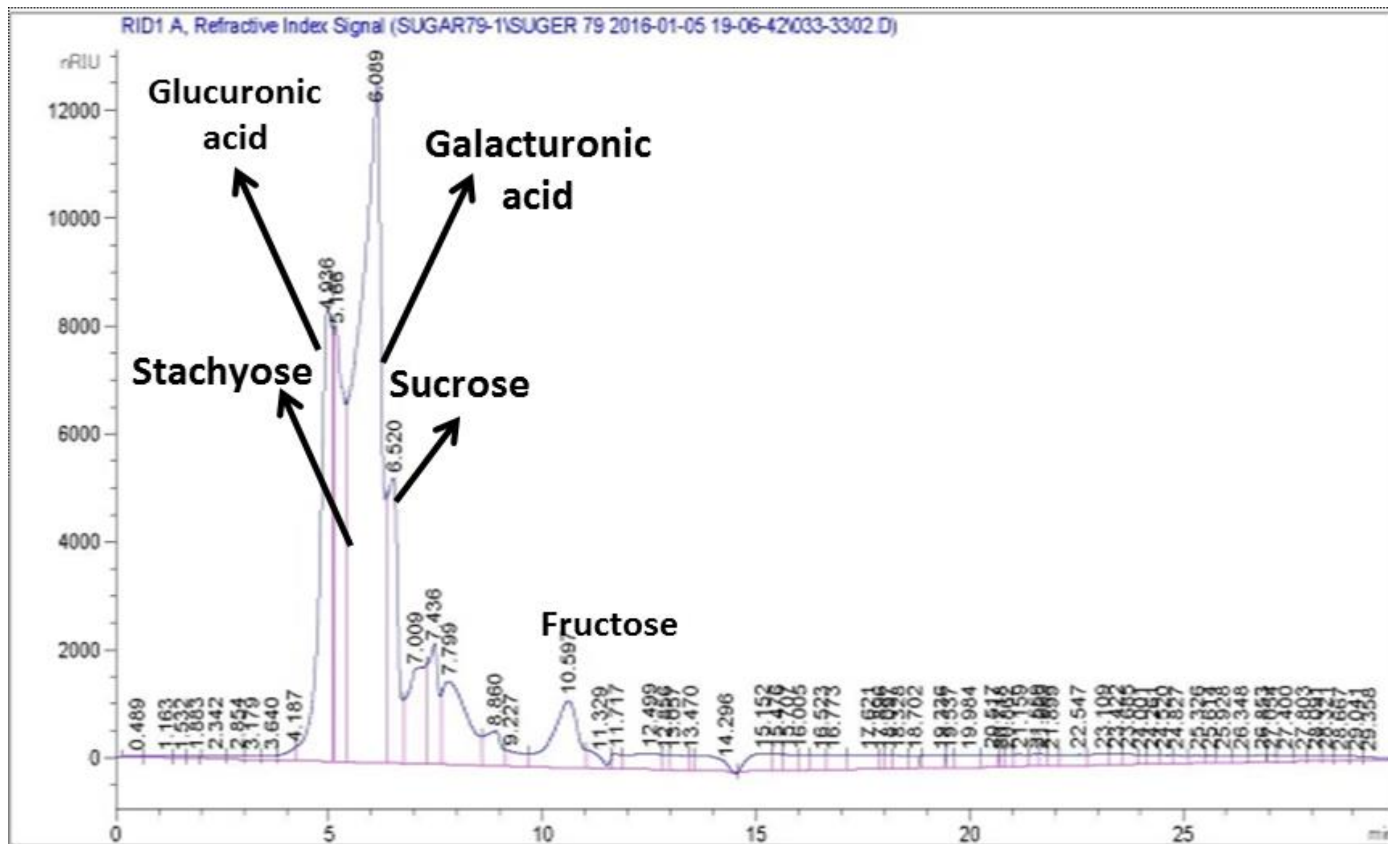

Figure 2D. Chromatogram of HPLC analysis of mucilage hydrolysate of *A. saponaria* (A7)

compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing counties (Tanak et al., 2006).

Conflict of Interests

The authors have not declared any conflict of interest.

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Full Length Research Paper

In vitro anthelmintic activity of stem and root barks of *Alstonia boonei* De Wild

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Alstonia boonei **De Wild ethanol extracts of the stem bark (ABSB) and root bark (ABRB) were evaluated for possible anthelmintic activity. Three different concentrations of each extract (50, 100 and 150 mg/ml) were evaluated for *in vitro* anthelmintic activity by determining the effects of the extracts on the paralysis and death times of *Pheretima posthuma.* Mebendazole (MBZ) 15 mg/mg was used as reference anthelmintic. ABSB and ABRB demonstrated a concentration dependent anthelmintic activity with a reduction in paralytic and death times upon increase in the concentration of the extracts. ABSB revealed better anthelmintic activity than ABRB at all concentrations tested. ABSB also revealed a significant paralytic time (*p<0.01*) at 150 mg/ml with reference to MBZ. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids saponins and glycosides in ABSB and ABRB.**

Key words: *Alstonia boonei*, anthelmintic, *Pheretima posthuma,* death time, paralytic time.

INTRODUCTION

Medicinal plants have been used over the decades to manage helminth infections. However, scientific evaluation of some of these traditional treatments has not been conducted so as to validate their usage. With the current increase in anthelmintic resistance amongst both human and farm animals (Vercruysse et al., 2011), it is imperative that the traditional usage of some of these medicinal plants be validated and novel molecules isolated in order to curb the menace. The use of medicinal plant as anthelmintic has been practised in many indigenous cultures for centuries. Studies have shown that in many developing countries, ethno medicine is still the primary treatment option for many parasitic diseases (Tanner et al., 2011; Gazzinelli et al., 2012). *Alstonia boonei* De Wild (Apocynaceae) is an indigenous African tree mostly found in the evergreen rain forest of tropical West Africa (Gosse et al., 1999).

The plant is well known by almost all traditional healers practising along the west coast of Africa (Adotey, 2012). The bark of the plant is known to possess antirheumatic, anti-inflammatory, anthelmintic and antidiabetic properties (Hadi and Bremner, 2001). Traditionally, a cold infusion of the fresh or dried bark is used as an anthelmintic and also to expel other intestinal parasites in children (Adotey, 2012). Studies conducted on the methanol extracts of the stem bark have demonstrated potent

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anti-inflammatory, analgesic and antipyretic properties (Olajide et al., 2000). Aqueous and ethanol extracts of the stem bark have also demonstrated antimicrobial and anthelmintic activities (Adomi, 2006; Danquah et al., 2012). The stem bark which is mostly used for traditional treatment of malaria is also known to possess very potent antioxidant compounds (Akinmoladun et al., 2007).

Extensive research conducted on this plant has resulted in the identification of various bioactive compounds of diverse pharmacological activity, including; Boonein: a 9 carbon terpenoid lactone possibly known to be the precursor in indole alkaloid biogenesis (Marini-Bottelo et al., 1983). Although much research has been conducted on this plant, there is limited information on the anti-infective potentials of the various parts of the plant. Current anti-infective information available is mostly related to the stem bark of the plant. In the face of high levels of microbial resistance to antibiotics as well as other anti-infective agents, it is important that new compounds be identified and developed. This study therefore aimed to determine and compare the anthelmintic potential of the stem and root barks of *A. boonei* in order to support its traditional uses.

MATERIALS AND METHODS

Collection of plant

The stem and root bark of *A. boonei* were identified and collected in the month of March, 2015 from the Aburi Botanic Gardens in the Eastern Region of Ghana. The plant samples were authenticated by Mr. Albert Asiedu Prempeh, a botanist and curator of the garden. Voucher specimens CUC/DPS/2015/A018 and CUC/DPS/2015/A019 were deposited in the Pharmacognosy Section of the Department of Pharmaceutical Science, Central University College. The samples were washed with distilled water to get rid of debris and then air dried for 3 weeks at room temperature (25 to 28°C).

Extraction of plant

The extraction process was carried out using the method described by Adu et al. (2015) with slight modifications. The dried plant samples were milled into coarse powder using laboratory mill equipment. A quantity of 250 g of both samples was weighed and extracted using 70% v/v ethanol by cold maceration for 72 h. The supernatant and the bulk extract was then filtered using a filter paper with the aid of a vacuum pump. The extracts obtained were concentrated using a rotary evaporator (Buchi, Germany, R210) at 40°C. The concentrates were later dried in a hot air oven at 40°C to obtain the solid extract. The extracts were then stored at 4°C until needed.

Phytochemical screening

Phytochemical tests for the presence of some plant secondary metabolites were performed on the powdered plant samples for the presence of tannins, alkaloids, flavonoids and glycosides (Trease and Evans, 2002).

Experimental organism

Adult Indian earthworms (*Pheretima posthuma*) which have anatomical resemblance to human intestinal roundworms were obtained from the nursery of a vegetable farm near Central University College, Accra, Ghana. Normal saline solution (0.9%) was used to wash the worms to remove all debris.

In vitro anthelmintic activity evaluation

The experiment was performed with slight modifications to the method described by Bhawar et al. (2009). Earthworms of lengths 3.0 to 6.0 cm were employed. Extract concentrations of 50, 100 and 150 mg/ml were prepared using distilled water. Mebendazole (MBZ) at a concentration of 15 mg/ml was used as the reference standard. Normal saline solution (0.9%) was used as a negative control.

Experimental procedure

Five earthworms were placed in each Petri dish into which the various extracts concentrations and reference drug were added. Observations were made for the time taken by the various extract concentrations to cause paralysis and death of the individual worms. Paralysis was observed when no movement was seen in the worms unless when shaken vigorously. Death was denoted by a lost in motility of the worms even upon pricking with a pin and placement in 50°C warm water, coupled with a fading away of body colour. Solutions within which worms demonstrated vigorous motility and life after ≥360 min of exposure where classified as not exerting anthelmintic activity (Na= No activity).

Statistical analysis

Results were presented as mean ± standard deviation (N=5). Analysis was done using GraphPad prism version 5 (GraphPad Software, San Diego, CA, USA) by two way analysis of variance (ANOVA) followed by bonferonni post-test analysis which recognises $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ as statistically significant.

RESULTS

Preliminary phytochemical screening

Results from the phytochemical screening revealed the presence of tannins, alkaloids, glycosides and flavonoids in both *Alstonia boonei* stem bark (ABSB) and *Alstonia boonei* root bark (ABRB) (Table 1).

Anthelmintic activity

The extracts demonstrated some level of anthelmintic activity at the concentrations tested. ABSB demonstrated more potent activity than ABRB in a concentration dependent manner with shorter paralysis and death times as compared to ABRB (Tables 2 and 3).

Table 1. Phytochemical screening.

Phytochemicals	RESULTS	
	ABRB	ABSB
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Flavonoids	+	+

(+) = Present; (-) = Absent; ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark.

Table 2. Paralysis time of ABSB and ABSB against *P. posthuma*.

Extract concentration (mg/ml)	Time (min)		
	ABSB	ABRB	0.9% saline
50	55.00 ± 3.43	Na	Na
100	22.00 ± 1.21*	127.00 ± 5.16	Na
150	17.00 ± 2.10**	93.00 ± 2.04	Na
MBZ 15 mg/ml	27.00 ± 2.23	-	-

ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark; MBZ: mebendazole; Na: No activity; *$p < 0.05$; **$p < 0.01$, values are mean ± SD (N=5)

Table 3. Death time of ABSB and ABRB against *P. posthuma* .

Extract concentration (mg/ml)	Time (min)		
	ABSB	ABRB	0.9% saline
50	Na	Na	Na
100	175.00 ± 4.52	Na	Na
150	100.00 ± 2.47	151.00 ± 2.27	Na
MBZ 15 mg/ml	102.00 ± 2.18	-	-

ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark; MBZ: mebendazole; Na: No activity; values are mean ± SD (N=5)

DISCUSSION

The results from the anthelmintic bioassay revealed that both extracts ABSB and ABRB have anthelmintic activity. These results correlate with research conducted by Danquah et al. (2012) reporting the possible anthelmintic potentials of the stem bark extracts of *A. boonei*. The ability of plants to exhibit anthelmintic activity has largely been attributed to the presence of tannins. Tannins are believed to exert anthelmintic activity by interfering with the energy generation of the helminth parasite by uncoupling oxidative phosphorylation or by binding to free proteins in the gastrointestinal tract of the helminth. This eventually results in death of the parasite (Danquah et al., 2012; Adu et al., 2015; Olusegun-Joseph et al., 2012). The results from the phytochemical screening revealed the presence of tannins in both ABSB and ABRB; this could have been responsible for the anthelmintic activity.

Studies conducted by Mute (2009) also reported the role of alkaloids in anthelmintic activity by causing paralysis of worms through their action on the central nervous system of the helminth. This therefore implies that the presence of alkaloids in the extract also contributed to their anthelmintic activity.

The results obtained however revealed that the anthelmintic activity was concentration dependent for both extracts with the higher concentrations demonstrating better anthelmintic activities (Tables 2 and 3). ABSB demonstrated better anthelmintic activity than ABRB. ABSB also demonstrated significant paralytic times ($p<0.05$) at 100 mg/ml and ($p<0.01$) at 150 mg/ml

with reference to MBZ. This could therefore imply that the stem bark of the plant possesses more bioactive compounds responsible for anthelmintic activity than the root bark, hence the higher activity. These findings support the folkloric use of *A. boonei* as an anthelmintic agent.

Conclusion

Ethanol extracts of stem and root barks of *A. boonei* possess anthelmintic properties. The stem bark exerts better anthelmintic activity than the root bark.

Conflict of interest

The authors have not declared any conflicts of interests.

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