Screening of hydroalcoholic leaf extract of *Ajuga parviflora* for antidiabetic potential

Semester progress report

Submitted by

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OVERVIEW

Indian medicines are used by about 60 % of the world's population. These are not only used for primary health care in rural areas of the developing countries, but also in the developed countries alongside modern medicines. While the traditional medicines are derived from medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only. In the present study, we investigated one of the antidiabetic herb of Himachal Pradesh, *Ajuga parviflora*, and aimed to provide an experimental justification to its traditional antidiabetic use. Further, through several *in-vitro* assays, like antioxidant and anti-inflammatory assay, we tried to explain whether or not this plant is capable of interfering with the mechanisms leading to development of diabetic complications.

Introduction

Plants have been used for the medicinal purposes long before prehistoric period. Evidence exist that Indian Vaids and European cultures were using herbs for over 4000 years as medicine. cultures in Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The main fact is that, use of herbal treatments is independent of any age groups and the sexes. The blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. It's time to promote them globally.

Ajuga parviflora

Common name: Small-Flowered Bugleweed

Family: Lamiaceae (Mint family)

A. parviflora is a type of bugleweed that is found in places like Afghanistan, India, Pakistan, and the disputed Kashmir region. Not a lot is known about this plant as it's kind of rare but it's likely an annual or short-lived perennial plant, it has green leaves, and it has small purple flowers.

Medicinal Uses

Research conducted on this plant's extract have shown that it may have numerous medicinal uses and the entire plant that grows above ground, or even its roots, may have medicinal properties of

one kind or another. That means the leaves might be chewed and eaten whole or, in other cases, a drink composed of this plant's root powder may be ingested.

In either case, some evidence exists for the fact that it has antimicrobial activities as it can actively target some types of bacteria. These bacteria include Citrobacter and Pseudomonas aurogenosa. It has also been used as an astringent, which is a substance that might help stop the flow of blood, and as an agent that helps stop headaches. There's also some evidence that some people have used it to manage hypertension, or high blood pressure, as well as hepatitis, or inflammation of the liver and any jaundice that may stem from it. Jaundice refers to yellowing of the skin, mucous membranes, and whites of the eyes sometimes as a result of liver disease. Finally, it has been used as a general tonic, as a substance that vitalizes a person who takes it.

What is Diabetes?

Diabetes is a disease that occurs when your blood glucose, also called blood sugar, is too high. Blood glucose is your main source of energy and comes from the food you eat. Insulin, a hormone made by the pancreas, helps glucose from food get into your cells to be used for energy. Sometimes your body doesn't make enough or any insulin or doesn't use insulin well. Glucose then stays in your blood and doesn't reach your cells.

Over time, having too much glucose in your blood can cause health problems. Although diabetes has no cure, you can take steps to manage your diabetes and stay healthy. Sometimes people call diabetes "a touch of sugar" or "borderline diabetes." These terms suggest that someone doesn't really have diabetes or has a less serious case, but every case of diabetes is serious.

What are the different types of diabetes?

The most common types of diabetes are type 1, type 2, and gestational diabetes. If you have type 1 diabetes, your body does not make insulin. Your immune system attacks and destroys the cells in your pancreas that make insulin. Type 1 diabetes is usually diagnosed in children and young adults, although it can appear at any age. People with type 1 diabetes need to take insulin every day to stay alive. If you have type 2 diabetes, your body does not make or use insulin well. You

can develop type 2 diabetes at any age, even during childhood. However, this type of diabetes occurs most often in middle-aged and older people. Type 2 is the most common type of diabetes.

Gestational diabetes

Gestational diabetes develops in some women when they are pregnant. Most of the time, this type of diabetes goes away after the baby is born. However, if you've had gestational diabetes, you have a greater chance of developing type 2 diabetes later in life. Sometimes diabetes diagnosed during pregnancy is actually type 2 diabetes.

Aim and Objectives

In the present study, we investigated one of the antidiabetic herb of Himachal Pradesh, *Ajuga parviflora*, and aimed to provide an experimental justification to its traditional antidiabetic use. Further, through several *in-vitro* assays, like antioxidant and anti-inflammatory assay, we tried to explain whether or not this plant is capable of interfering with the mechanisms leading to development of diabetic complications.

Review of Literature

Species belonging to genus Ajuga (Labiatae) have been used as folk medicinal plants as anthelmintic, antifungal, hypoglycaemic, antitumor and antimicrobial agents (Rodriguez-Hahn, Esquivel & Cardenas, 1994; Wessner, Champion, Girault, Kaouadji, Saidi & Lafont, 1992).

Withanolides

Since the isolation of the first withanolides in the mid-1960s, over 600 new members of this group of compounds have been described, with most from genera of the plant family Solanaceae. Many withanolides have shown a variety of interesting biological activities ranging from antitumor, cytotoxic and potential cancer chemopreventive effects, to feeding deterrence for several insects as well as selective phytotoxicity towards monocotyledoneous and dicotyledoneous species. Trypanocidal, leishmanicidal, antibacterial, and antifungal activities have also been reported (Misico et al,1999).

Two withanolides, Ajugin E and F, had been isolated from the defatted methanolic extract of Ajuga parviflora. (Nawaz et al, 1999). Two other withanolides, ajugin A and ajugin B were also found (Khan et al., 1999). The plant has found diverse medicinal uses in indigenous systems of medicine. It has been used as an astringent and for treatment of swollen wounds, diarrhea, rheumatic fever, eye trouble, and diseases of bladder.

Withanolides have been considered a monopoly of Solanaceous plants. There are only rare reports on their isolation from a marine organism (a soft coral) and from members of the Taccaceae and the Leguminosae (Ray & Gupta, 1994). Ajugin is the first report of naturally occurring withanolides in Labiatae. (Khan et al,1999). Ajugins C and D had also been found. (Khan et al,1999).

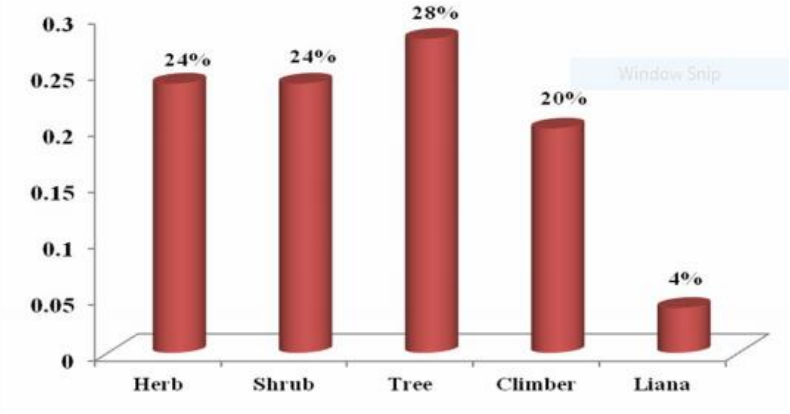
Method of preparation

Various methods of preparation of these herbal remedies have been recorded. Decoction of (24%) species followed by juice of (20%), powder (16%), cooked and paste (4% each) were practiced by the inhabitants. In addition to this, 16% species were consumed as such and 20% were chewed to

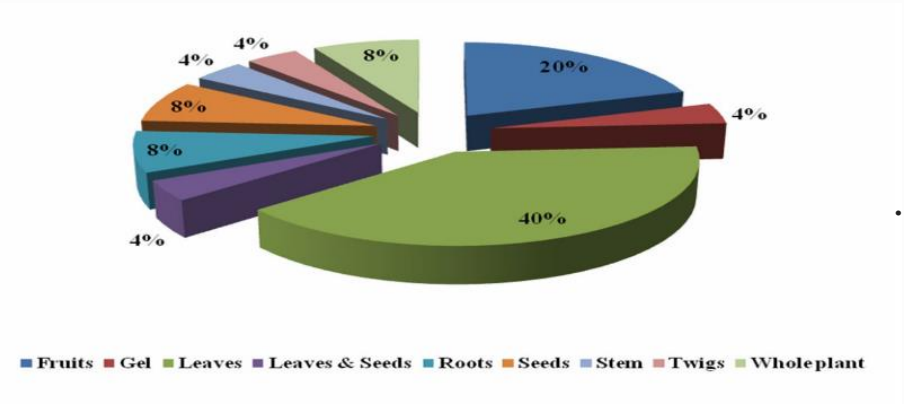
manage the diabetes. Most of the remedies were prepared from fresh materials. Some others were prepared from dry as well as fresh materials. The materials of only four species have been used in dry form. The additives such as water, salt, fruits of *Piper nigrum* have been used in some preparations. Different plant parts such as fruits, leaves, roots, seeds, whole plant, twigs etc. were used for the preparation of traditional medicines. It has been observed that 40% species were harvested for their leaves, followed by fruits (20%), roots, seeds, whole plant (8% each) and others (such as gel, leaves as well as seeds and so on with 4% each) (Sidhu et al, 2015).

Sample was collected from mandi and there are other plants in that area also. Some of them are listed below along with how they are used. *Ajuga parviflora*, *Syzygium cumini*, *Eleusine coracana*, *Tinospora sinensis*, *Berberis aristata* and *Momordica charantia* were some of the most cited plants. Other species like *Cuscuta reflexa*, *Cynodon dactylon*, *Ocimum tenuiflorum*, *Cordia dichotoma* and *Dalbergia sissoo* have also been utilized as antidiabetic plants. Fourteen of these species are growing wild whereas the remaining are cultivated. It has been observed that all the formulations have been prepared from the single plant species. The use of these species in polyherbal forms likely to be more effective. The dosage of particular medicines may vary as it depends upon the age and general health of the patients (Sidhu et al, 2015).

Many recorded species have also been used traditionally for the same purpose by inhabitants of other regions or countries including India. Many of these species have been proved scientifically for their antidiabetic potential. However, as per the available literature, Clematis virginiana, Cornus capitata, Cucumis sativus var. hardwickii have been reported for the first time. These species are likely to have an antidiabetic potential. Further studies are required to prove their antihyperglycaemic activity. This can provide some new, alternate or modified materials to the pharmaceutical sectors (Sidhu et al, 2015).



Plant species used for treatment of diabetes in mandi



Percentage(s) of part used in preparation of medicines.

Acute and chronic toxicological studies of *Ajuga parviflora* in experimental animals

It was previously observed that single oral doses (2–14 g/kg) of a lyophilized aqueous extract of *Ajuga parviflora* (AP-extract) in mice or daily oral administration of 10 mg/kg of AP-extract in rats for 2 weeks did not result in any adverse effects. It has been evaluated AP-extract for its behavioral and pharmaco-toxicological effects after acute and chronic administration by the oral and intraperitoneal routes in rats and mice. No toxicity was observed in mice after single oral doses of as high as 14 g/kg of the AP-extract. However, single intraperitoneal injections of the AP-extract (1500–5500 mg/kg BW) produced a dose-dependent increase in adverse effects in the general behavior and the mortality rate; In chronic toxicological studies in rats, the AP-extract

(administered orally at daily doses of 100, 300 and 600 mg/kg for 3 months), did not cause any changes in haematological and biochemical parameters, with the exception of a short rise in platelet counts and a short-term decrease in serum glucose levels (Hilaly et al, 2004).

DIABETES

The term diabetes mellitus describes a metabolic disorder of multiple causes characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to coma and in absence of effective treatment death (Alberti et al,1998).

Type 1

Type 1 indicates the processes of beta-cell destruction that may ultimately lead to diabetes mellitus in which 'insulin is required for survival' to prevent the development of ketoacidosis, coma and death. Type 1 is usually characterized by the presence of anti-GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction (Alberti et al,1998).

Type 2

Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. The specific reasons for the development of these abnormalities are not yet known (Alberti et al,1998).

Other Specific Types

Other Types are less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include, for example, fibrocalculous pancreatopathy, a form of diabetes which was formerly classified as one type of malnutrition-related diabetes mellitus (Alberti et al,1998).

Expenditure on health care incurred by diabetic subjects in a developing country

It is estimated that there are approximately 20 million diabetic patients in India and the annual estimated cost could be Rs. 90200/– million (US$ 2.2 billion) for diabetes health care. There are many different clinics in India where patients have to pay for the services. Those who can afford prefer treatment from private centers. The government-run free hospitals are often crowded. Therefore, even some low and middle-income groups of patients preferred private hospital care. low-income group patients attending the private center spent as much as 25% of their income. The annual cost of diabetes care was significantly higher in patients with long duration (55 years' vs 5 years). The differences were seen in almost all aspects of treatment. Therefore, treatment of diabetes becomes more expensive as time increases (Shobhana et al., 2000).

Medicinal plants of with anti-diabetic potential

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human diseases. India has about 45 000 plant species and among them, several thousands have been claimed to possess medicinal properties. 45 such plants and their products have been mentioned in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity (Grover et al, 2002).

Due to economic problems, providing modern medical healthcare in developing countries such as India is still a far goal. The most commonly used drugs of modern medicine such as aspirin, anti-malarial, anti-cancers, digitalis, etc. originated from plant sources. Out of around 250 000 higher plants, less than 1% have been screened and very few in regard to DM. Therefore, it is obvious to look for options in plant medicine for diabetes. The goals of medicines are the same i.e. the welfare of the patient. We should look towards a future of integrated medicine and hope that research in alternative medicine will help identify what is safe and effective (Grover et al, 2002).

Material and methods

Plant used

A. parviflora was used in this experiment for basically finding about its anti-diabetic properties along with its capacity to quench free radicals. Its phytochemicals were also screened.

Plant collection

Plant was collected from mandi district of Himachal Pradesh near shikari mata temple in the month of June.

Materials

All the chemicals used in this study were procured from Sigma-Aldrich, Loba Chemie, Merck, SD Fine-Chem, Himedia, and Spectrochem.

Plant extraction

Leaves were cleaned under running water and dried in shade for 7 days. Dry weight of leaves was measured-46. 5gm.The dried leaves were then made into a coarse powder and were subjected to hydro-alcoholic (30:70) extraction using Soxhlet extractor. Extraction process was run for 6 days for 6 hours each day. Collected extract was filtered while hot, concentrated under reduced pressure using rotary evaporator, lyophilized and stored at 4°C until used.

Preliminary phytochemical screening

The lyophilized hydro-alcoholic leaf extract was qualitatively tested for the presence of phytochemical constituents such as alkaloids, flavonoids as per the previously well-defined and widely used standardized methods.

Determination of total flavonoid content

Total flavonoids present in the extract were determined using simple and previously well-established spectrophotometric method by taking quercetin as a standard compound. 0.5 ml of 200 mg/ml extract was transferred to the test tube containing 75 µL of 5% $NaNO_2$ solution. Mixture was allowed to stand for 10 minutes after which 150 µL of a 10% $AlCl_3.6H_2O$ solution was added

to the reaction tube. A reaction mixture was allowed to stand for 5 more minutes after which 0.5 ml NaOH (1 M) and 2.5 ml of distilled water were added to it. Absorbance was then measured at 510 nm using the UV spectrophotometer. Entire procedure was performed in triplicate. Standard curve of quercetin was prepared using the same procedure. Total flavonoid content present in the *Ajuga parviflora* was expressed as mg querctine equivalent (QE)/g dried extract using the linear regression equation obtained from the standard curve of quercetin.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging was used to determine the free radical scavenging potential of the AP according to the previously defined method, with slight modifications. Briefly, 0.4 mM solution of DPPH was prepared 95% methanol. Different concentrations (250-1000 μg/ml) of extract and ascorbic acid (standard) were also prepared in 95% methanol and 10 ml of sample was then transferred into the reaction test tubes. 3 ml of DPPH solution was added to each tube and mixed vigorously followed by 30 minutes' incubation in dark at 37°C. For the purpose of control reaction, equivalent volume of 95% methanol in DPPH was used. Absorbance of all the samples was recorded in triplicate at 517 nm using the UV spectrophotometer. % DPPH radical scavenging activity was calculated using following equation.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control-absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS radical scavenging assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, ABTS·+ scavenging effect (%) = ((AB–AA)/ AB)×100 , where, AB is absorbance of ABTS radical

+ methanol; AA is absorbance of ABTS radical + sample extract/standard. Trolox was used as standard substance.was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank.

$$\text{ABTS scavenging activity (\%)} = \frac{\text{Absorbance of control-absorbance of sample}}{\text{Absorbance of control}} \times 100$$

In-vitro evaluation of anti-inflammatory activity

Heat-induced hemolysis

Aliquots (5 ml) of the isotonic buffer containing 2.0mg/mL of different extractives of plant were put into centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54oC for 20min in a water bath. The other pair was maintained at0-5oC in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation where OD1 is

$$\text{\% Inhibition of hemolysis} = 100 \times [1 - (OD2 - OD1 / OD3 - OD1)]$$

Red blood cell (RBC) membrane stabilization assay

Potential of the plant extract to stabilize cellular membrane of RBCs was evaluated according to protocol previously described by Asanuma et al., Sadique et al. and Shinde et al. with slight modification. Sufficient amount of goat blood was obtained from slaughterhouse in the glass tubes having 1.8 mg/ml 5% ethylenediaminetetraacetic acid solution and mixed gently. Blood was centrifuged at 5000 rpm for 15 minutes and washed thrice with equal volumes of saline. Further, 10% v/v suspension of RBC was prepared in normal saline; which was stored at 4°C and used within 6 hrs of preparation. Reaction mixture was prepared by mixing 1 ml of 10% RBCs

suspension and 1 ml of different concentrations (250-1000 μg/ml) of AP, diclofenac sodium, or distilled water (blank) in the test tubes. All the samples were incubated at 57°C for 30 minutes over water bath and were immediately cooling under running tap water. Samples were centrifuged at 2500 rpm for 5 minutes, and absorbance of the supernatant was measured spectrophotometrically at 560 nm. Entire experimentation was performed in triplicate, and percent inhibition of RBC membrane was determined using the following equation.

$$\% \text{ Membrane stabilization} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Result

Phytochemical screening

- ACIDIC COMPOUNDS----------NEGATIVE.
- ALEURONE GRAINS (specialized dry vacuole where storage protein accumulates for seeds) ----------POSITIVE.

- ALKALOIDS-

1) DRAGENDOFF'S TEST----------POSITIVE

2) TANNIC ACID TEST----------POSITIVE

- AMINO ACIDS-

1) MILLIONS TEST----------NEGATIVE

2) NIN HYDRIN TEST----------NEGATIVE

- CARBOHYDRATES-

1) MOLISH TEST----------NEGATIVE

2) BORFOED'S TEST----------NEGATIVE

- GLYCINE----------POSITIVE

- FLAVANOIDS-

1) ALKALINE REAGENT TEST----------POSITIVE

2) ZINC HYDROCHLORIDE TEST----------POSITIVE

- GLYCOSIDES-

1) ANTHROQUIONE GLYCOSIDE

BORNTRAGER'S TEST----------POSITIVE

2) CARDIAC GLYCOSIDES-

LEGAL'S TEST----------NEGATIVE

BAILET'S TEST----------NEGATIVE

3) COURMARIAN GLYCOSIDES----------POSITIVE

4) SAPONIN GLYCOSIDES----------POSITIVE

- INULIN----------POSITIVE
- TANNINS-

1) FERRIC CHLORIDE TEST----------POSITIVE

2) TEST FOR CATECHIN----------NEGATIVE

3) TEST FOR CHLORGENIC ACID----------POSITIVE

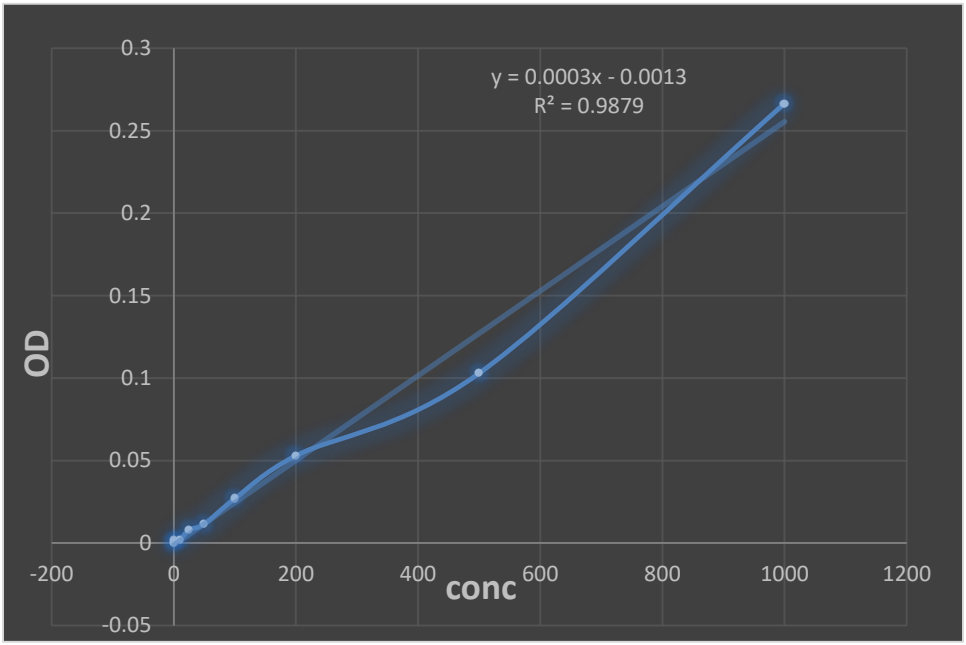
- STARCH----------NEGATIVE
- STEROIDS & TERPENOIDS-

SALKWOSKI TEST----------POSITIVE

- PROTEINS----------NEGATIVE.

Total flavonoid content

	Total flavanoid content								
	CONC(µm)	1000	500	200	100	50	25	10	1
	BLANK								
	0.05	0.051	0.051	0.058	0.063	0.077	0.107	0.155	0.311
	0.05	0.052	0.053	0.058	0.06	0.077	0.099	0.151	0.322
AVG	0.05	0.0515	0.052	0.058	0.0615	0.077	0.103	0.153	0.3165
BLNK SBS	0	0.0015	0.002	0.008	0.0115	0.027	0.053	0.103	0.2665
CONC	0	1	10	25	50	100	200	500	1000
OD	0	0.0015	0.002	0.008	0.0115	0.027	0.053	0.103	0.2665

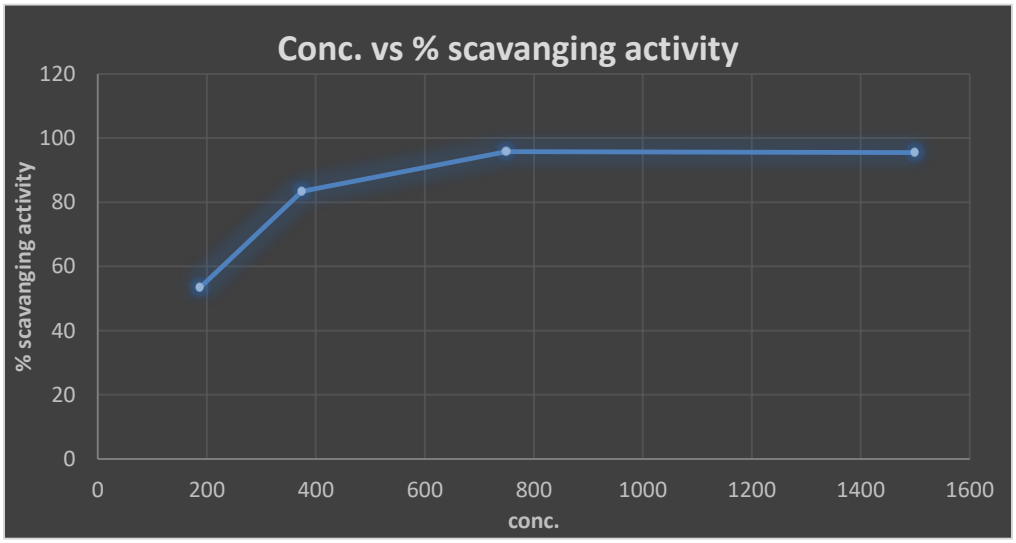


DISCUSION

Total flavonoid content of the crude extract was determined in term of QE from the standard curve of quercetin. Linear regression equation of (y = 0.0003x - 0.0013) and high degree of correlation (R^2 = 0.9879) between concentration and corresponding absorbance was observed from the calibration curve. High levels of total flavonoids (96µg QE/g crude extract) were observed to be present. Antioxidant properties possessed by the plant or extract are generally attributed to the presence of total phenolic and flavonoids in it.

DPPH assay result

	Solvent blank	AA Blank	Blank1500	B750	B375	B187.5
	0.067	0.061	0.265	0.162	0.102	0.068
	0.044	0.044	0.29	0.156	0.101	0.069
	0.0555	0.0525	0.2775	0.159	0.1015	0.0685
	DPPH	AA+D	1500	750	375	187.5
	0.849	0.075	0.316	0.196	0.25	0.449
	0.85	0.073	0.315	0.193	0.235	0.479
AVG	0.8495	0.074	0.3155	0.1945	0.2425	0.464
blank substraction			0.038	0.0355	0.141	0.3955
scavanging %			95.52678	95.82107	83.402	53.4432



DISCUSSION

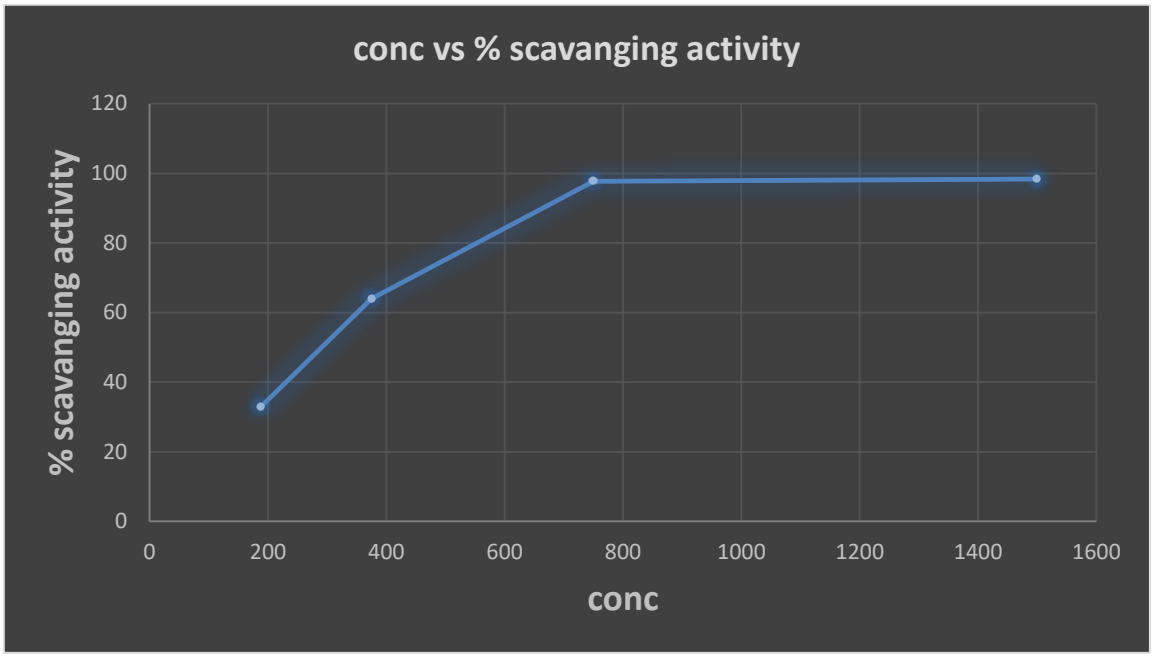
As I demonstrated that AP possessed high levels of flavonoid compounds, which may be responsible for the high potential of AP to scavenge DPPH free radicle. DPPH radical scavenging assay is a well-established assay to determine the antioxidant potential of the herbal extracts with

high reliability and accuracy. DPPH is converted to more stable and colorless molecule after it gains electrons from the antioxidant substance. Therefore, stronger the antioxidant potential of plant extract, more discoloration of DPPH solution it will produce; which is recorded spectrophotometrically. In the present study, hydroalcoholic extract was subjected to DPPH radicle scavenging assay at different concentrations and radicle scavenging activity was compared to ascorbic acid. Concentration depended scavenging activity was observed for both standard and plant.

Hydroalcoholic extract resulted in 95.52% DPPH radicle scavenging activity at the highest concentration while ascorbic acid demonstrated 97.46% inhibition at the same concentration.

ABTS assay result

	Solvent blank	AA Blank	B1500	B750	B375	B187.5
	0.04	0.042	0.255	0.146	0.079	0.06
	0.04	0.044	0.247	0.149	0.08	0.059
Average	0.04	0.043	0.251	0.1475	0.0795	0.0595
	ABTS	AA+ABTS	1500	750	375	187.5
	0.964	0.074	0.27	0.167	0.436	0.698
	0.982	0.075	0.264	0.172	0.424	0.727
Average	0.973	0.0745	0.267	0.1695	0.43	0.7125
blank substraction		0.0315	0.016	0.022	0.3505	0.653
scavanging %		96.76259	98.3556	97.73895	63.97739	32.88798



DISCUSION

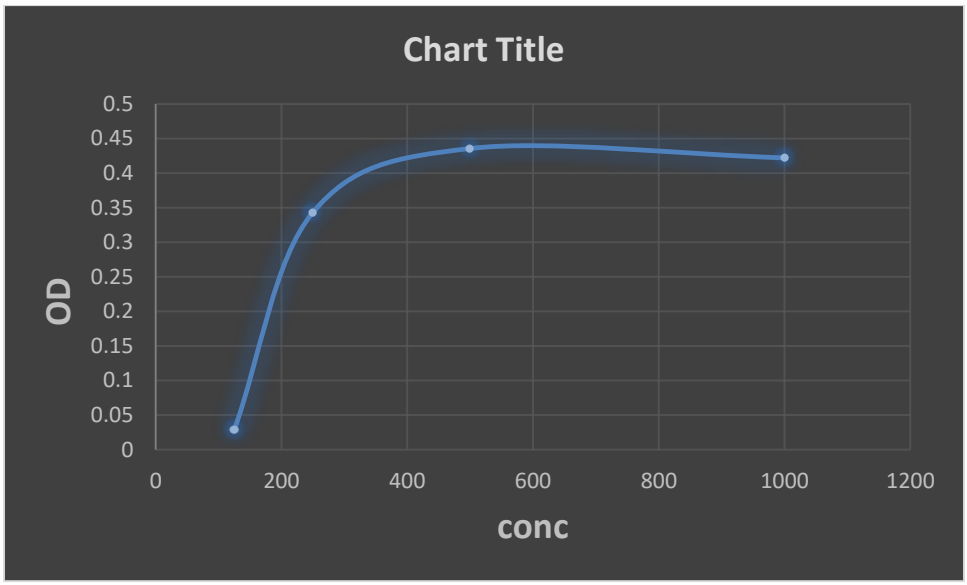
As I demonstrated that AP possessed high levels of flavonoid compounds, which may be responsible for the high potential of AP to scavenge ABTS free radicle. ABTS radical scavenging assay is a well-established assay to determine the antioxidant potential of the herbal extracts with high reliability and accuracy. In the present study, hydroalcoholic extract was subjected to ABTS radicle scavenging assay at different concentrations and radicle scavenging activity was compared to ascorbic acid. Concentration depended scavenging activity was observed for both standard and plant.

Hydroalcoholic extract resulted in 98.35% ABTS radicle scavenging activity at the highest concentration while ascorbic acid demonstrated 96.76% inhibition at the same concentration.

Heat induced hemolysis results

	CTRL	1000µg/ml	500µg/ml	250µg/ml	125µg/ml
	0.59	0.519	0.5	0.386	0.077
	0.62	0.596	0.529	0.418	0.08
AVG	0.605	0.5575	0.5145	0.402	0.0785
T-B		0.5575	0.5145	0.402	0.0785
		0.1355	0.079	0.059	0.05
OD		0.422	0.4355	0.343	0.0285
			GRAPH		
OD		1000	500	250	125
		0.422	0.4355	0.343	0.0285
% Inhibition of hemolysis		69.75	71.98	56.69	4.71

T1B	T2B	T3B	T4B
0.12	0.077	0.059	0.05
0.151	0.081	0.059	0.05
0.1355	0.079	0.059	0.05



DISCUSSION

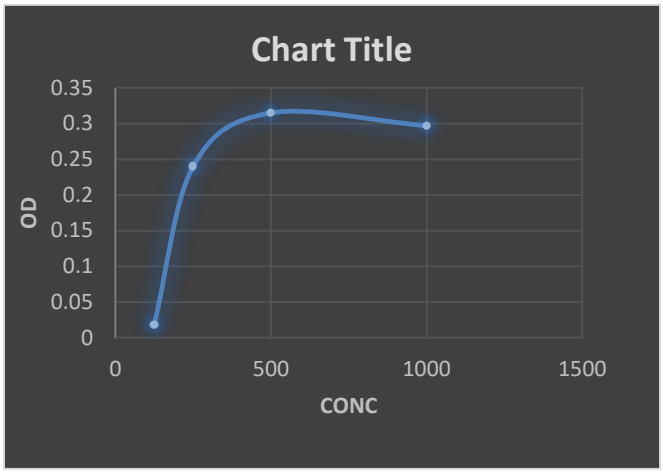
The extract of AP at highest concentration was able to show 69.75% of % Inhibition of hemolysis of erythrocytes from heat lysis. Whereas at the lowest concentration our extract was able to show 4.71% Inhibition of hemolysis from heat.

RBC membrane stabilization

	CTRL	1000µg/ml	500µg/ml	250µg/ml	125µg/ml
	0.54	0.409	0.387	0.29	0.069
	0.57	0.467	0.408	0.312	0.071
AVG	0.555	0.438	0.3975	0.301	0.07
T-B	0.555	0.438	0.3975	0.301	0.07
	0.061	0.141	0.0825	0.061	0.0515
OD	0.494	0.297	0.315	0.24	0.0185

T1B	T2B	T3B	T4B
0.125	0.08	0.061	0.051
0.157	0.085	0.061	0.052
0.141	0.0825	0.061	0.0515

CONC	1000	500	250	125
OD	0.297	0.315	0.24	0.0185
%Membrane stabilization	60.12	63.76	48.58	3.74



DISCUSION

The extract of AP at highest concentration was able to show 60.12% Membrane stabilization. Whereas at the lowest concentration our extract was able to show 3.74% Membrane stabilization.

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