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Evaluation of antidiabetic and antioxidant activities of *Achyranthes aspera* leaf extracts: An *in vitro* study

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Abstract
The present investigation aimed to screen the phytochemicals in the methanolic and petroleum ether leaf extracts of *Achyranthes aspera* and evaluate their antidiabetic and antioxidant activities by using an *in vitro* model. Phytochemical screening of the extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, phenols and steroids. The results of α–amylase and α–glucosidase enzymes inhibition activities were found in a dose-dependent manner. The strongest activity was showed by methanolic fraction (55.0±0.50% for α–amylase and 53.06±0.23% for α–glucosidase inhibition at 160 µg/ml) compared to petroleum ether extract (51.87±00% for α–amylase and 46.0±0.22% for α–glucosidase inhibition at 160 µg/ml). The plant extracts were also examined for its antioxidant activities by using DPPH scavenging method. The DPPH assay exhibited significant antioxidant activity of isolated phytochemical compounds'. The DPPH radical scavenging activity of the methanolic extract (68±0.44% at 250 µg/ml) was higher than that of petroleum ether extract (63.06±0.56% at 250 µg/ml). Thus, in conclusion this study can recommend this plant due to the presence of antioxidant components which have potential prospective for the control of diabetes and the related condition of oxidative stress. This knowledge will be helpful in exploring more potent antidiabetic principle from the natural resources for the clinical development of antidiabetic therapeutics.

Keywords: Diabetes mellitus, antidiabetic, antioxidant, hyperglycemia, postprandial blood glucose, insulin, α-amylase, α-glucosidase

Introduction
Diabetes mellitus is a leading cause of morbidity and mortality worldwide and a major economic burden. It is a chronic metabolic disorder manifested by hyperglycemia in which blood sugar levels are elevated either because of the insufficient production of insulin from the β-cells of pancreas or inactivity of body cells to respond the insulin properly [1]. About 90% of all cases of diabetes in developed and developing countries are non-insulin-dependent diabetes mellitus, also known as type-2 diabetes (T2D), or adult-onset diabetes [2, 3]. Persons with diabetes have lipid disorders and an increased risk of coronary heart disease, peripheral vascular disease and cerebrovascular disease.

In the process of digestion of food in the alimentary canal, α-amylase catalyses the hydrolysis of α-1,4-glycosidic linkages of starch and glycogen and α-glucosidase further breaks down the disaccharides into simple sugars making it available for intestinal absorption. The α-glucosidase enzyme catalyzes the cleavage of glycosidic bond and subsequently liberates glucose from the non-reducing end of the oligosaccharide chain [4]. The postprandial blood glucose levels have been found to play an important role in the onset and developing complications of T2D [5]. The postprandial rise in blood glucose level is associated with the activity of α-amylase and α-glucosidase enzymes in the small intestine [6]. The reduction of the activities of these two enzymes could play a role in managing postprandial hyperglycemia by slowing down the digestion of starch and extending intestinal carbohydrate holding time. This would result in decrease in the rate of glucose absorption and a subsequent reduction in the rate of increase of postprandial blood glucose. This could be a promising therapeutic strategy for the control of T2D, and forms the basis of many current clinical antidiabetic agents [7-9].

In diabetes, chronic hyperglycemia is associated with dyslipidemia, increased oxidative stress and consequently an alteration within the body's antioxidant defense system [10]. There is increasing proof that complications associated with diabetes are related to oxidative stress induced by the generation of free radicals. The antioxidant defense system protects the cells against free radicals. Once formation of free radicals overtakes the antioxidant defense system, the free radicals begin attacking the cells leading to several physiological disorders including diabetes.

In diabetes, an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses have been observed to be the cause of oxidative stress. Hence, compounds with antioxidative properties would be useful antidiabetic agents [11]. Synthetic antioxidants, such as tert butyl hydroquinone, butylated hydroxyanisole, propyl gallate, and butylated hydroxytoluene are extensively used to avoid the oxidative stress and autoxidation process [12]. However, the use of such compounds are restricted due to their side effects on human health; therefore, efforts have been undertaken to search for natural agents as alternatives to synthetic antioxidants [12, 13].

In clinical practice, oral hypoglycemic agents and insulin are used for the treatment of diabetes mellitus; the former being reported to be endowed with characteristic profiles of serious side effects. Several synthetic inhibitors or antidiabetic agents, such as acarbose, miglitol and voglibose may reduce the absorption rate of glucose by slowing down carbohydrate digestion, inflicting a decrease in postprandial serum glucose level [14]. For example, acarbose is an inhibitor of α-glucosidase enzyme that commonly used to decrease glucose absorbance by reducing the production of this enzyme in the small intestine [15]. However, several synthetic hypoglycemic agents are non-specific, fail to prevent the development of diabetic complications and induce side effects [16]. Some of these side effects include abdominal discomfort, gastrointestinal bloating, diarrhea and flatulence [17].

An alternative approach which has attracted considerable attention in recent years is using medicinal plants and their phytoconstituents as natural sources because of their well-known ability to scavenge free radicals. Effectively, plants are one of the best sources of natural antioxidants compounds such as flavonoid, phenolic, and alkaloid compounds that possess various pharmacological properties with little or no side effects and protect human health from many diseases [18-21]. These compounds have ability to remove free radicals, chelate catalytic metals, and scavenge oxygen [12, 22]. Components of plants, with naturally occurring antioxidant activity can be used to control the balance between free radicals and antioxidant stress in diabetes patients, and may be a less harmful alternative to synthetic antioxidant products [23].

The investigators observed that the plant *Achyranthes aspera* belonging to Amaranthaceae family has several medicinal properties. However, the antidiabetic and antioxidant activities of this medicinal plant is not scientifically validated and authenticated. Hence, the present investigation was aimed to evaluate the *in vitro* antidiabetic and antioxidant activities of methanolic and petroleum ether extract of leaves of *A. aspera* so that it can be used for human welfare.

Materials and Methods

1. **Plant material:** *Achyranthes aspera* Linn. (Amaranthaceae) is an annual herb found as weed throughout India. The plants were collected from campus of the Regional Institute of Education (NCERT), Bhubaneswar. The plant was identified on the basis of flower and inflorescence part of *Achyranthes Aspera* and taxonomically identified by the Department of Botany, Utkal University, Bhubaneswar, Odisha.
2. **Preparation of Plant Extract:** The fresh leaves of the plant were collected and washed properly with distilled water so as to remove dust and other foreign particles. The leaves were shade dried in the laboratory with proper ventilation and at an ambient temperature. The air dried leaves were further dried to remove moisture content in a hot air oven at 40^0C for 24 hours. The completely dried leaves were pulverized to a coarse powder by using mechanical grinder. Then the dried powdered material was stored in air tight bottles. 100 gm of powdered material was extracted with different solvents like, methanol and petroleum ether separately using Soxhlet apparatus (40-45 hours each). All the crude extracts obtained were filtered and distilled to evaporate the solvent from the extracts. The liquid extracts were concentrated separately under vaccum and the resulting dried extracts were preserved in desiccator until further use. The dried extracts were weighed and the percent yield was calculated by the formula mentioned below [24].

$$\text{Percent yield of extract} = \frac{\text{Weight of the extract after evaporating solvent and drying x 100}}{\text{Dry weight of the plant material}} \quad \text{…Eq. 1}$$

$$\text{Percent yield of petroleum ether extract} = \frac{6.334}{160} \times 100 = 3.96\% \text{ w/w} \quad \text{…Eq. 2}$$

$$\text{Percent yield of methanolic extract} = \frac{6.31}{160} \times 100 = 10.19\% \text{ w/w} \quad \text{…Eq. 3}$$

Colour of petroleum ether extract was yellowish-green while that of methanolic extract was greenish-black. The percentage yield of petroleum ether extract, i.e. 3.96% was less than methanolic extract, i.e. 10.19%w/w.

3. **Preliminary phytochemical analysis:** Preliminary phytochemical screening to check the presence or absence of primary and secondary metabolites such as alkaloids, saponins, tannins, terpenoids, steroids, phenol and flavonoids of both methanolic and petroleum ether extracts were subjected to a number of chemical tests by following standard methods [25-30]. The different chemical tests performed for phytochemical screening were: (i) steroids – Liebermann-burchard test, (ii) alkaloids – Hager's test, (iii) saponins – Foam test, (iv) tannins – Gelatin-lead acetate test, (v) phenols – Ferric chloride test, (vi) flavonoids – Shinoda test and (vii) terpenoids – Liebermann-burchard test.
4. **α-amylase inhibition assay:** The leaf extracts were examined for its α-amylase inhibitory activity by adopting the method of Hara and Honda [31] with little modifications. Three Test tubes were taken and labeled as blank, test (T) and control (C). To each test tube 2.5 mL of phosphate buffer of pH 6.8 was added. 1mL of starch and sodium chloride was added to all the three test tubes. The test tubes were incubated at 37 ^{0}C for 10 minutes. After incubation, 0.5 mL of extract and 0.2 mL of enzyme (α-amylase) was added to the test tube 'T'. The contents of the test tube were mixed well and incubated at 37^0C for 10 minutes. After incubation, 0.5 mL of 2N NaOH was added to the test tube T and C. 0.2 mL of enzymes was added to the control 'C'. Ascorbic acid serves as blank for positive control. 0.2 mL of dinitro-salicylic acid was added to all the test tubes. The contents were mixed well and kept in a boiling water bath for 15 minutes. The intensity of reddish orange colour was read at 540 nm by UV-spectrophotometer. A known α-amylase inhibitor, acarbose was used for comparison studies. The percentage of inhibitory action of serum α-amylase was calculated by using the following formula.

$$\text{Percentage inhibition} = \frac{\text{O.D. of control} - \text{O.D. of test}}{\text{O.D. of control}} \times 100$$

5 **α-glucosidase inhibition assay:** The α–glucosidase inhibitory activity of *A. aspera* was examined by using the method of Li *et al.* [32] with slight modification. Three Test tubes were taken and labeled as blank, test (T) and control (C). 200 µL of starch and 200 µL of enzyme (α-glucosidase) were added to the test tubes 'T' and 'C'. This was followed by the addition of 0.5 mL of extract to T. The contents were thoroughly mixed and incubated at 37 ^{0}C for 30 minutes. Trichloro-acetic acid was added to all the test tubes after incubation. The test tubes were then centrifuged for 10 minutes. The liberated glucose from the starch by the action of enzyme reacted with 1.5 mL of anthrone reagent. The contents were boiled for 15 minutes. Ascorbic acid was taken as a positive control. A known α-amylase inhibitor, acarbose was used for comparison studies. The intensity of color developed was read at 540 nm by UV-spectrometer. The percentage of inhibitory action of α-glucosidase was calculated by the following formula.

$$\text{Percentage inhibition} = \frac{\text{O.D. of control} - \text{O.D. of test}}{\text{O.D. of control}} \times 100$$

6. **DPPH radical scavenging activity:** DPPH radical scavenging activity was performed using the method employed by Fargere *et al.* [33] and Saeed *et al.* [34] DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was utilized as free radicals. The reducing ability of antioxidants towards DPPH was evaluated by recording the decrease of its absorbance at 515 nm by UV spectrophotometer. The degree of decolorization of the purple coloured solution of DPPH indicates the free radical scavenging activity.
   a. **Qualitative analysis:** The ethanol extract was applied on a TLC plate as a spot (100µg/ml) for chromatographic separation of the extract using the mobile phase methanol: chloroform (95:5, v/v). It was allowed to develop the chromatogram for 30 minutes. After completion of the chromatogram the whole plate was sprayed with DPPH (0.15% w/v) solution using an atomizer. The color changes (yellowish color development on pinkish background on the TLC plate) were noted as an indicator of the presence of antioxidant substances.
   b. **Quantitative analysis:** The free radical scavenging capacity of both petroleum ether as well as methanolic extract was determined by using DPPH. An ethanolic DPPH solution (0.004%) was mixed with serial dilutions (10 to 100 µg/ml) of crude extract and after 30 minutes, the absorbance was read at 515 nm using UV spectrophotometer. Ascorbic acid was used as a positive control and the ethanol and pet ether was used as blank. The inhibition curve was plotted and IC50 value was determined [35]. The DPPH scavenging ability of plant extracts was calculated using the following equation:

$$\% \text{ scavenging activity} = [(\text{Abs control} - \text{Abs sample})]/ (\text{Abs control})] \times 100$$

7. **Statistical Analysis:** All the experiments were performed in triplicates, and complete randomized designs were used. The variables were tested using one-way analysis of variance (ANOVA), and the multiple mean comparisons were performed in SAS V8, Cary, NS, USA) with Turkey's test. The data are expressed as a mean ±standard deviation.

Results

The present study was designed to investigate the bioactive properties of the aforementioned plant. These properties include α-amylase and α-glucosidase inhibition activity and DPPH radical scavenging activity. Phytochemical screening of the methanolic and petroleum ether leaves extracts of *A. aspera* revealed the presence of major metabolites (Table 1). Methanolic extracts showed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and phenols, whereas petroleum ether extract contained tannins, flavonoids, terpenoids, steroids and phenol (Table 1), This could be attributed to the solubility of these phytochemicals in solvents of different polarity. Presence of these phytochemicals in the plant suggest the potential use of *A. aspera* as a source of antidiabetic and antioxidant compounds.

Table 1: The phytochemical profile of the prepared plant leaf extracts

Phytochemical compounds	Petroleum ether extract	Methanolic extract
Alkaloids	–	+
Tannins	+	+
Saponins	–	+
Flavonoids	+	+
Terpenoids	+	+
Steroids	+	+
Phenols	+	+

+: present, –: not present

The % inhibitions of α-amylase activity of *A. aspera* petroleum ether leaves extract were determined along with the standard acarbose. The concentration of acarbose and the extracts varied from 10 to 160 µg/mL. There was a dose-dependent increase in percentage inhibitory activity against α-amylase with 6.77±0.56% at a concentration of 10 µg/ ml and 51.87±0.0% at 160 µg/ml, at 540 nm, whereas in case of methanolic extract the % inhibition of α-amylase activity of *A. aspera* aerial part extract with 14.9±0.35% at a concentration 10 µg/ ml and 55.0±0.50% at 160µg/ml at 540 nm. The positive control, acarbose has exerted the highest potent inhibitory action against α-amylase 66.53±0.34% in petroleum ether and 68.8% in methanol Table 2). The concentration of the extracts resulting in 50% inhibition of the enzyme activity (IC_{50}) was determined graphically (Figure 1).

Table 2: The % inhibition of α-amylase activity at different concentrations

Sample	Concentration (in µg/ml)	% Inhibition	IC50 (µg/ml)
Acarbose	10	20.2±0.03	68.5
	20	34.2±0.028	
	40	42.05±0.67	
	80	53.5±0.66	
	160	66.5±0.34	
Petroleum ether extract	10	6.77±0.56	157.2
	20	14.34±0.87	
	40	28.19±0.10	
	80	39.79±0.10	
	160	51.87±0.0	
Methaanolic extract	10	14.9±0.35	125
	20	19.07±0.65	
	40	27.0±0.87	
	80	47.5±0.49	
	160	55.0±0.50	

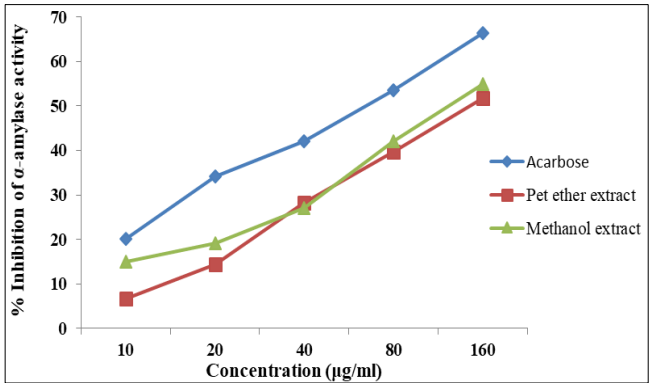

Fig 1: α-amylase inhibitory activity of *A. aspera* leaves extract

The % inhibition of α-glucosidase activity of *A. aspera* methanolic leaves extract was determined along with the standard acarbose. The concentration of acarbose and the extracts varied from 10 to 160 µg/mL. There was a dose-dependent increase in percentage inhibitory activity against α-glucosidase with 11.25±0.14% at a concentration of 10 µg/ ml and 53.06±0.23% at 160 µg/ml, at 540 nm, whereas in case of petroleum ether the % inhibition of α-glucosidase activity of *A. aspera* aerial part extract with 4.07±0.78% at a concentration 10 µg/ ml and 46.0±0.22% at 160 µg/ml at 540nm. The positive control, acarbose has exerted the highest potent inhibitory action against α-glucosidase is 60.05±0.25% (Table 3). The concentration of the extracts resulting in 50% inhibition of the enzyme activity (IC_{50}) was determined graphically (Figure 2).

Table 3: The % inhibition of α-glucosidase activity at different concentrations

Sample	Concentration (in µg/ml)	% Inhibition	IC50
Acarbose	10	15.2±0.98	120.04
	20	26.5±0.45	
	40	33.9±0.56	
	80	48.5±0.35	
	160	60.5±0.25	
Petroleum ether extract	10	4.07±0.78	173.05
	20	9.65±0.66	
	40	21.78±0.02	
	80	33.3±1.09	
	160	46.0±0.22	
Methaanolic extract	10	11.25±0.14	147.5
	20	16.20±0.65	
	40	24.09±0.76	
	80	37.43±0.56	
	160	53.06±0.23	

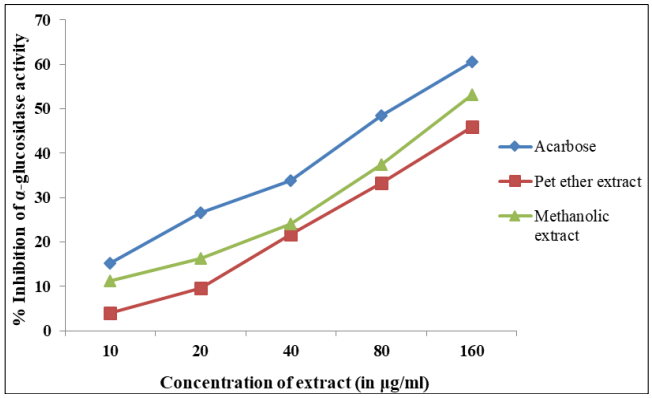

Fig 2: α-glucosidase inhibitory activity of *A. aspera* leaves extract

Antioxidant activities of the methanolic and petroleum ether extracts of *A. aspera* were tested for antioxidant activity using DPPH free radical. DPPH is a stable free radical largely used for evaluating scavenging activity of antioxidants available in plant extracts. The DPPH solution loses the characteristic dark purple colour and becomes yellow and this was used as a parameter to evaluate the *in vitro* antioxidant activity of leaf extract of *A. aspera*. The extracts showed antioxidant activity as shown in Table 4. There was a dose-dependent increase in the percentage of antioxidant activity for all concentrations tested. Ascorbic acid was used as the standard drug for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid and the extracts varied from 50 to 250 µg/mL. The highest inhibitory activities showed by the methanolic extract was 68±0.44% inhibition at 250 µg/ mL and that of the petroleum ether extract was 63.06±0.56% inhibition at 250 µg/ mL. The standard positive control ascorbic acid showed 77±0.45% inhibitions at 250 µg/mL.

Table 4: Free radical scavenging activity of prepared plant leaf extracts

Sample	Concentration (in µg/ml)	% DPPH radical scavenging activity	IC_{50} (in µg/ml)
Ascorbic acid	50	35±0.09	110.0
	100	47±0.67	
	150	56±1.01	
	200	69±0.45	
	250	77±0.45	
Petroleum ether extract	50	19. 5±0.30	207.05
	100	34.2±0.87	
	150	43.1±0.45	
	200	49.07±0.57	
	250	63.06±0.56	
Methaanolic extract	50	29.05±0.36	167.98
	100	39±0.56	
	150	48±0.78	
	200	55±0.34	
	250	68±0.44	

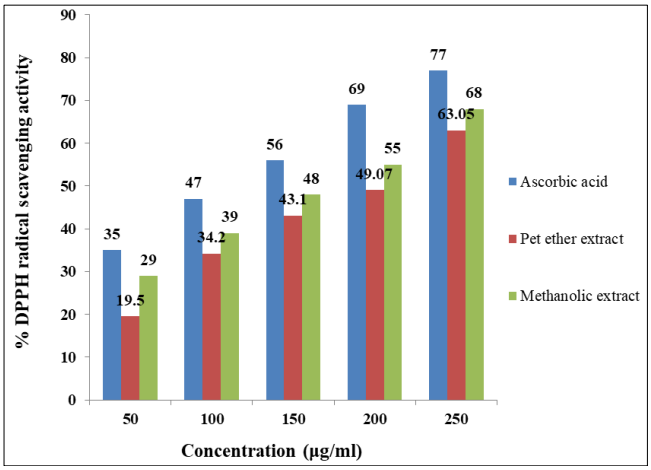

Fig 3: DPPH radical scavenging activity of the *A. aspera* methanolic and petroleum ether extracts

Discussion

The use of ethno-botanicals has a long history for the treatment of diabetic conditions as well as oxidative stress-related conditions [1, 34, 36] however, extensive scientific validation is still awaited. Therefore, the search for more effective and safer antidiabetic and antioxidant agents remain

as an important area of research. The present study is an attempt to evaluate the antidiabetic and antioxidant activity of the leaves of *Achyranthes aspera*. The phytochemical analysis of methanolic and petroleum ether extracts of *Achyranthes aspera* leaves revealed the presence of several phytochemicals such as alkaloids, tannins, saponins, flavonoids, terpenoids, phenols and steroids.[21] Several studies have shown that most of these compounds have antioxidant properties [37-40].

The postprandial blood glucose levels have been found to play an important role in the onset and developing complications of T2D [5]. (Inhibition of α-amylase and α-glucosidase activities is one of the therapeutic strategies for managing postprandial hyperglycemia [8]. Though synthetic drugs like acarbose and miglitol strongly inhibit the action of α-amylase and α-glucosidase, however, they produce side effects like vomiting, abdominal distention, flatulence, and diarrhea [41]. Recently, the interest in using medicinal plants and their phytoconstituents as natural substances has considerably increased, because these substances are intended to be used as drugs to replace synthetic compounds to avoid side effects. In recent years, the hunt for natural sources has increased significantly because of their well-known ability to scavenge free radicals. Several researchers have reported that plants are the sources for natural antioxidants compounds that possess various pharmacological properties with little or no side effects and protect human health from many diseases [19, 42]. The phytochemicals prevent oxidative stress related diseases by delaying the oxidation of lipids or other molecules by inhibiting the propagation of oxidative chain reaction. A number of studies have reported that phytochemicals offer potential therapeutic benefits in alleviating diabetes and inhibitory effects against α-amylase and α-glucosidase [41, 43-45]. The medicinal plants are a safer alternative for management of T2D as they help to maintain low blood glucose and prevent high blood pressure, as well as boost body antioxidant system and insulin regulation [46]. The plants containing antioxidant compounds can protect β-cells from reactive oxygen species (ROS) and therefore, can prevent diabetes induced by ROS [46].

The result of the present study revealed that both petroleum ether and methanolic leaf extracts showed inhibitory effects on both α-amylase and α-glucosidase enzymes (Table 1 and 2) which increased with increased concentration. Indeed, at the concentration of 160 µg/ml, petroleum ether extract has the highest inhibitory activity against α-amylase (51.87% ±0.0and 55.0% ±0.5). However, the inhibitory effect of methanolic extract on α-glucosidase enzyme is much stronger (53.06% ±0.23) than petroleum ether extract (46.0% ±0.22) at concentration of 160 µg/ml. Some researchers also reported that the methanolic extract showed α-glucosidase inhibitory activity which increased with increased concentration [40, 42, 47]. To calibrate the effectiveness of inhibition, IC_{50} was used which represents the concentration of an inhibitor required for 50% inhibition of its targeted enzyme. The petroleum ether and methanolic extracts showed a stronger inhibitory capacity against α-amylase with IC_{50} values of 157.2 µg/ml and 125.0 µg/ml respectively which are greater than that of acarbose (68.5 µg/ml). Similar trend was observed for α-glucosidase. The acarbose α-glucosidase inhibitory capacity was 120.04 µg/ml, which was quite lower than petroleum ether and methanolic extracts inhibitory capacity i.e.173.05 µg/ml and 147.5 µg/ml respectively. The obtained results of the present study are in accordance with earlier reports [40, 42, 48-50].

The difference in α-amylase and α-glucosidase inhibition capacity may be due to structural difference in enzymes or phytochemical composition of extracts or both. The past study on *Aristolochia longa* reported that difference between ethyl acetate and methanolic extracts inhibitory activities of α-glucosidase was due to phytochemical composition of the plant [42]. In *Eleusine coracana*, the phenolics compounds inhibit the activity of carbohydrases because of their ability to bind with proteins [51]. The flavonoids possesses high inhibitory potential on α-glucosidase and can reduce postprandial blood glucose level [52]. In the present study, the phytochemical profile of both petroleum ether and methanolic extract showed the presence of both phenols and flavonoids which may be the reason for inhibition of α-amylase and α-glucosidase activity. The result of the present study demonstrated stronger inhibitory effect of methanolic extract on both α-amylase and α-glucosidase activity which may be due to presence of other phytochemicals like alkaloids and saponins that are absent in petroleum ether extract. Similar views have been suggested by some recent studies in *Gymnema montanum* [53] in mice [54] in *Ocimum canom* [55] and in *Clausena excavate* [56] on α-amylase and α-glucosidase activity.

In recent years, a number of studies have shown that complications associated with diabetes are related to oxidative stress induced by the generation of free radicals [9, 56, 57]. The elevated free radical level in the body as a result of a failure in endogenous antioxidant defense mechanism or exposure to environmental oxidants or damage to cell structures enhances the risk [58, 59]. The free radicals (associated with one or more unpaired electrons) are highly unstable and attain stability by extracting electrons of other molecules. The unstable antioxidants eliminate free radicals by inhibiting the rate of oxidation [57]. The antioxidant drugs used for treatment of diabetes, heart attack, artherosclerosis and cancer etc which are due to oxidative stress produce side effects [60]. In the current study, DPPH assay was used to explore the antioxidant activity of leaf extract of *A. aspera*. Both petroleum ether and methanolic leaf extracts showed scavenging effect which increased with the concentration of the samples. At 250 µg/ml concentration, both petroleum ether and methanolic leaf extracts exhibited maximum DPPH radical scavenging activity of 63.06±0.56% and 68±0.44% respectively. The findings of the study showed stronger DPPH radical scavenging capacity of petroleum ether and methanolic extracts with IC_{50} values of 207.05 µg/ml and 167.98 µg/ml respectively (Table 4) which are higher than that of standard drug, ascorbic acid (110.0 µg/ml). The results of the present study are in analogy with the studies in various Algerian plants, in *Aristolochia indica* [61] in 11 herbal plants [40] and in *Aristolochia longa* [42]. By using DPPH assay, they all showed that the respective plant extracts had higher free radical scavenging activity than the standard drug used in their studies. In the current research, the observed superior free radical scavenging activities of petroleum ether and methanolic leaf extracts of plants *A. aspera* could be correlated to their high flavonoid and phenolic compounds. Moreover, the antiradical activity relies on the availability and capacity of these extracts to provide hydrogen or electron [59]. In the present research, the findings acquired indicated the free radical stabilizing ability of extracts by giving them electron or hydrogen. The difference in antiradical activity observed amongst the extracts (63.06±0.56% for petroleum ether and 68±0.44% for methanol) in the present study may

be due to the presence of different level of several bioactive compounds (such as phenols and flavonoids) which have the ability to donate hydrogen atoms to stabilize the free radicals [62, 63] or may be due to presence of other phytochemicals like alkaloids and saponins that are absent in petroleum ether extract. However, further comprehensive studies are required to isolate the antioxidant components of these extracts and determine their *in vivo* biological activities.

Conclusion

The preliminary phytochemical analysis indicated that, the plant *Achyranthes aspera* leaf extracts contain supportive phytochemicals such as alkaloids, tannins, saponins, flavonoids, terpenoids, phenols and steroids. The obtained results revealed that both methanolic and petroleum ether leaf extracts showed significant *in vitro* antioxidant and antidiabetic activity when compared with standard drugs. The methanolic leaf extract of *Achyranthes aspera* exhibited superior *in vitro* free radical scavenging and antidiabetic activity than petroleum ether extract. This superior activity might be due to presence of different level of several bioactive compounds or may be due to presence of other phytochemicals like alkaloids and saponins that are absent in petroleum ether extract but present in methanolic extract. However, further research is needed to determine the study-specific phytochemicals with the mechanisms behind these activities.

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Conflicts of Interest

The authors declare no conflicts of interest.

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