

In vitro* Assessment of Total Bioactive Contents, Antioxidant, Anti-Alzheimer and Antidiabetic Activities of Leaves Extracts and Fractions of *Aloe vera

**Samira Bendjedid^{1*}, Radia Djelloul¹, Aicha Tadjine¹,
Chawki Bensouici², and Abbas Boukhari³**

¹Department of Biology, Faculty of Natural Sciences and Life, Research Laboratory of Functional and Evolutionary Ecology, Chadli Bendjedid University, El Tarf 36000, Algeria

²Biotechnology Research Center, Constantine 25000, Algeria

³Department of Chemical Sciences, Research Laboratory of Organic Synthesis Modelling and Optimization of the Chemical Processes, Badji Mokhtar University, Annaba 23000, Algeria

*Corresponding author. E-mail: samiraphyto@gmail.com
<https://doi.org/10.12982/CMUJNS.2020.0031>

Received: August 14, 2019

Revised: September 21, 2019

Accepted: September 24, 2019

ABSTRACT

*The present work was conducted to evaluate the quantitative analysis of the leaves of *Aloe vera* and to evaluate their potential antioxidant, anti-Alzheimer and antidiabetic properties in vitro. The acetone extract, methanolic extract and its four fractions were subjected to quantitative determination of polyphenol, flavonoid, flavonol, condensed tannin and hydrolysable tannin contents. Then, the antioxidant properties of all extracts and fractions were evaluated by using DPPH free radical scavenging, ABTS cation radical decolorization, Cupric Reducing Antioxidant Capacity and Metal Chelating Activity assays. Anti-Alzheimer activity was tested against acetylcholinesterase and butyrylcholinesterase enzymes using the Ellman method. The antidiabetic activity was evaluated by using Alpha-Glucosidase Inhibition Assay. The acetone extract showed the highest amount of total phenolic content (TPC), total flavonoid content (TFC), flavonol content (FLC) and total condensed tannins content (TCTC) compared to others extracts and fractions. The water fraction exhibited the low content of TFC, TPC and FLC. The content of total hydrolysable tannins (THTC) varied from 0.94 ± 0.01 to 1.22 ± 0.02 $\mu\text{g TAE/mg}$ extract. The methanolic extract exhibited highest antioxidant activity in all tests (IC_{50} value: 24.21 ± 0.30 $\mu\text{g/mL}$ in DPPH assay, IC_{50} value : 30.75 ± 1.67 $\mu\text{g/mL}$ in ABTS assay, IC_{50} value: 140.99 ± 2.95 $\mu\text{g/mL}$ in metal chelating activity, and*

IC₅₀ value: 17.50±0.47 µg/mL in CUPRAC assay). Furthermore, the n-butanol fraction indicated the highest BChE inhibitory activity (IC₅₀ value: 79.61±2.41 µg/ml) and the good α-glucosidase inhibitory activity (IC₅₀ value: 40.75±0.06 µg/ml). These results showed that Aloe vera leaves, can be used in food and pharmaceutical industries as natural antioxidants, as well as moderate anti-Alzheimer and antidiabetic agents.

Keywords: *Aloe vera*, Antidiabetic, Anti-Alzheimer, Antioxidant, Extract, Phenolic

INTRODUCTION

The use of traditional medicine is as old as human, civilization and in many regions of the world is still the primary source of health care. Ayurvedic medicine in India, for example, is still commonly practiced, with approximately 85% of Indians using crude plant preparations for the treatment of various ailments and diseases (Kamboj, 2000). Even in Western civilizations, plants play an important role in medicine. At least 25% of pharmaceuticals prescribed worldwide are directly obtained from plants with many more drugs being semi-synthetic derivatives of natural plant precursors (Hostettmann and Hamburger, 1993; Walsh, 2003). Approximately 119 pure chemical substances extracted from higher plants are used in medicine throughout the world (Hoareau and Dasilva, 1999). Examples of medicinally important plant-derived compounds include the anticholinergic drug atropine derived from plants of the family *Solanaceae* (*Atropa belladonna*, *Datura stramonium* and *Mandragora officinarum*), the analgesics morphine and codeine (from *Papaver somniferum*), the anti-malarial drug quinine and its derivatives (from *Cinchona* spp.), the anticancer drug taxol (derived from *Taxus brevifolia*) (Gilani and Rahman, 2005).

Plants have the capacity to synthesize a large variety of secondary metabolites that are used to perform important biological functions. Since ancient times, many plants have been used for the treatment of many diseases related to the toxic effects of oxidants. Oxidative stress is responsible for several diseases, such as atherosclerosis, arthritis, cardiovascular disorders, Alzheimer's disease and cancer (Halliwell, 1999). Alzheimer's disease is the severe form of dementia, and the acetylcholinesterase (AChE) inhibitor drugs are used to treat Alzheimer's disease. Most of these drugs cause liver and bradycardia, intestine toxicities and stomach (Dökmeçi, 2000). The synthetic antioxidants can induce many side effects, for these reasons, the improvement and handling of safe anticholinesterases and antioxidants from nature are desired. As one of the most important secondary metabolites, phenolic compounds are widely investigated in many medicinal plants. The beneficial effects of phenolic compounds in human life is attributed to their antioxidant activity that mainly due to their redox

properties, which allow them to act as reducing agents, hydrogen donators (Rice-Evans et al., 1996), radical scavengers, and metal chelators.

The relative or absolute deficiency of insulin or the resistance of the β cells to the action of insulin causes an abnormal increase in the concentration of glucose in the blood that induces an endocrine disorder, it is the diabetes mellitus. The fast uptake of glucose in the intestine by the action of α -amylase and α -glucosidase helps in the breakdown of complex carbohydrates into simple sugars is an important factor which results in postprandial hyperglycemia (Nagmoti and Juvekar, 2013). The synthetic antidiabetic drugs are commonly used for treating diabetes mellitus by inhibiting α -amylase and α -glucosidase activity and thus, reduce glucose absorption in the body. These drugs can induce many side effects, such as renal tumors, adverse gastrointestinal symptoms and liver toxicity (Gray and Flatt, 1997; Fujisawa et al., 2005). For this reason, it is necessary to replace them with natural antidiabetic.

Plants from the genus *Aloe* belonging to *Asparagaceae* family have a long history of medicinal use in the treatment of many diseases. Among these plants we are interested by the *Aloe vera* which is an ornamental and medicinal plant, it is a perennial succulent plant. The leaves of *Aloe vera* consist of two products, first one is yellow bitter juice from cut leaf base and the second is mucilaginous juice from the leaf parenchyma. This plant has several biological properties: antimicrobial (Ferro et al., 2003; Cock, 2008), redox-state maintenance bioactivities (Sirdarta and Cock, 2008; Sirdarta and Cock, 2010), anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-dermatological, nutritional, cosmetic, digestive (Hamman, 2008; Mariappan and Shanthi, 2012).

The major constituents of *Aloe vera* leaves can be classified into five groups, namely, phenolics, saccharides, vitamins, enzymes, and low molecular weight substances (Ray et al., 2013). A large part of the pharmacological properties are due to the presence of various polysaccharides and the phenolic compounds as well (Rodriguez et al., 2010). *Aloe vera* polysaccharides have also been speculated to enhance immunity activity and exert antioxidant effects in oral ulcer animal models (Yu et al., 2009). Faster wound closure has also been demonstrated in rats treated with isolated and characterised *Aloe vera* polysaccharides (Oryan et al., 2016). The biological activities of *Aloe vera* leaves is the results of synergism among the inherent component compounds of leaves, rather than a single ingredient (Hamman, 2008). The bioactivity of *Aloe vera* with reference to the anti-oxidative potential, has been demonstrated by different groups of authors, and the phenolic components and acetylated polysaccharides present in *Aloe vera* leaves have been assigned to the pertained free radical scavenging activities (Hu et al., 2003; Lui et al., 2007; El-Shemy et al., 2010; Ray et al., 2012). In our another investigation, it has been observed that the phenolic content, antioxidant, antidiabetic and anti-Alzheimer of *Aloe vera* are interlinked phenomena, and phenolic content was found to be critical in the regulation of pertained antioxidant activities, the antioxidant activity of aloin

was also found to be significant in the investigation (Ray et al., 2012). Furthermore, *Aloe vera* leaves has shown its potential in the management of diabetes mellitus. Clinical trials have shown that, in obese individuals with prediabetes or early untreated diabetes mellitus, *Aloe vera* gel complex reduced body weight, body fat mass, and insulin resistance (Choi et al., 2013). Abo- Youssef and Messiha (2013) also proved the antidiabetic effect of *Aloe vera* leaf pulp extract *in vivo* and *in vitro* as compared to glimepiride.

The present study aims to determine the total polyphenol, flavonoid, flavonol, and tannin contents and to investigate the antioxidant, anti-Alzheimer and antidiabetic activities of different extracts and fractions of *Aloe vera*: methanol, chloroform, ethyl acetate, n-butanol, water and acetone.

MATERIALS AND METHODS

Plant material

The leaves of *Aloe vera* were collected in October 2016 from the *Aloe vera* farm at Paraje Retamar in the interior of the municipality of Almaria (southeast of Spain). The leaves of the plant were identified at the Laboratory of Functional and Evolutionary Ecology of Chadli Bendjedid University (El Tarf, Algeria), where the voucher specimen has been deposited. The leaves of the plant were crushed into a fine powder using an electric blender and then was stored in glass vials protected from light.

Spectral measurements and chemicals used

The measurements and calculations of the activity results were evaluated by using bioactivity measurements, they were carried out on a 96-well microplate reader, Perkin Elmer Multimode Plate Reader EnSpire at Center of Biotechnology Research. 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylatedhydroxylanisole (BHA), butylatedhydroxyltoluene (BHT), α - Tocopherol, Folin-ciocalteu's reagent (FCR), 3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid disodium salt (Ferrene), Ethylenediaminetetraacetic acid (EDTA), Neocuproine, 2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonicacid)diammoniumsalt (ABTS), p-nitrophenyl- α -D-glucopyranoside, 5,50-dithiobis (2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide, butyrylthiocholine chloride, and galantamine were obtained from Sigma Chemical Co (Sigma-Aldrich GmbH, Stern-heim, Germany), horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg) and electric eel AChE (Type-VI-S, EC 3.1.1.7, 425.84 U/mg) were obtained from Sigma, St. Louis, MO, Copper (II) chloride, Potassium persulfate, Sodium Carbonate, Aluminum Nitrate, Iron (II) chloride, Potassium acetate, Ammonium acetate were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade.

Preparation and fractionation of methanolic extract

The extraction was carried out according to the method of Yanlun et al. (2016) with minor modifications. 10 g of leaf powder was mixed with 100 mL of MeOH/H₂O (80:20, v/v) for 24 h at room temperature. The extraction was performed 3 times with renewal of the solvent. The contents were then filtered through Whatman filter paper n°1. The filtrate was evaporated at 40° C by a rotary evaporator. After evaporation, the obtained crude methanol extract was dissolved with 30 mL of water. The remaining solution was washed with 30 mL of petroleum ether to remove lipid soluble substances. Then the organic phase was eliminated. The remaining aqueous solution was successively extracted with chloroform, ethyl acetate and n-butanol. After evaporation, four fractions were obtained: chloroform, ethyl acetate, n-butanol and water.

Preparation of acetone extract

The preparation of the acetone extract was carried out according to the method of Zhang et al. (2008). 5 g of leaf powder was mixed with 100 mL of acetone and distilled water (70/30, v/v) for 72 h at room temperature. Then, the mixture was filtered through Whatman filter paper n°1. The filtrate was evaporated at 40° C by a rotary evaporator. The remaining solution was washed with 30 mL of dichloromethane to remove lipid soluble substances. The aqueous phase was extracted with 30 mL of ethyl acetate. After evaporation of the solvent, the acetone extract was obtained.

The extraction yield was calculated and expressed as the percentage of the extract in relation to the mass of crushed leaves (% w/w).

Determination of total polyphenol content

The TPC of extracts and fractions was determined on the microplate reader according to a method described by Müller et al. (2010). A volume of 20 µL of extract solution (1 mg/ml in methanol) was mixed with 100 µL of Folin–Ciocalteu reagent (1:10) and 75 µL of sodium carbonate 7.5%. The mixture was incubated at room temperature in the dark for 2 h. A blank is prepared in the same way by replacing the extract by the used solvent. The absorbance was read at 765 nm using a 96-well microplate reader. The TPC was calculated from the linear regression equation of standard curve of gallic acid ($y=0.003x+0.104$, $R^2=0.997$) and expressed as gallic acid equivalents in micrograms per milligram of extract (µg GAE/mg extract).

Determination of total flavonoid content

The TFC was determined using the aluminum chloride (AlCl₃) method developed by Topçu et al. (2007) with some modifications. A volume of 50 µL of extract solution (1 mg/ml in methanol) was mixed with 30 µL of methanol, 10 µL of potassium acetate (1M) and 10 µL of aluminium nitrate (10%). The mixture was allowed to stand at room temperature for 40 min. A blank is prepared in the same way by replacing the extract by the used solvent. The absorbance was read at 415 nm using a 96-well microplate reader. The TFC was calculated

from the linear regression equation of standard curve of quercetin ($y=0.004x$, $R^2=0.997$) and expressed as quercetin equivalents in micrograms per milligram of extract ($\mu\text{g QE/mg extract}$).

Determination of total flavonol content

The FLC was determined using the aluminum chloride (AlCl_3) method developed by Kumaran and Karunakaran (2007). 50 μL of extract solution (1 mg/ml in methanol) was mixed with 50 μL (20 mg/mL) aluminum chloride and 150 μL (50 mg/mL) sodium acetate. The mixture was incubated for 150 min at room temperature in the dark, the absorbance was measured at 440 nm using a 96-well microplate reader. The FLC was calculated from the linear regression equation of standard curve of quercetin ($y=0.012x+0.017$, $R^2=0.999$) and expressed as quercetin equivalents in micrograms per milligram of extract ($\mu\text{g QE/mg extract}$).

Determination of total condensed Tannins content

The TCTC was determined using the vanillin assay (Julkunen-Titto, 1985). To 50 μL of sample, 1,500 μL of vanillin/methanol solution (4%, w/v) were added, and the solution was homogenized. Then, 750 μL of concentrated HCl were added and left at room temperature around 20 min. The absorbance was measured against the blank at 550 nm. The TCTC was calculated from the linear regression equation of standard curve of catechin ($y=0.004x-0.038$, $R^2=0.994$) and expressed as catechin equivalents micrograms per milligram of extract ($\mu\text{g CE/mg Extract}$).

Determination of total hydrolysable Tannins content

The THTC was determined using potassium iodate assay (Çam and Hişil, 2010). A volume of 1 mL of sample was mixed with 5 mL of the potassium iodate (4%), the mixture was homogenized and left at room temperature around 15 min. The absorbance was measured against the blank at 550. The THTC was calculated from the linear regression equation of standard curve of tannic acid ($y=0.144x-0.133$, $R^2=0.989$) and expressed as tannic acid equivalents micrograms per milligram of extract ($\mu\text{g TAE/mg Extract}$).

Antioxidant activity

DPPH free radical scavenging activity. The free radical scavenging activity of the extracts, fractions and standards (BHT, BHA and α tocopherol) was determined spectrophotometrically by the DPPH assay described by Blois (1958) with slight modifications. 40 μL of various concentrations (12.5-800 $\mu\text{g/mL}$) was added to 160 μL of freshly prepared DPPH solution (0.1M) in methanol. The mixture was incubated for 30 min at room temperature in the dark, the absorbance was measured at 517 nm using a 96-well microplate reader. BHT, BHA and α -tocopherol were used as antioxidant standards for comparison of the activity.

DPPH free radical scavenging activity in percentage (%) was calculated using the following equation :

$$\% \text{ Inhibition} = [(Abs_{CN} - Abs_{Ext}) / Abs_{CN}] \times 100$$

Where Abs_{CN} is the absorbance of the control reaction and Abs_{Ext} is the absorbance of the extract.

ABTS cation radical decolorization assay. The $ABTS^{\cdot+}$ scavenging activity was done by the method of Re et al. (1999) with slight modifications. The $ABTS^{\cdot+}$ was produced by the reaction between 7 mM ABTS in distilled water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. The $ABTS^{\cdot+}$ solution was diluted to get an absorbance of 0.703 ± 0.025 at 734 nm with ethanol. 40 μ L of various concentrations (12.5-800 μ g/mL) was added to 160 μ L of $ABTS^{\cdot+}$ solution, After 10 min the absorbance was measured at 734 nm using a 96-well microplate reader. BHT and BHA were used as antioxidant standards for comparison of the activity. The scavenging capability of $ABTS^{\cdot+}$ was calculated using the following equation :

$$\% \text{ Inhibition} = [(Abs_{CN} - Abs_{Ext}) / Abs_{CN}] \times 100$$

Where Abs_{CN} is the absorbance of the control reaction and Abs_{Ext} is the absorbance of the extract. The results were given as IC_{50} value (μ g/mL) corresponding the concentration of 50% inhibition.

Cupric reducing antioxidant capacity assay. The cupric reducing antioxidant capacity of the extracts and fractions was determined by the CUPRAC method (Apak et al., 2004) with slight modifications. 40 μ L of various concentrations (12.5-800 μ g/mL) was added to 60 μ L of Ammonium Acetate buffer (1 M, PH 7.0) solution, 50 μ L of copper (II) chloride solution (0.01 M prepared from $CuCl_2 \cdot 2H_2O$) and 50 μ L of neocaproine solution (0.0075 M). The mixture was incubated for 60 min at room temperature. The absorbance of the solution was measured at 450 nm by the use of 96-well microplate reader. BHT, BHA and α -tocopherol were used as antioxidant standards for comparison of the activity. The results were given as $A_{0.50}$ (μ g/mL) corresponding the concentration indicating 0.50 absorbance intensity.

Metal chelating activity on ferrous ions. The metal chelating activity of *Aloe vera* extracts and fractions on Fe^{2+} was measured spectrophotometrically (Decker and Welch, 1990) with slight modifications. 40 μ L of various concentrations (12.5-800 μ g/mL) was added to 40 μ L of methanol and 40 μ L of 0.2 mM $FeCl_2$. The reaction was initiated by the addition of 80 μ L 0.5 mM ferene, after 10 min the absorbance was measured at 593 nm using a 96-well microplate reader. EDTA was used as chelating standard for comparison of the activity.

The metal chelating activity was calculated using the following equation. The results were given as IC₅₀ (µg/mL):

$$\% \text{ Inhibition} = [(Abs_{CN} - Abs_{Ext}) / Abs_{CN}] \times 100$$

Where Abs_{CN} is the absorbance of the control reaction and Abs_{Ext} is the absorbance of the extract.

Anti-Alzheimer activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts and fractions was determined by the method of Ellman et al. (1961) with slight modifications. 150 µL of sodium phosphate buffer (100 mM, pH 8.0), 10 µL of the sample solution dissolved in ethanol at different concentrations (3.125-200 µg/mL) and 20 µL AChE or BChE solution in buffer were mixed and incubated for 15 min at 25 °C, and 10 µL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 10 µL of acetylthiocholine iodide (0.5 mM) or 10 µL of butyrylthiocholine chloride (0.2 mM). The absorbance of the solution was measured at 412 nm by the use of 96-well microplate reader. The galantamine was used as the reference compound. The results were given as IC₅₀ value (µg/mL) corresponding the concentration of 50% inhibition.

Antidiabetic activity

Alpha-glucosidase inhibition assay. Alpha-glucosidase inhibitory activity was performed according to Nampoothiri et al. (2011). 50 µL of various concentrations (15.62-1000/mL) was added to 100 µl of enzyme solution (0.1U/ml) and 50 µl substrate solution (using 5 mM p-nitrophenyl- α-D-glucopyranoside in 100 mM sodium phosphate buffer, pH 6.9). The mixture was incubated for 5 min at 37°C, the absorbance was measured at 405 nm each 10 min, for 30 min using a 96-well microplate reader. Acarbose (1 mg/ml) was used as a positive control. The results were given as IC₅₀ value (mg/mL) corresponding to the concentration shows 50% inhibition.

Statistical analysis

All assays were estimated in triplicates and the results were presented as means ± standard deviation (SD). Statistical analysis was performed using Variance Analysis (ANOVA). The results are considered to be significant when $P < 0.05$.

RESULTS

Extraction yield

The extraction yield was calculated for the different extracts and fractions of *Aloe vera*. The highest yield was 20 % for methanolic extract while its fractions; chloroform, ethyl acetate, n-butanol, water were 3.50 %, 1.35 %, 14.63 %, 12.72 %

respectively. It was noted that the acetone extract has the low yield 1.16 %. These results are presented in Table 1.

Table 1. Extraction yields of *Aloe vera* in the organic solvents.

Extracts/ Fractions	Yields (%)
Methanol	20.97 ± 0.36
Chloroform	3.50 ± 0.11
Ethyl acetate	1.35 ± 0.12
n-Butanol	14.63 ± 0.12
Water	12.72 ± 0.75
Acetone	1.16 ± 0.5

Total polyphenol, total flavonoid, total flavonol, total condensed and total hydrolysable tannin contents

The quantification of TPC, TFC, FLC, TCTC and THTC of *Aloe vera* extracts and fractions are presented in Table 2. The total phenol content was significantly ($P < 0.05$) higher than total flavonoid and total flavonol. The ethyl acetate fraction showed the highest TPC (258.78 µg GAE/mg extract) followed by acetone extract (225.33 µg GAE/mg extract), chloroform (199.89 µg GAE/mg extract), methanol (187.55 µg GAE/mg extract) and n-butanol (149.22 µg GAE/mg extract). Whereas, the water fraction showed the lowest level (125.67 µg GAE/mg extract). While, for the TFC, the acetone extract was found to have the highest TFC (175.58 µg QE/mg extract) compared to chloroform fraction (90.25 µg QE/mg extract), n-butanol fraction (77.5 µg QE/mg extract), methanolic extract (76.25 µg QE/mg extract) and water fraction (75.75 µg QE/mg extract). However, the lower rate of TFC was found in the ethyl acetate fraction (67µg QE/mg extract).

The acetone extract exhibited the highest FLC with (87.30 µg QE/mg extract) followed by ethyl acetate, chloroform, methanol, n-butanol and water extracts (50.13 µg QE/mg extract, 39.69 µg QE/mg extract, 39.58 µg QE/mg extract, 32.38 µg QE/mg extract and 27.86 µg QE/mg extract respectively).

The TCTC of extracts and fractions ranged from 11.66 to 38.16 µg CE/mg extract. This study showed that the acetone extract of leaves exhibited the highest TCTC with (38.16 µg CE/mg extract) followed by ethyl acetate, methanol, chloroform, n-butanol and water extracts (36.66 µg CE/mg extract, 19.41 µg CE/mg extract, 16.83 µg CE/mg extract, 13.41µg CE/mg extract and 11.66 µg CE/mg extract respectively). The THTC varied from 0.94 to 1.22 µg TAE/mg extract. Among the extracts, the water fraction was found to contain the highest amount of THTC (1.22 µg TAE/mg extract).

Table 2. Total polyphenol, total flavonoid, total flavonol, total condensed tannin and total hydrolysable tannin contents of *Aloe vera* extracts and fractions.

Extracts/ Fractions	TPC ¹	TFC ²	FLC ³	TCTC ⁴	THTC ⁵
Methanol	187.55± 0.09	76.25±0.03	39.58±0.88	19.41±0.76	0.94±0.01
Chloroform	199.89±0.04	90.25±0.00	39.69±0.01	16.83±0.38	1.02±0.00
Ethyl acetate	225.33±0.05	67±0.04	50.13±0.02	36.66±0.62	1.04±0.02
n-Butanol	149.22±0.00	77.5±0.01	32.38±0.00	13.41±0.46	0.97±0.01
Water	125.67±0.2	75.75±0.16	27.86±0.25	11.66±0.14	1.22±0.02
Acetone	258.78±0.09	175.58±0.02	87.30±0.01	38.16±0.87	1.00±0.04

Note: Each value represents a means±SD of three parallel measurements ($P < 0.05$).¹: µg gallic acid/mg Extract, ²: µg quercetin/mg Extract, ³: µg quercetin/mg Extract, ⁴: µg catechin/mg Extract, ⁵: µg tannic acid/mg extract.

Antioxidant activities

The free radical scavenging activity of extracts and fractions is presented in Table 3. The results reveal that the DPPH• Radical scavenging activity increased linearly with increasing of concentration. In this study The methanolic extract exhibited the highest activity (IC_{50} : 24.21 µg/mL) in comparison with the other extracts and closer activity to that of BHT (IC_{50} : 12.99 µg/mL), α-Tocopherol (IC_{50} : 13.03 µg/mL), and further to the BHA (IC_{50} : 6.15 µg/mL), followed by acetone extract, n-butanol, ethyl acetate and chloroform fractions (IC_{50} : 92.20, 119.63, 297.15, 298.20 µg/mL, respectively). The methanolic extract also exhibited a good activity but less than those of antioxidant standards, however Water fraction showed weak activity ($IC_{50} > 800 \mu\text{g/mL}$).

The results of ABTS Radical Scavenging Assay of the extracts and fractions are compared with those of BHT and BHA (Table 3). The methanolic extract exhibited the highest activity (IC_{50} : 30.75 µg/mL) very close to the n-butanol fraction and water fraction (IC_{50} : 34.16 and 37.60 µg/mL, respectively) and is further to BHA and BHT (IC_{50} : 1.82 and 1.30 µg/mL, respectively), followed by chloroform fraction (IC_{50} : 42.14 µg/mL) and acetone extract (IC_{50} : 81.84 µg/mL), these last extracts and fractions also exhibited a good activity but less than those of antioxidant standards, however the ethyl acetate showed weak activity (IC_{50} : 128.98 µg/mL).

The results of CUPRAC of the extracts and fractions are compared with those of BHT and BHA (Table 3). Activity (absorbance) increased linearly with the increasing amount of extracts and fractions. In this assay the methanolic extract exhibited highest activity ($A_{0.50}$: 17.50 µg/mL) among the extracts, followed by acetone extract ($A_{0.50}$: 22.82 µg/mL), ethyl acetate fraction ($A_{0.50}$:

41.09 µg/mL), n-butanol fraction ($A_{0.50}$: 158 µg/mL), water ($A_{0.50}$: 162.50 µg/mL) and chloroform ($A_{0.50}$: 172.50 µg/mL). However, none of the extracts exhibited higher activity than those of antioxidant standards.

The chelating effect of the extracts and fractions on ferrous ions is shown in Table 3, compared with EDTA. The methanolic extract also showed the highest metal chelating activity (IC_{50} : 140.99 µg/mL) followed by chloroform fraction (IC_{50} : 156.17 µg/mL), water fraction (IC_{50} : 165.38 µg/mL), n-butanol fraction (IC_{50} : 220.87 µg/mL), ethyl acetate fraction (IC_{50} : 545.31 µg/mL) and acetone extract (IC_{50} > 800 µg/mL).

Table 3. Antioxidant activity of *Aloe vera* extracts and fractions by DPPH, ABTS, CUPRAC and metal chelating assays.

Extracts/ Fractions	DPPH assay IC_{50} (µg/ml)	ABTS assay IC_{50} (µg/ml)	CUPRAC assay $A_{0.50}$ (µg/ml)	Metal chelate assay IC_{50} (µg/ml)
Methanol	24.21±0.30	30.75±1.67	17.50±0.47	140.99±2.95
Chloroform	298.20±1.54	42.14±0.66	172.50±2.12	156.17±1.85
Ethyl acetate	297.15±4.84	128.98±1.62	41.09±1.29	545.31± 2.21
n-Butanol	119.63±0.23	34.16±0.64	158.±2.83	220.87± 1.76
Water	>800	37.60±0.67	162.50±0.71	165.38±0.31
Acetone	92.20± 7.09	81.84±0.39	22.82±2.47	>800
BHA ^{a,b}	6.15±0.42	1.82±0.11	5.35±0.71	NT ^e
BHT ^{a,c}	12.99±0.45	1.30±0.31	8.97±3.94	NT ^e
α-Tocopherol ^a	13.03±5.20	NT ^e	NT ^e	NT ^e
EDTA ^{a,d}	NT ^e	NT ^e	NT ^e	8.80±0.47

Note: IC_{50} and $A_{0.50}$ values represent the means±SD of three parallel measurements ($P < 0.05$).

^a Reference compounds, ^b Butylatedhydroxyanisole, ^c Butylatedhydroxytoluene,

^d Ethylenediaminetetraacetic acid, ^e Not tested.

Anti-Alzheimer activity

Table 4 shows the AChE and BChE inhibitory activities of the extracts and fractions of *Aloe vera*, compared with that of galantamine. The n-butanol and chloroform fractions exhibited moderate BChE inhibitory activity (IC_{50} value: 79.61±2.41 and 94.04±1.41 µg/mL, respectively) but this BChE inhibitory activity is lower than the galantamine standard (IC_{50} value : 34.75±1.99 µg/mL), whilst they showed mild AChE inhibitory activity. However, acetone extract showed weak inhibitory activity against BChE (IC_{50} value : 102.32±0.24 µg/mL). Methanolic extract, acetate ethyl and water fractions showed mild BChE inhibitory activity, whilst they exhibited no activity against AChE enzyme.

Table 4. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of *Aloe vera* extracts and fractions.

Extracts/Fractions	AChE assay IC ₅₀ (µg/mL)	BChE assay IC ₅₀ (µg/mL)
Methanol	NA ^b	>200
Chloroform	>200	94.04±1.41
Ethyl acetate	NA	>200
n-Butanol	>200	79.61±2.41
Water	NA ^b	>200
Acetone	>200	102.32±0.24
Galantamine ^a	6.27±1.15	34.75±1.99

Note: IC₅₀ values represent the means±SD of three parallel measurements ($P < 0.05$).

^a Reference compounds, ^bNot active.

Antidiabetic activity

Table 5 shows the α -Glucosidase inhibitory activity of the extracts and fractions, compared with that of Acarbose used as a positive control. The n-butanol fraction (IC₅₀ value : 40.75±0.06 µg/ml) exhibited highest α -glucosidase inhibitory activity, even higher than Acarbose (IC₅₀ value : 151.30±0.08). Acetate ethyl fraction showed mild α -Glucosidase Inhibitory activity (IC₅₀ >1,000 µg/mL). The methanol extract, chloroform and aqueous fractions, however, were inactive at all concentrations.

Table 5. α -Glucosidase Inhibitory activity of *Aloe vera* extracts and fractions.

Extracts/ Fractions	α -Glucosidase Assay IC ₅₀ (µg/mL)
Methanol	NA ^b
Chloroform	NA ^b
Ethyl acetate	>1,000
n-Butanol	40.75±0.06
Water	NA ^b
Acetone	NA ^b
Acarbose ^a	156.17±1.85

Note: IC₅₀ values represent the means±SD of three parallel measurements ($P < 0.05$).

^a Reference compounds, ^bNot active.

DISCUSSION

The results of extraction yields are close to those of the work carried out by Saritha et al. (2010) who recorded an extraction yield equal to 28.14 %, and 3.05 % for the methanol and chloroform extracts respectively. According to the results obtained by Ejoba (2012) they recorded an extraction yield of 8.6 % for chloroform extract and 5.4 % for the water extract. Concerning total polyphenol, flavonoid and flavonol contents, the results suggest that polyphenol, flavonoid and flavonol are important components of the crude methanolic extract. These results are in disagreement with The studies conducted by Anirban et al. (2013) which showed that the methanolic extract had values equal to 35.77 µg GAE/mg extract, 29.75 µg QE/mg extract, and 13.66 µg QE/mg extract for total polyphenol, flavonoid and flavonol respectively. While, Taukoorah and Mahomoodally (2016) found that *Aloe vera* gave a value of 66.06 µg GAE/ mg extract for polyphenol and 60.95 µg QE/mg extract for flavonoid. Several factors can influence the polyphenol and flavonoid contents such as the development period or the age of the plant (Okamura et al., 1996; Park and Kwon, 2006; Rodriguez et al., 2010). In term of DPPH-scavenging potential of methanolic extract, our results are in accordance with the studies carried out by Saritha et al. (2010), the inhibition percentages of methanolic, acetone and chloroform extracts were 93.14 %, 74.03 %, 57.68 % respectively at 250 µg/mL, in this studies the methanol extract showed better radical scavenging activity than the other extracts. Anirban et al. (2013) have also reported higher DPPH scavenging activities for methanolic extract (IC₅₀ 58 µg/mL). This result is in agreement with the statement, *Aloe vera* extracted in different solvents obtained highest DPPH inhibition in methanol extract (Saritha et al., 2010). In the metal chelating activity on ferrous ions, the results of our investigation are in accordance with those published earlier, which mentioned that the methanolic extract showed more chelating ability than acetone extract of *Aloe vera* leaves. However, the chelating ability was relatively lower than that of EDTA (Saritha et al., 2010). The BChE inhibitory activity of chloroform extract may be due the presence of ursolic acid (Kolak et al., 2009) found in large amounts. The presence of flavonoids in the butanol extract may have caused BChE inhibitory activity.

In the present study, leaves of *Aloe vera* were assessed in terms of its ability to inhibit key carbohydrate hydrolysing enzymes. Significant inhibition was found explaining that the antidiabetic property of *Aloe vera* leaves may be due to enzyme inhibition. These results are in disagreement with The studies conducted by Taukoorah and Mahomoodally (2016), their results from α -glucosidase inhibition assay showed no enzyme inhibitory activity for the *Aloe vera* leaves. Importantly, some researchers have indicated that there is a positive relationship between polyphenol content, total flavonoid and the ability to inhibit α -Glucosidase (Ramkumar et al., 2010). The phenolic compounds are known by their capacity to inhibit the activities of carbohydrate-hydrolyzing enzymes

because of their ability to bind to proteins (Shobana et al., 2009). Moreover, flavonoids have been known to possess high inhibitory potential towards α -Glucosidase in both *in vitro* and *in vivo* studies (Adefegha and Oboh, 2012).

CONCLUSION

In this study, antioxidant, anti-Alzheimer and antidiabetic activities of leaves extracts and fractions of *Aloe vera* were determined. The methanolic extract exhibited the highest antioxidant activities in all assays. The n-butanol fraction indicated the highest BChE inhibitory activity and the good α -glucosidase inhibitory activity.

These results showed that *Aloe vera* would be suggested as a new potential source of natural antioxidative phenolic compounds, which can replace the synthetic antioxidant in the field of cosmetics, pharmaceuticals and foods. In addition, the results revealed that the leaves of *Aloe vera* can be exploited in the discovery of bioactive natural products for the treatment of diabetes mellitus and Alzheimer's disease. However, this study can be complemented by isolation and identification of several polyphenolic components for a therapeutic purpose.

ACKNOWLEDGEMENTS

The authors wish to express thanks to Algerian minister of higher education and scientific research (MESRS) for the support, to Mr. Manuel Navarro (Agricultural engineer, Owner of *Aloe vera* farm in Almaria, Spain) for the harvest of plant, Mr. Bouhedjar Khalid (Biotechnology Research Center, Constantine, Algeria.) for the statistical analysis.

REFERENCES

- Abo-Youssef, A.M.H., and Messiha, B.A. 2013. Beneficial effects of *Aloe vera* in treatment of diabetes: comparative *in vivo* and *in vitro* studies. Bulletin of Faculty of Pharmacy, Cairo University. 51(1): 7-11. <https://doi.org/10.1016/j.bfopcu.2012.03.002>
- Adefegha, S.A.A., and Oboh, G. 2012. *In vitro* inhibition activity of polyphenol-rich extracts from *Syzygium aromaticum* (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe²⁺-induced lipid peroxidation in rat pancreas. Asian Pacific Journal of Tropical Biomedicine. 2(10): 774-781. [https://doi.org/10.1016/S2221-1691\(12\)60228-7](https://doi.org/10.1016/S2221-1691(12)60228-7)
- Anirban, R., Dutta Gupta, S., and Sampad, G. 2013. Evaluation of anti oxidative activity and UV absorption potential of the extracts of *Aloe vera* L. gel from different growth periods of plants. Industrial Crops and Products. 49: 712-719. <https://doi.org/10.1016/j.indcrop.2013.06.008>

- Apak, R., Güclü, K., Özyürek, M., and Karademir, S.E. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupricion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*. 52(26): 7970-7981. <https://doi.org/10.1021/jf048741x>
- Blois, M.S.1958. Antioxidant determinations by the use of a stable free radical. *Nature*.181: 1199-1200. <https://doi.org/10.1038/1811199a0>
- Çam, M., and Hişil, Y. 2010. Pressurised water extraction of polyphenols from pomegranate peels. *Food Chemical*. 123(3): 878-885. <https://doi.org/10.1016/j.foodchem.2010.05.011>
- Choi, H.C., Kim, S.J., Son, K.Y, Oh, B.J., and Cho, B.L. 2013. Metabolic effects of aloe vera gel complex in obese prediabetes and early non-treated diabetic patients: randomized controlled trial. *Nutrition*. 29(9): 1110-1114. <https://doi.org/10.1016/j.nut.2013.02.15>
- Cock, I.E. 2008. Antimicrobial activity of *Aloe barbadensis* Miller leaf gel components. *Journal of Microbiology*. 4: 2.
- Decker, E.A., and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle *Journal of Agricultural and Food Chemistry*. 38(3): 674-677. <https://doi.org/10.1021/jf00093a019>
- Dökmeçi, I. 2000. *Fundamentals of Pharmacology*. Istanbul, Turkey: Nobel Tıp Kitabevi Press.
- Ejoba, R. 2012 Phytochemical constituents of some leaves extract of *Aloe vera* and *Azadirachta indica* plant species. *Global Advanced Research Journal of Environmental Science and Toxicology*. 1(2): 014-017
- El-Shemy, H., Aboul-Soud, M.A., Aboul-Enein, K.M., Kabash, A., and Yagi, A. 2010. Anti-tumor properties and modulation of antioxidant enzymes activity by Aloe vera leaf active principles isolated via supercritical carbon dioxide extraction. *Current Medicinal Chemistry*. 17(2): 129-138. <https://doi.org/10.2174/092986710790112620>
- Ellman, G.L., Courtney, K.D., Andres, V., and Featherston, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*. 7: 88-95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- Ferro, V.A., Bradbury, F., Cameron, P., Shakir, E., Rahman, S.R., and Stimson, W.H. 2003. *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. *Antimicrobial Agents and Chemotherapy*. 47(3): 1137-1139. <https://doi.org/10.1128/AAC.47.3.1137-1139.2003>
- Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., and Ogihara, T. 2005. Effect of two α -glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism*. 54(3): 387-390. <https://doi.org/10.1016/j.metabol.2004.10.004>

- Gilani A.H., and Rahman A.U. 2005. Trends in ethnopharmacology. *Journal of Ethnopharmacology*. 100(1-2): 43-49. <https://doi.org/10.1016/j.jep.2005.06.001>
- Gray, M., and Flatt, R. 1997. Nature's own pharmacy: the diabetes perspective. *Proceedings of the Nutrition Society*. 56: 507-517. <https://doi.org/10.1079/PNS19970051>
- Halliwell, B. 1999. Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutrition Reviews*. 57(4): 104-113. <https://doi.org/10.1111/j.1753-4887.1999.tb06933.x>
- Hamman, J.H. 2008 Composition and applications of *Aloe vera* leaf gel. *Molecules*. 13(8): 1599-1616. <https://doi.org/10.3390.molecules13081599>
- Hoareau, L., and Dasilva E.J. 1999. Medicinal plants: a re-emerging health aid. *Electronic Journal of Biotechnology*. 2(2): 3-4.
- Hostettmann, K., and Hamburger M. 1993. Search for new lead compounds of natural origin. In: Testa, B., Kyburz, E., Fuhrer, W., Giger, R., editors. *Perspectives in Medical Chemistry*. Verlag Helvetica Acta, Basel.
- Hu, Y., Xu, J., and Hu, Q. 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. *Journal of Agricultural and Food Chemistry*. 51(26): 7788-7791. <https://doi.org/10.1021/jf034255i>
- Julkunen-Titto, R. 1985. Phenolic constituents in the leaves of northern Willows methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry*. 33(2): 213-217. <https://doi.org/10.1021/jf00062a013>
- Kamboj, V.P. 2000. Herbal medicine. *Current Science*. 78(1): 35-39.
- Kolak, U., Hacibekiroğlu, I., Öztürk, M., Özgökçe, F., Topçu G., and Ulubelen, A. 2009. Antioxidant and anticholinesterase constituents of *Salvia pocolata*. *Turkish Journal of Chemistry*. 33(6): 813-823. <https://doi.org/10.3906/kim-0902-1>
- Kumaran, A., and Karunakaran, R.J. 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Science and Technology*. 40(2): 344-352. <https://doi.org/10.1016/j.lwt.2005.09.011>
- Lui, C.H., Wang, C.H., Xu, Z-L., and Wang, Y. 2007. Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochemistry*. 42(6): 961-970. <https://doi.org/10.1016/j.procbio.2007.03.004>
- Mariappan, V., and Shanthi, G. 2012. Antimicrobial and phytochemical analysis of *Aloe vera* L. *International Research Journal of Pharmacy*. 3(10): 158-161.
- Müller, L., Gnoyke, S., Popken, A.M., and Böhm, V. 2010. Antioxidant capacity and related parameters of different fruit formulations. *LWT - Food Science and Technology*. 43: 992-999. <https://doi.org/10.1016/j.lwt.2010.02.004>

- Nagmoti, D., and Juvekar, A. 2013. *In vitro* inhibitory effects of *Pithecellobium dulce* (Roxb.) Benth. seeds on intestinal α -glucosidase and pancreatic α -amylase. *Journal of Biochemical Technology*. 4(3): 616-621.
- Nampoothiri, S.V., Prathapan, A., Cherian, O.L., Raghu, K.G., Venugopalan, V.V., and Sundaresan, A. 2011. *In vitro* antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against LDL oxidation and key enzymes linked to type 2 diabetes. *Food and Chemical Toxicology*. 49(1): 125-131. <https://doi.org/10.1016/j.fct.2010.10.006>
- Okamura, N., Asai, M., Hine, N., and Yagi, A. 1996. High-performance liquid chromatographic determination of phenolic compounds in *Aloe* species. *Journal of Chromatography A*. 746(2): 225-231. [https://doi.org/10.1016/0021-9673\(96\)00342-1](https://doi.org/10.1016/0021-9673(96)00342-1)
- Oryan, A., Mohammadalipour, A., Moshiri, A., and Tabandeh, M.R. 2016. Topical application of *Aloe vera* accelerated wound healing, modeling, and remodeling: an experimental study with significant clinical value. *Annals of Plastic Surgery*. 77(1): 37-46. <https://doi.org/10.1097/SAP.0000000000000239>
- Park, J.H., and Kwon, S.W. 2006. Chemical components of Aloe and its analysis: an epit-ome of chemical components and low molecular compounds. In: Park YI, Lee SK, editors. *New Perspectives on Aloe*. Boston, MA: Springer. p.19-34. <https://doi.org/10.1007/0-387-34636-8-3>
- Ramkumar, K.M., Thayumanavan, B., Palvannan, T., and Rajaguru, P. 2010. Inhibitory effect of *Gymnema montanum* leaves on α -glucosidase activity and α -amylase activity and their relationship with polyphenolic content. *Medicinal Chemistry Research*. 19(8): 948-961. <https://doi.org/10.1007/s00044-009-9241-5>
- Ray, A., Dutta Gupta, S., and Ghosh S. 2012. Isolation and characterization of potent bioactive fraction with antioxidant and UV absorbing activity from *Aloe barbadensis* Miller gel. *Journal of Plant Biochemistry and Biotechnology*. 22(4): 483-487. <https://doi.org/10.1007/s13562-012-0178-2>
- Ray, A., Dutta Gupta, S., and Ghosh, S. 2013. Evaluation of anti-oxidative activity and UV absorption potential of the extracts of *Aloe vera* L. gel from different growth periods of plants. *Industrial Crops and Products*. 49: 712-719. <https://doi.org/10.1016/j.indcrop.2013.06.008>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. 1999. Antioxidant activity aplying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 26(9): 1231-1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
- Rice-Evans, C., Miller, N., and Paganga, G. 1996. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biology and Medicine*. 20(3): 933-956. [https://doi.org/10.1016/0891-5849\(95\)02227-9](https://doi.org/10.1016/0891-5849(95)02227-9)

- Rodriguez, E.R., Martin, J.D., and Romero, C.D. 2010. *Aloe vera* as a functional ingredient in foods. *Critical Reviews in Food Science and Nutrition*. 50(4): 305-326. <https://doi.org/10.1080/10408390802544454>
- Saritha, V., Anilakumar, K.R., and Khanum, F. 2010. Antioxidant and antibacterial activity of *Aloe vera* gel extracts. *International Journal of Pharmaceutical and Biological Archive*. 1(4): 376-384.
- Shobana, S., Sreerama, Y.N., and Malleshi, N.G. 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α -glucosidase and pancreatic amylase. *Food Chemistry*. 115(4): 1268-1273. <https://doi.org/10.1016/j.foodchem.2009.01.042>
- Sirdaarta, J., and Cock, I. 2008. Vitamin E and Trolox™ reduce toxicity of *Aloe barbadensis* miller juice in *Artemia franciscana* nauplii but individually are toxic at high concentrations. *International Journal of Toxicology*. 5: 1.
- Sirdaarta, J., and Cock, I. 2010. Effect of *Aloe barbadensis* Miller juice on oxidative stress biomarkers in aerobic cells using *Artemia franciscana* as a model. *Phytotherapy Research*. 24(3): 360-364. <https://doi.org/10.1002/ptr.2946>
- Taukoorah, U., and Mahomoodally, M.F. 2016. Crude *Aloe vera* gel shows antioxidant propensities and inhibits pancreatic lipase and glucose movement *In Vitro*. *Advances in Pharmacological Sciences*. 2016: Article ID 3720850. <https://doi.org/10.1155/2016/3720850>
- Topçu, G., Ay, A., Bilici, A., Sarıkürkcü, C., Öztürk, M., and Ulubelen, A. 2007. A new flavones from antioxidant extracts of *Pistacia terebinthus*. *Food Chemistry*. 103(3): 816-822. <https://doi.org/10.1016/j.foodchem.2006.09.028>
- Walsh, G. 2003. *Biopharmaceuticals: Biochemistry and Biotechnology*. 3rd ed. Chinchester: Wiley. Irland.
- Yanlun, J., Ang, Z., Yulin, F., Min, L., Xianfang, Z., Hua, W., and Zhenwen, Z. 2016. Phenolic compounds and antioxidant activities of grape cane extracts from vineyards. *Spanish Journal of Agricultural Research*. 14(3): 1-13. <https://doi.org/10.5424/sjar/2016143-8951>
- Yu, Z., Jin, C., Xin, M., and JianMin, H. 2009. Effect of *Aloe vera* polysaccharides on immunity and antioxidant activities in oral ulcer animal models. *Carbohydrate Polymers*. 75(2): 307-311. <https://doi.org/10.1016/j.carbpol.2008.07.029>
- Zhang, S.Y., Zheng, C.G., Yan, X.Y., and Tian, W.X. 2008. Low concentration of condensed tannins from catechu significantly inhibits fatty acid synthase and growth of MCF-7 cells. *Biochemical and Biophysical Research Communications*. 371(4): 654-658.