

PHYTOCHEMICAL INVESTIGATIONS OF 10 EDIBLE PLANTS AND THEIR ANTIOXIDANT AND ANTIDIABETIC ACTIVITY

A. Hassan, F. Al-Salman, A. Ali Redha*, M. Salem and Z. Saeed

Department of Chemistry, College of Science,
University of Bahrain, Sakhir, Kingdom of Bahrain.

ABSTRACT

Ten edible plant parts, tamarind (*Tamarindusindica*), chia (*Salvia hispanica L.*), scarlet gourd (*Cocciniagrandis*), neem (*Azadirachtaindica*), quinoa (*Chenopodium quinoa*), leek (*Aliumporrum*), bittermelon (*Momoridicacharantia*), and fenugreek (*Trigonellafoenum-graceum*) seeds and leaves were extracted with a range of solvents (water, ethanol, ethyl acetate, acetone and petroleum ether) and investigated for their phytochemical content by qualitative phytochemical screening and quantitative determination of total phenolic content and flavonoid content. The antioxidant and antidiabetic activity of the plant extracts was also evaluated. Based on the phytochemical screening, flavonoids were present in chia, scarlet gourd, neem seeds, neem leaves, quinoa, bitter melon, fenugreek leaves and fenugreek seeds. Saponins were present in quinoa and tannins were present in neem seeds, neem leaves, quinoa, bitter melon, fenugreek leaves and fenugreek seeds. Sterols and terpenes were detected in scarlet gourd, neem seeds, neem leaves, quinoa, leek, bitter melon, fenugreek leaves and fenugreek seeds. Anthraquinone glycosides and alkaloids were not detected in any of the plants. According to the quantitative analysis, the highest phenolic content was determined in fenugreek leaves water extract (8.21 ± 0.22 mg GAE/g) and the highest flavonoid content was determined in fenugreek leaves ethanol extract (6.29 ± 0.19 mg RE/g). The highest antioxidant activity was revealed by neem leaves water extract ($28.43 \pm 0.92\%$) and the highest antidiabetic activity was shown by quinoa ethyl acetate extract ($87.95 \pm 3.72\%$).

Keywords: Edible plants, phytochemicals, phenolics and antioxidant activity.

1. INTRODUCTION

Diabetes mellitus is a non-communicable chronic disease which can negatively affect the lifespan of a person, including children and adults of different ages^{1,2}. Low levels of insulin in blood, will result in an increase in the concentration of glucose in blood which ultimately led to diabetes (hyperglycemia). Diabetic patients who intake insulin, as a temporary solution for the disease, can also suffer from other medical issues. Upon the increase of insulin in the body, or unbalanced intake of insulin, patients can start suffering of severe hypoglycemia, when the amount of glucose in the blood drops below 54 mg/dl¹. According to the International Diabetes Federation (IDF), 425 million people are

suffering from diabetes and around 75-80% of diabetic patients die as a result of cardiovascular issues^{2,3}. Diabetic patients can also experience blindness and kidney failure as a result of diabetic retinopathy and diabetic nephropathy³.

A important enzyme linked to diabetes is α -amylase, it is a vital enzyme in the metabolism of carbohydrates in both humans and plants, it breaks down starch which is a polymer of glucose units linked by α 1-4 bond to yield maltose as the end product⁴. In humans, upon consumption of a rich starchy meal, α -amylase will start to breakdown the macromolecule of starch into simple sugars such as glucose, and ultimately increase the level of glucose in blood. Diabetic patients face a challenge upon

elevation of glucose level in their blood, as they have reduced insulin levels and thus the glucose molecules cannot be cleared in their blood. Therefore, it is vital to inhibit the enzymatic activity of α -amylase, to a certain extent, in diabetic patients to maintain a low level of glucose in their blood⁵. Typical pharmaceutical α -amylase drugs are available, however herbal based drugs have started to become more favoured by people due to their economic price and lower side effects⁵. Plant sources with historical and traditional medical and anti-diabetic properties can be a possible source of discovering novel natural product molecules to treat diabetes. Edible plant products with significant phenolic content and antioxidant potential have shown possible anti-diabetic potential. Continuous researches are conducted to discover novel compounds to treat diabetes, the current plant extracts used for diabetes treatment include cinnamon, bitter melon, tea, fenugreek and bitter melon. The mechanism of the anti-diabetic activity in each case differs based on the plant source and biochemistry of enzyme inhibition (i.e. respected enzyme to be inhibited).

In this research ten edible plants selected due to being potential remedy for diabetes according to published scientific literature: tamarind (*Tamarindusindica*), chia (*Salvia hispanica L.*), scarlet gourd (*Cocciniagrandis*), neem (*Azadirachtaindica*), quinoa (*Chenopodium quinoa*), leek (*Aliumporrum*), bittermelon (*Momordicacharantia*), and fenugreek (*Trigonellafoenum-graceum*) seeds and leaves. Prior to the antidiabetic investigation, phytochemical investigation was conducted which involved, phytochemical screening, quantitative determination of phenolic compound and flavonoids and determination of antioxidant activity.

Tamarind (*Tamarindusindica*), a tropical edible plant, widely spread in Asia and Africa. Tamarind has been widely used in the traditional Indian medication to treat diabetes, inflammation, constipation and indigestion⁶. The presence of some phytochemicals in tamarind, such as carvacrol, tartaric acid, cinnamaldehyde, epicatechin and lupeol give tamarind several medical potentials, such as being considered as an antioxidant, antibacterial, antifungal and antiviral⁷. In a research considering the antibacterial potential of tamarind, it was found that tamarind has the potential to significantly inhibit the growth of several bacteria such as *Aeromonashydrophila*, *Hafniaalvei* and *Pseudomonas putida*⁷. In modern medicine, the polysaccharides (and their derivatives) present in tamarind has been using in drug delivery medications⁶.

Chia(*Salvia hispanica*) seeds are nutritionally rich and contains an extensive range of biologically active compounds including phenolic compounds, carotenoids, sterols, stanols and phytoestrogens⁸. Chia seeds were traditionally used to lower risks of obesity (including diabetes) and cardiovascular issues⁹. Chia seeds positively influence the cardiac system (i.e. cardio-protective), control hypertension and dyslipidaemia, antidepressant, antiviral, anti-carcinogenic, lowers LDL cholesterol, increases HDL cholesterol and repairs damaged tissues¹⁰. Scarlet gourd (*Cocciniagrandis*) has been previously used to treat bronchitis, asthma, skin diseases and anorexia¹¹. Currently, scarlet gourd is also used in the treatment of diabetes in India¹². Due to presence of some phytochemicals in scarlet gourd, it has shown the antihyperglycemic properties and the potential to inhibit glucose-6-phosphate¹². Based on medicinal investigations of scarlet gourd extracts, the antioxidant and antimicrobial potential was proved and the cell proliferative ability with MDA-MA 321 breast cancer cells was also verified¹¹.

All parts of the neem (*Azadirachtaindica*) have a characteristic bitter taste due to the presence of high quantities of alkaloids, phenolic compounds, flavonoids, terpenoids, steroids and carotenoids which give the neem plant important antioxidant, antimicrobial and anti-inflammatory characteristics¹³. The essential oil extracted from the leaves, seeds and the fruit of neem tree is widely used in pharmaceutical products up to today¹⁴. The extracted oil has low toxicity and can significantly inhibit tyrosinase and lipase enzymes¹⁴.

Quinoa (*Chenopodium quinoa*) is rich in polyphenols, specifically saponins and tannins¹⁵. The biologically active component of quinoa, provide several medicinal properties to the plant, such as having antioxidant, anti-inflammatory, anti-carcinogenic, antitumor and antimicrobial properties¹⁶.

Leek (*Aliumporrum*) is an edible vegetable which grows widely around the world. Leek contains numerous biologically active compounds, including phenolic compounds, organic acids, organo-sulfur compounds, amino acids and carbohydrates¹⁷. Leek phenolic compounds are of high medical value due to their anti-atherosclerotic, anti-tumorigenic and antimicrobial potential¹⁷. In addition, the presence of organo-sulfur compounds in leek, enhance the antioxidant potential of leek¹⁸. Nevertheless, the phenolic content (polyphenols) and the presence of microelements also play a vital role in the antioxidant potential of leek¹⁸.

Bitter melon (*Momordica charantia*) contains a mixture of bioactive compounds including phenolic acids, flavonoids, alkaloids, triterpenes and essential oil¹⁹. The presence of β -carotene, lycopene and zeaxanthin in bitter melon provide a good antioxidant activity for the plant constituents¹⁹. Traditionally, bitter melon plant parts have been used to treat toothache, diarrhoea, piles, eczema, jaundice, psoriasis and furuncle²⁰. In addition, bitter melon has been found to have vital pharmacological properties and used as a contraceptive, laxative, abortifacient and antimalarial herb²⁰. Some clinical studies have suggested the bitter melon can lower blood glucose levels and helps glucose tolerance, however the exact mechanism of reducing blood glucose levels is unknown yet²¹. Recently, bitter melon has been used in anticancer research to determine its efficacy against several malignancies²¹. Fenugreek (*Trigonella foenum-graceum*), an aromatic plant, that has a unique value in folk Indian, Chinese and African medicine and has been commonly used for treatment of acne, wounds, dehydration, stomach ache, elevated blood pressure and constipation²². Fenugreek is mainly comprised on polyphenols and flavonoids which have shown enhanced mitochondrial function in 3T3-L1 adipocytes and insulin sensitivity²³.

2. MATERIALS AND METHODS

2.1 CHEMICALS AND INSTRUMENTS

2.1.1 CHEMICALS

Organic solvents: petroleum ether, ethyl acetate, chloroform, acetone, methanol and ethanol, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide, starch, maltose, α -amylase from pancreas (~ 25 IU/mg), 3,5-dinitrosalicylate, sodium potassium tartrate, reagents: Folin-Ciocalteu's reagent, and aluminum chloride were all purchased from Fluka Chemie AG, Buchs, Switzerland. Gallic acid, rutin hydrate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were all purchased from Sigma Chemicals Co., St Louis, MO, USA. The chemicals were used without any kind of treatment or purification.

2.1.2 INSTRUMENTS

Rotary evaporator (Buchi, Rotavapor R-200, Switzerland), freeze dryer (Labconco, Kansas City, USA.), water bath (Grant Instruments, GR150 High Performance, Cambridge, UK), double beam PerkinElmer Lambda 25VU/VIS spectrometer, and hot plate (Thermo Scientific, Cimarec Digital Hotplate, Cambridge, UK).

2.2 Preparation of Plant Samples

All plant samples were obtained from the local market and washed thoroughly with water. Tamarind (fruit), scarlet gourd (fruit), leek (whole plant) and bitter melon (fruit) were dried by freeze drying (temperature = -83°C and pressure = 0.100 mBar). Chia, neem leaves and seeds, quinoa, fenugreek seeds and leaves were air dried at ambient room temperature. All the respected plant samples were grinded using a commercial grinder to obtain fine powder. Samples were stored in airtight bags for further assays.

2.3 Extraction methods

2.3.1 Extraction for phytochemical screening

Acid Extract

To prepare the acidic plant extract, 1g of plant sample was mixed with 6.0ml of 0.1M hydrochloric acid. The test tube was covered and kept for 20 minutes. After 20 minutes, the extract obtained by filtration on cotton.

Aqueous Extract: Five grams of the dry plant powder were weighted and placed in a beaker; about 70.0 mL of deionized water were added. The tube was placed in a hot water bath to boil, then the plant extract was obtained by filtration on cotton.

Methanolic Extract

Ten grams of the dry plant powder were weighted and placed in a conical flask, 80.0 mL of methanol were added. The conical flask was covered and allowed to extract for 30 minutes at ambient room temperature. Methanolic extract of the respected plant was obtained by filtration on cotton. The extract poured into a porcelain dish and placed on the steam bath to evaporate the solvent. The extract residue was suspended in 10 mL of chloroform.

2.3.2 Extraction for quantitative assays

Five portions of 0.1 g of plant powder were weighed accurately. Each portion placed in a clean conical flask. To each flask, 10.0 mL of one of the following solvents were added (water, ethanol, acetone, ethylacetate, and petroleum ether), and kept for 24 hours at room temperature. Then the extract was obtained by filtration. The plant extract was obtained by filtration, the residue was placed in another 10.0 mL of the same solvent for 10 minutes for further extraction, it was filtered again. The two extracts were combined. The organic extracts were placed in rotary evaporator to evaporate the solvents, while the aqueous extract was lyophilized in freeze dryer. Finally, all extracts were suspended in 10.0 mL methanol.

2.4 Phytochemical screening²⁴⁻²⁷

Test for Flavonoids: The acidic extract of the plant was equally divided into three test tubes. In one test tube, magnesium turnings were added, and the colour was observed after 2-3 minutes. The presence of flavonoids in the extract is indicated by the formation of a red colour. In the second test tube, sodium hydroxide (1.0 M) was added until pH = 10. The presence of flavonoids in the extract is indicated by the formation of a yellow colour. The last test was conducted by the addition of amyl alcohol to the plant extract. The presence of flavonoids in the extract is indicated by the formation of a yellow colour in the organic layer.

Test for Saponins

In a test tube, about 3.0 mL of aqueous extract were shaken vigorously to froth. The test tube was allowed to stand for 15 – 20 minutes. The presence of saponins in the plant extract is indicated by the formation of a froth layer.

Test for Tannins

In a test tube, 6 drops of ferric chloride (FeCl₃) were added to 2.0 mL of the aqueous plant extract. Blue colour indicates hydrolysable tannins, and green colour indicates condensed tannins.

Test for Sterols and Terpenes

To two test tubes, 2.0 mL of the methanolic extract were added. To the first test tube, (Salkowski test), about 5 drops of concentrated sulfuric acid were added. Red-brown colour in the bottom of the test tube indicates that the plant contains sterol and/or terpenes. In the second test tube, (Liebermann test), 5-10 drops of Liebermann-Burchardt reagent (conc. H₂SO₄ and acetic acid 19:1) were added slowly on the wall of the test tube, if red-violet zone forms, then the plant contains triterpenes, if blue or blue-green colour forms then plant contains steroidal compounds.

Test for Anthraquinone glycosides

To 1.0 mL of ammonia, 2 mL of methanolic extract and mixed well in a test tube. In positive test, red rose colour should appear in aqueous layer.

Test for Alkaloids

Two mL of the methanolic extract were added to three clean test tubes. The first test tube was treated with 5-10 drops of Mayer reagent, the second with 5-10 drops of Buchardat reagent, and the third with 5-10 drops of Dragendorff reagent. Then the sample observed for the presence of coloured precipitate. Positive test will give a white precipitate with Mayer reagent, orange

precipitate with Buchardat reagent, and brown precipitate with Dragendorff reagent.

2.5 Determination of phenolic content⁴

In a test tube, 1.0 mL of the plant extract prepared for the quantitative assays was added to 5.0 mL of Folin-Ciocateu reagent (diluted 10-folds), and after 5 minutes 4 mL of Na₂CO₃ (75 g/L) were added. After passing 30 minutes, the absorbance of the sample was measured at 680 nm using UV/VIS spectrometer. A standard curve of gallic acid was also prepared to determine the concentration of gallic acid equivalents in plant extracts. Measurements were conducted in triplicates for each plant extract. Then gallic acid equivalents (GAE) were calculated in (mg gallic acid/g dry plant) by the equation shown below

$$\text{GAE} = \frac{\text{conc. of gallic acid in the sample (mg/mL)}}{\text{conc. of plant extract (g/mL)}}$$

2.6 Determination of flavonoid content²⁸

In a test tube, 1.0 mL of the plant extract prepared for the quantitative assays was added to 1.0 mL of AlCl₃ (20 g/L in EtOH) and 2.0 mL of ethanol. After 40 minutes the absorbance of the coloured solution was measured at 415 nm using UV/VIS spectrometer. A standard curve of rutin was also prepared to determine the concentration of rutin equivalents in plant extracts. Measurements were conducted in triplicates for each plant extract. Then rutin equivalents (RE) were calculated in (mg rutin/g dry plant) by the equation below

$$\text{RE} = \frac{\text{conc. of rutin in the sample (mg/mL)}}{\text{conc. of plant extract (g/mL)}}$$

2.7 Determination of the antioxidant activity of plant extracts

A solution of 45 mg/L of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was freshly prepared using methanol, the absorbance of the solution was measured at 517 nm using a UV/VIS spectrometer (to be considered as the blank sample)²⁹. In a test tube, 3.00 mL of DPPH were added to 50.0 μL of the previously prepared extract. After 30 minutes, the absorbance was measured at 517 nm using UV/VIS spectrometer²⁹. Measurements were conducted in triplicates for each plant extract. The percentage of the scavenging activity of DPPH radical by the plant extract was determined by the equation below

$$\% \text{Scavenging activity of DPPH radical} = \left(\frac{\text{Abs.blank} - \text{Abs.sample}}{\text{Abs.blank}} \right) \times 100$$

2.8 Determination of antidiabetic activity

2.8.1 Construction of Maltose Calibration Curve⁴

Standard solutions of maltose of varying concentrations were prepared. To each standard solution, 1.0 mL of prepared 3,5-dinitrosalicylate maltose coloring reagent (DNSA reagent) was added and boiled for 5 minutes. The absorbance of each standard solution was then measured at 540 nm by a UV/VIS spectrometer.

2.8.2 Determination of Amylase Activity under Optimum Conditions⁴

Into 4 test tubes, a volume of 2.5 mL phosphate buffer (pH = 6.66), 1.0 mL of 2.5% starch, 0.5 mL of 2% sodium chloride solution and 0.1 mL methanol was added and placed in a 37°C water bath at for 15 minutes (incubation process). After equilibration, a volume 0.05 mL of enzyme amylase was added into each test tube quickly at the same time. Upon the addition of amylase to the first test tube, the enzymatic reaction was terminated by the addition of 0.5 mL of 2M sodium hydroxide and 1.0 mL of maltose colouring reagent, which was then immediately boiled for 5 minutes in boiling water bath. Then, the absorbance of the coloured solution was measured at 540 nm using UV/VIS spectrometer and noted as the base value for maltose concentration at zero minute. The other remaining three test tubes were removed from the water bath after 10 minutes (of addition of amylase). Again, to terminate the enzymatic reaction of those test tubes, 0.5 mL of 2M sodium hydroxide and 1.0 mL of maltose colouring reagent was added and were boiled for 5 minutes in boiling water bath. Then, the absorbance of the coloured solutions was measured at 540 nm using UV/VIS spectrometer. Average results were considered. Preparation of blank sample followed the same procedure but excluding the addition of the enzyme amylase.

To calculate the exact absorbance (OD) of 10 minutes reaction test tube it was subtracted from the 0 minute, as shown in the equation below:

$$OD \text{ of } (10 \text{ min}) = Abs.(10 \text{ min}) - Abs.(0 \text{ min})$$

Then maltose concentration [Maltose] was determined in (mg/mL) from the calibration curve and then calculated based on the following equation:

$$\text{Enzyme Activity (mg/mL.min)} = \frac{[\text{Maltose}]}{10 \text{ minute}}$$

2.8.3 Determination of Amylase Activity in the presence of Plant Extracts⁴

The same method described in 2.8.2 was followed with the replacement of the 0.1 mL

methanol with 0.1 mL of each plant extract. By this, the effect of presence of plant extract on the activity of amylase on starch hydrolysis (to maltose) was determined. The exact absorbance, maltose concentration, percentage of relative activity of enzyme were calculated to determine the percentage inhibition of enzyme. The relative enzyme activity and thus the percentage inhibition were calculated based on the following equations:

%Relative Enzyme Activity =

$$\frac{\text{Enzyme Activity with the extract}}{\text{Enzyme Activity of normal reaction}} \times 100$$

%Inhibition = 100 - % Relative Enzyme Activity

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical Screening of Edible Plants

Based on the phytochemical screening done (Table 3.1), fenugreek seeds and leaves, neem seeds and leaves and quinoa are relatively richer in phytochemical content to the other edible plants tested. Flavonoids were detected in tamarin, chia, scarlet gourd, neem seeds and leaves, quinoa, bitter melon and fenugreek leaves and seeds. Saponins were only detected in neem leaves and fenugreek leaves and seeds. Tannins were present in neem seeds and leaves, quinoa, bitter melon and fenugreek leaves and seeds. The presence of sterols and terpenes was confirmed in scarlet ground, neem seeds and leaves, quinoa, leek, bitter melon and fenugreek leaves and seeds. Anthraquinone glycosides and alkaloids were not present in any of the plants.

3.2 Phenolic content of edible plants

The amount phenolic content of different extracts of the 10 edible plants was determined (Table 3.2 and Figure 3.1) based on Folin-Ciocateu method. Quinoa, neem leaves and fenugreek leaves had the highest phenolic content compared to the other plants investigated. Extracts with the most significant amount of phenolic content were fenugreek leaves water extract (8.21 ±0.22 mg GAE/g), neem leaves ethanol extract (7.74 ±0.10 mg GAE/g), fenugreek leaves ethanol extract (7.30 ±0.27 mg GAE/g) and quinoa ethanol extract (6.96 ±0.08 mg GAE/g). Polar extracts showed higher phenolic content compared to non-polar extracts which suggests that extraction of phenolic compounds and solvent polarity are related.

3.3 Flavonoid content

The flavonoid content of different extracts of the 10 edible plants was determined (Table 3.3 and Figure 3.2) based on aluminium chloride method. Quinoa, neem leaves and fenugreek leaves had the highest phenolic content compared to the other plants investigated. Extracts with the most significant amount of flavonoid content were fenugreek leaves ethanol extract (6.29 \pm 0.19 mg RE/g), fenugreek leaves water extract (5.52 \pm 0.71 mg RE/g), quinoa ethanol extract (5.20 \pm 0.06 mg RE/g) and neem leaves ethanol extract (4.88 \pm 0.17 mg RE/g). Polar extracts showed higher flavonoid content compared to non-polar extracts.

3.4 Antioxidant activity

The antioxidant activity of different extracts of the 10 edible plants was determined (Table 3.4 and Figure 3.3) based on DPPH radical scavenging activity. The highest antioxidant activity was observed in neem leaves, bitter melon and fenugreek seeds. Extracts with the most significant radical scavenging potential were neem leaves water extract (28.43 \pm 0.92%), neem leaves acetone extract (27.72 \pm 0.41%), neem leaves ethanol extract (26.41 \pm 0.92%) and bitter melon ethanol extract (24.55 \pm 1.55%). The antioxidant activity of plant extracts of higher polarity is more significant. The antioxidant properties of the extracts are usually associated with the phenolic content of the extract. Phenolic compounds are capable of undergoing oxidation-reduction reactions and become oxidized (i.e. reducing agents)¹⁸. Phenolic compounds can reduce alkoxy and peroxy radicals, quench reactive species of oxygen and prevent peroxidation¹⁸.

The values of phenolic compounds content and antioxidant activity in a set of samples are correlated very well¹⁸. Thus, measuring the phenolic content could be suggested as an indicator of antioxidant properties. The relation of phenolic content and antioxidant capacity was expressed by plotting a graph between them for each plant and obtain the R²- Square value. The obtained R² values were very close to 1, which means phenolic content and antioxidant capacity are proportionally related.

3.5 Antidiabetic activity

The antidiabetic activity of different extracts of the 10 edible plants was investigated by determining their potential in inhibiting the activity of amylase based on the reaction of hydrolysis of starch to maltose. All plants showed antidiabetic activity, but with different

potentials. Extracts with the most significant amylase inhibition activity were quinoa ethyl acetate extract (87.95 \pm 3.72%), scarlet ground petroleum ether extract (82.20 \pm 2.29%), tamarin petroleum ether extract (80.34 \pm 5.39%) and fenugreek seeds ethanol extract (79.83 \pm 3.45%) and chia petroleum ether extract (78.81 \pm 2.39%). Although in petroleum ether extracts did not show significant phenolic content, flavonoid content or antioxidant activity; however, their antidiabetic activity is clearly significant.

Based on these analyses, it could be concluded that these plants are potent inhibitors of α -amylase enzyme. In the present study, amylase inhibition was not found to be related to antioxidant activity or phenolic content of the plant extract. The highest inhibition was found in petroleum ether extracts, which are poor in their antioxidant activity and phenolic contents. Sabu and Kuttan related the anti-diabetic activity of some plant extracts to their anti-oxidant activity, their method of measuring antidiabetic activity was very different from the method used in this study, they used direct measurement of blood glucose level in the normal and alloxan diabetic rats³⁰. It was reported, based on clinical trials that scarlet gourd leaves resulted in significant reduction of plasma glucose level³¹. The current findings can now support the clinical trial which required studies for validating their findings.

4. CONCLUSION

Ten edible plant parts, tamarind (*Tamarindusindica*), chia (*Salvia hispanica L.*), scarlet gourd (*Cocciniagrandis*), neem (*Azadirachtaindica*), quinoa (*Chenopodium quinoa*), leek (*Aliumporrum*), bittermelon (*Momoricacharantia*), and fenugreek (*Trigonellafoenum-graceum*) seeds and leaves were investigated for their phytochemical content, the total phenolic and total flavonoid content of their extracts were determined quantitatively. The antioxidant and antidiabetic activity of the plant extracts was also evaluated. The highest phenolic content was determined in fenugreek leaves water extract (8.21 \pm 0.22 mg GAE/g) and the highest flavonoid content was determined in fenugreek leaves ethanol extract (6.29 \pm 0.19 mg RE/g). The highest antioxidant activity was revealed by neem leaves water extract (28.43 \pm 0.92%) and the highest antidiabetic activity was shown by quinoa ethyl acetate extract (87.95 \pm 3.72%).

Table 3.1: Phytochemical Screening Results of 10 Edible Plants

Phytochemical		Tamarin	Chia	Scarlet gourd	Neem seeds	Neem leaves	Quinoa	Leek	Bitter melon	Fenugreek leaves	Fenugreek seeds
Flavonoids	Test A	-	+	+	+++	+++	+++	-	+	+++	+++
	Test B	-	-	-	++	+	+	-	-	-	-
	Test C	+	-	-	-	++	-	-	-	-	-
Saponins		-	-	-	-	-	++	-	-	+	++
Tannins		-	-	-	+	++	++	-	+	+++	+++
Sterols & Terpenes	Lieberman's Test	-	-	+++	+++	+++	+	+++	+	+	+++
	Salwonski's Test	-	-	-	-	-	-	-	-	-	-
Anthraquinone glycosides		-	-	-	-	-	-	-	-	-	-
Alkaloids	Dragendoff	-	-	-	-	-	-	-	-	-	-
	Buchardat	-	-	-	-	-	-	-	-	-	-
	Mayer	-	-	-	-	-	-	-	-	-	-
Key: (+++) = Copiously present; (++) = moderately present; (+) = slightly present; (-) = absent											
Note: Tannins present in neem seeds and leaves and bitter melon are condensed tannins, while those in quinoa and fenugreek leaves and seeds are hydrolysed tannins.											

Table 3.2: Phenolic content of extracts of 10 edible plants

Edible Plant	Phenolic Content as Gallic Acid Equivalents [GAE] (mg GAE/g)									
	Water Extract		Ethanol Extract		Acetone Extract		Ethyl acetate Extract		Petroleum Ether extract	
Tamarin	2.75	±0.08	1.82	±0.08	1.37	±0.10	1.24	±0.06	0.31	±0.07
Chia	1.77	±0.09	1.57	±0.04	1.26	±0.11	1.95	±0.04	0.34	±0.08
Scarlet gourd	2.89	±0.04	2.15	±0.07	1.50	±0.14	1.29	±0.11	0.36	±0.03
Neem seeds	3.94	±0.13	3.00	±0.06	1.74	±0.16	1.33	±0.02	0.97	±0.09
Neem leaves	6.66	±0.07	7.74	±0.10	6.06	±0.00	3.53	±0.13	0.31	±0.02
Quinoa	5.37	±0.16	6.96	±0.08	3.26	±0.02	1.67	±0.10	0.40	±0.04
Leek	3.34	±0.02	2.48	±0.03	1.47	±0.14	1.00	±0.19	0.31	±0.04
Bitter melon	1.97	±0.05	3.58	±0.02	1.07	±0.07	0.97	±0.06	0.38	±0.15
Fenugreek leaves	8.21	±0.22	7.30	±0.27	4.02	±0.08	1.92	±0.12	0.54	±0.00
Fenugreek seeds	3.77	±0.88	6.82	±0.18	2.33	±0.27	0.80	±0.14	0.25	±0.11

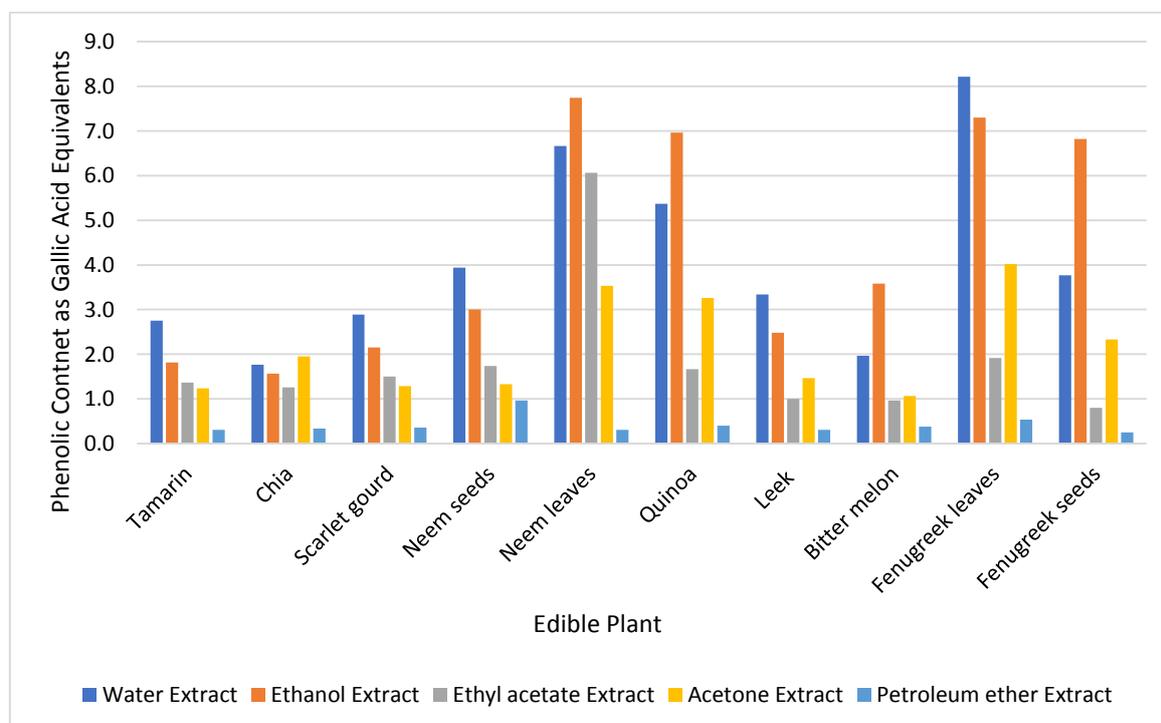
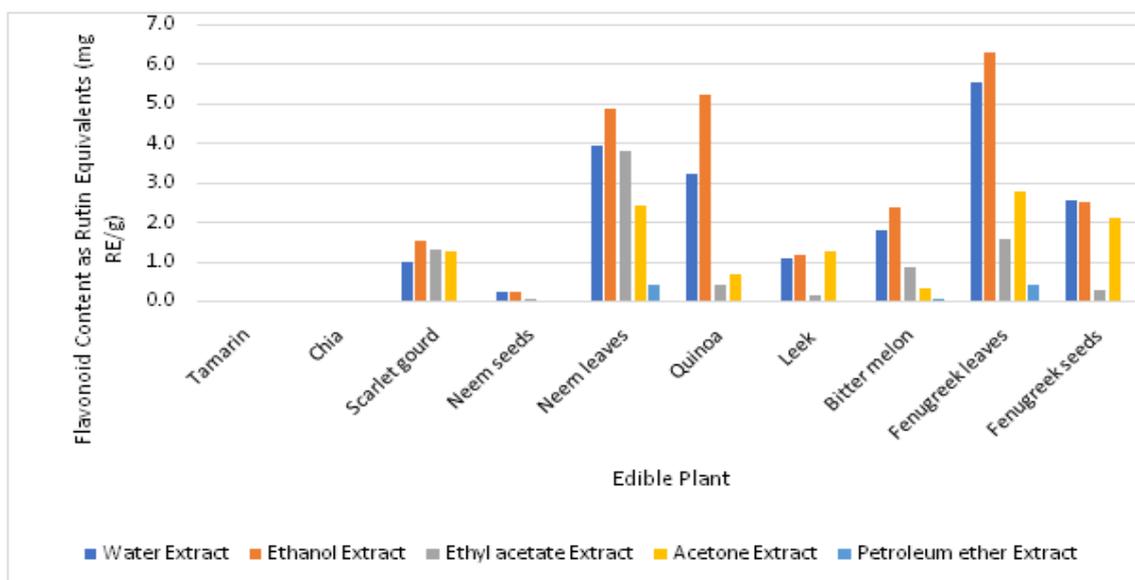


Fig. 3.1: Phenolic content of extracts of 10 edible plants

Table 3.3: Flavonoid content of extracts of 10 edible plants

Edible Plant	Flavonoid Content as Rutin Equivalents [RE] (mg RE/g)									
	Water Extract		Ethanol Extract		Ethyl acetate Extract		Acetone Extract		Petroleum ether Extract	
Tamarin	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00
Chia	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00
Scarlet gourd	0.99	±0.03	1.53	±0.17	1.26	±0.03	1.30	±0.05	0.02	±0.01
Neem seeds	0.23	±0.03	0.22	±0.02	0.02	±0.01	0.05	±0.02	0.00	±0.00
Neem leaves	3.91	±0.22	4.88	±0.17	2.42	±0.18	3.78	±0.18	0.40	±0.11
Quinoa	3.22	±0.04	5.20	±0.06	0.67	±0.05	0.39	±0.03	0.00	±0.05
Leek	1.07	±0.09	1.17	±0.62	1.26	±0.08	0.13	±0.05	0.00	±0.04
Bitter melon	1.78	±0.05	2.37	±0.04	0.32	±0.04	0.86	±0.06	0.06	±0.09
Fenugreek leaves	5.52	±0.71	6.29	±0.19	2.78	±0.27	1.54	±0.18	0.42	±0.03
Fenugreek seeds	2.54	±0.07	2.51	±0.06	2.09	±0.03	0.27	±0.02	0.00	±0.01

**Fig. 3.2: Flavonoid content of extracts of 10 edible plants****Table 3.4: Radical scavenging activity of extracts of 10 edible plants**

Edible Plant	Percentage Radical Scavenging Activity (%)									
	Water Extract		Ethanol Extract		Ethyl acetate Extract		Acetone Extract		Petroleum ether Extract	
Tamarin	10.76	±0.46	8.98	±0.67	5.76	±0.36	7.98	±0.84	3.98	±0.92
Chia	7.90	±0.13	7.87	±1.09	5.28	±1.47	7.56	±0.57	2.72	±1.00
Scarlet gourd	10.95	±0.64	9.43	±1.17	5.92	±0.25	7.06	±0.63	4.84	±0.93
Neem seeds	11.60	±0.43	10.06	±1.00	6.03	±0.05	8.03	±1.44	3.67	±0.33
Neem leaves	28.43	±0.92	26.41	±0.92	11.51	±0.60	27.72	±0.41	4.14	±0.53
Quinoa	9.40	±0.84	9.70	±0.13	7.92	±0.18	5.37	±1.08	1.97	±0.92
Leek	9.93	±0.80	8.20	±1.61	7.20	±0.26	4.89	±0.29	3.09	±0.38
Bitter melon	14.91	±0.27	24.55	±1.66	6.48	±0.21	5.31	±0.43	3.14	±0.13
Fenugreek leaves	11.57	±0.21	10.62	±0.34	7.29	±3.10	4.73	±0.21	3.81	±1.06
Fenugreek seeds	7.78	±0.34	15.71	±0.17	8.12	±1.12	5.00	±0.25	4.23	±0.26

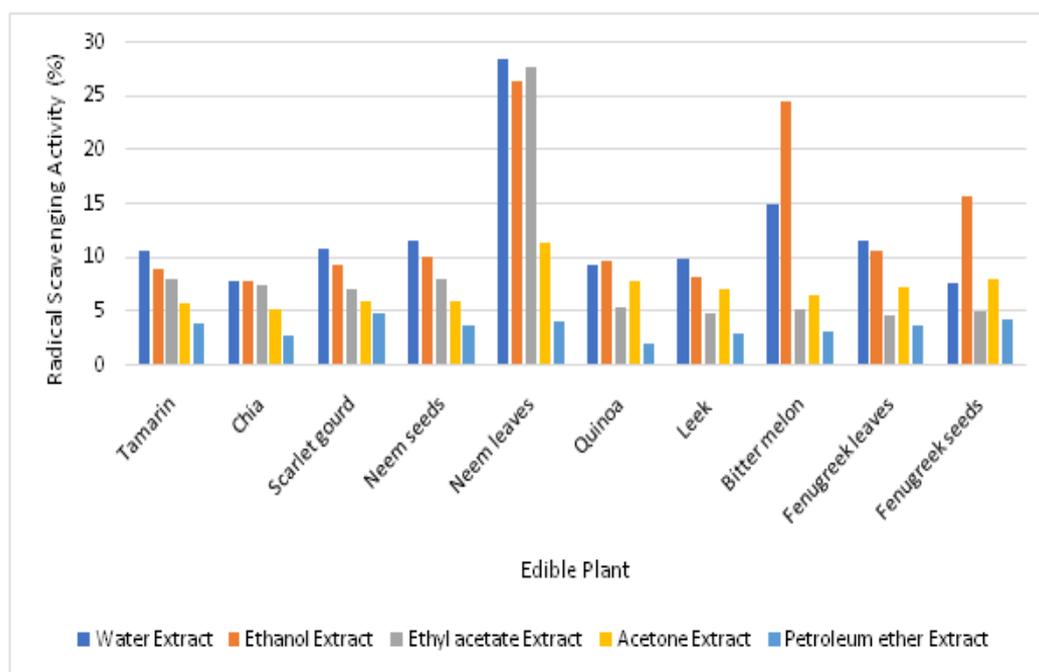


Fig. 3.3: Radical scavenging activity of extracts of 10 edible plants

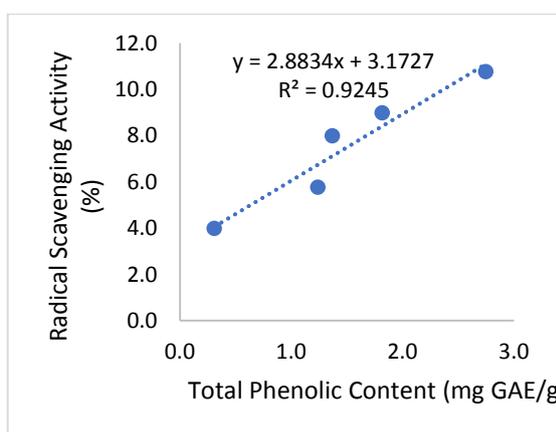


Fig. 3.4: Linear correlation between the total phenolic content and antioxidant activity of Tamarin

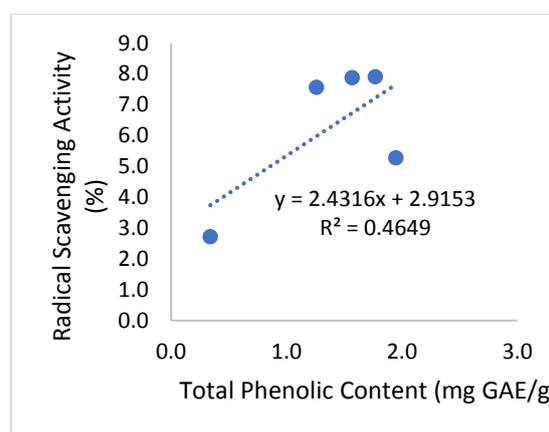


Fig. 3.5: Linear correlation between the total phenolic content and antioxidant activity of Chia

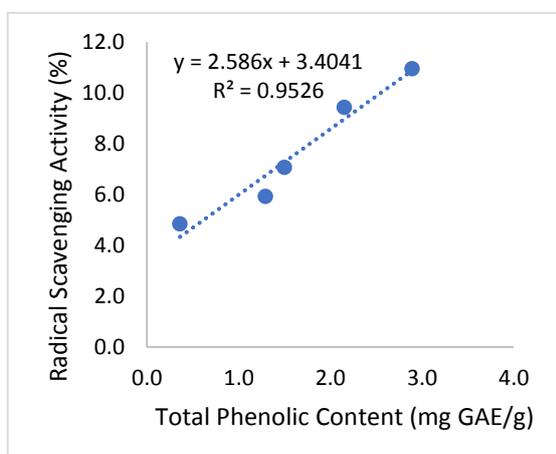


Fig. 3.6: Linear correlation between the total phenolic content and antioxidant activity of Scarlet Gourd

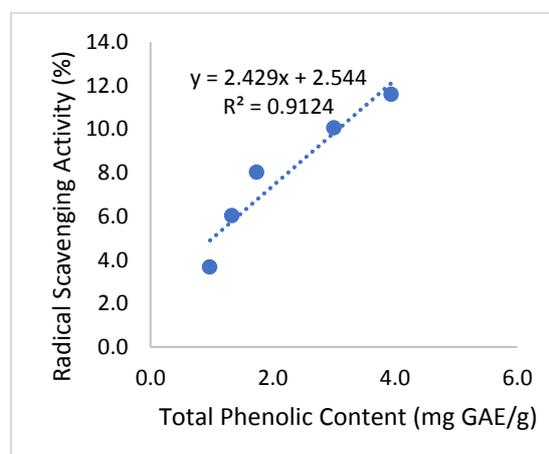


Fig. 3.7: Linear correlation between the total phenolic content and antioxidant activity of Neem seeds

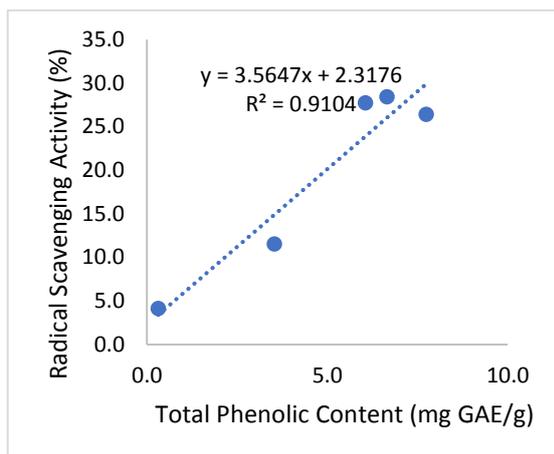


Fig. 3.8: Linear correlation between the total phenolic content and antioxidant activity of Neem Leaves

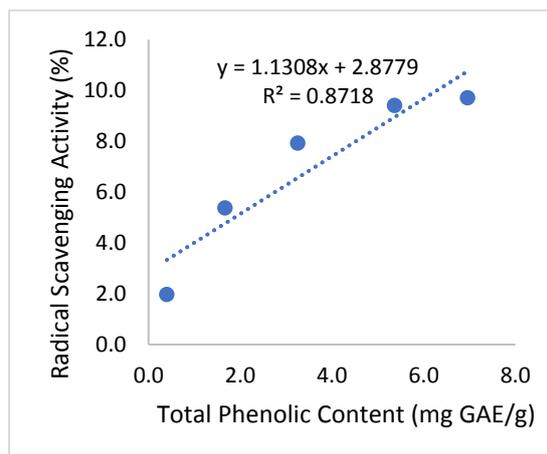


Fig. 3.9: Linear correlation between the total phenolic content and antioxidant activity of Quinoa

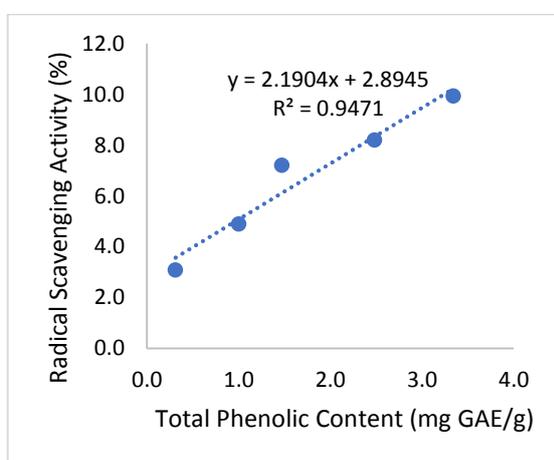


Fig. 3.10: Linear correlation between the total phenolic content and antioxidant activity of Leek

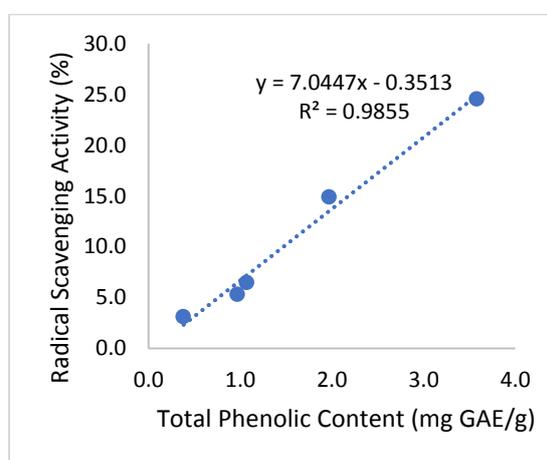


Fig. 3.11: Linear correlation between the total phenolic content and antioxidant activity of Bitter Melon

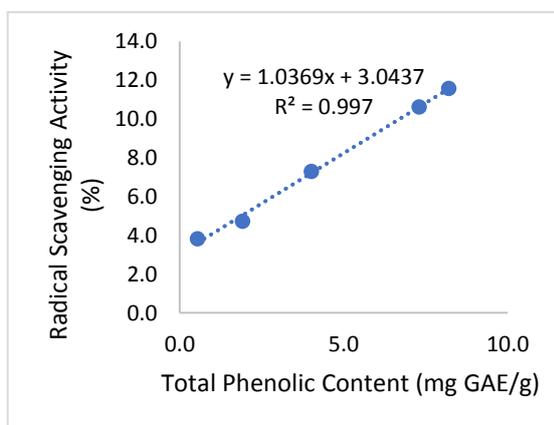


Fig. 3.12: Linear correlation between the total phenolic content and antioxidant activity of Fenugreek Leaves

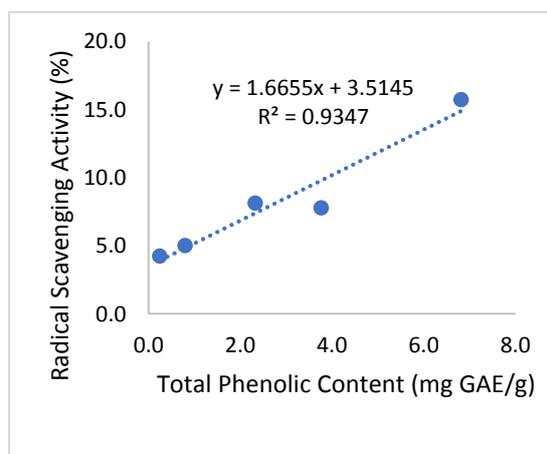
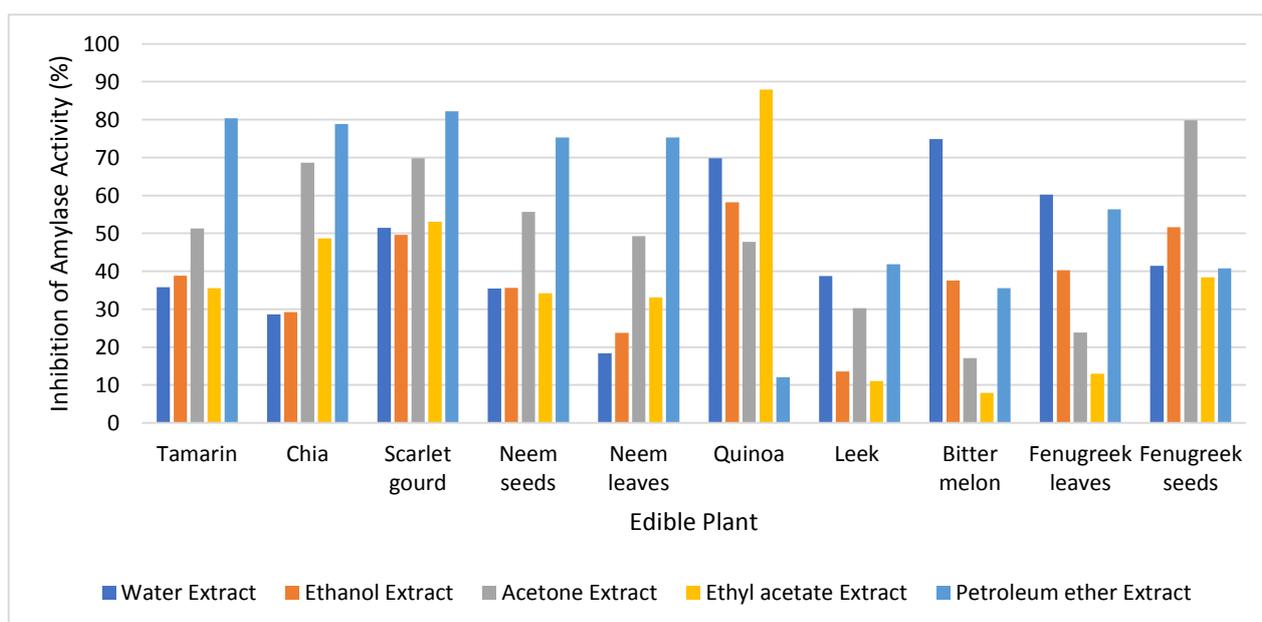


Fig. 3.13: Linear correlation between the total phenolic content and antioxidant activity of Fenugreek Seeds

Table 3.5: Antidiabetic activity of extracts of 10 edible plants

Edible Plant	Percentage Inhibition of Amylase Activity (%)									
	Water Extract		Ethanol Extract		Ethyl acetate Extract		Acetone Extract		Petroleum ether Extract	
Tamarin	35.82	3.62	38.83	5.70	51.31	4.64	35.58	6.01	80.34	5.39
Chia	28.64	5.21	29.22	6.68	68.62	3.29	48.68	4.57	78.81	2.39
Scarlet gourd	51.49	7.90	49.63	6.35	69.84	2.25	53.05	6.56	82.20	2.29
Neem seeds	35.49	8.65	35.62	4.63	55.69	6.67	34.16	5.54	75.33	6.60
Neem leaves	18.35	4.53	23.77	4.57	49.26	3.90	33.08	8.70	75.33	6.60
Quinoa	69.85	1.87	58.18	2.22	47.79	1.27	87.95	3.72	12.05	3.72
Leek	38.72	5.01	13.56	5.90	30.22	2.11	11.01	5.65	41.86	1.93
Bitter melon	74.84	5.91	37.53	1.80	17.13	1.98	7.88	7.00	35.52	3.30
Fenugreek leaves	60.22	1.85	40.23	1.95	23.85	6.72	12.99	8.60	56.33	1.18
Fenugreek seeds	41.45	4.56	51.59	1.27	79.83	3.45	38.44	5.58	40.73	6.79

**Fig. 3.14: Antidiabetic activity of extracts of 10 edible plants****REFERENCES**

- Eelco VD and Ryan CM. Diabetes mellitus in the young and the old : Effects on cognitive functioning across the life span. *Neurobiol Dis.* 2019;134:1-10.
- Animaw W and Seyoum Y. Increasing prevalence of diabetes mellitus in a developing country and its related factors. *PLoS One.* 2017;12(11):1-11.
- Wang Q, Zhang X, Fang L, Guan Q, Guan L and Li Q. Prevalence , awareness , treatment and control of diabetes mellitus among middle-aged and elderly people in a rural Chinese population: A cross-sectional study. *PLoS One.* 2018;13(6):1-11.
- Hasan AM, Redha AA and Mandeel Q. Natural Products Chemistry & Phytochemical Investigations of Pomegranate (*Punica granatum*) Rind and Aril Extracts and their Antioxidant , Antidiabetic and Antibacterial Activity. *Nat Prod Chem Res.* 2018;6(4):1-10.
- Oyedemi SO, Oyedemi BO, Ijeh II, Ohanyerem PE, Coopoosamy RM and Aiyegoro OA. Alpha-Amylase Inhibition and Antioxidative Capacity of Some Antidiabetic Plants Used by the Traditional Healers in Southeastern Nigeria. *Sci World J.* 2017;1-11.
- Gomathi AC, Xavier Rajarathinam SR and Mohammed Sadiq A. Phytochemical screening of aqueous extract of Tamarind (*Tamarindus indica* L.) Shell. *Int J Basic Appl Res.* 2018;7(11):65-70.
- Adeniyi OV, Olaifa FE, Emikpe BO and Ogunbanwo ST. Phytochemical Components and Antibacterial Activity of *Tamarindus indica* Phytochemical Components and Antibacterial Activity of *Tamarindus indica* Linn . Extracts

- against Some Pathogens. *Biotechnol J Int.* 2017; 17(2):1–9.
8. Kobus-cisowska J, Taczanowski M and Kmiecik D. The Chemical Composition and Nutritional Value of Chia Seeds — Current State of Knowledge. *Nutrients.* 2019;11:1–16.
 9. Ali NM, Yeap SK, Ho WY, Beh BK, Tan SW and Tan SG. The Promising Future of Chia , *Salvia hispanica L . J Biomed Biotechnol.* 2012;1-9.
 10. Ullah R, Nadeem M, Khalique A, Imran M, Mehmood S and Javid A. Nutritional and therapeutic perspectives of Chia (*Salvia hispanica L .*): a review. *J Food Sci Technol.* 2016;53:1750–1758.
 11. Sakharkar P and Chauhan B. Antibacterial , antioxidant and cell proliferative properties of *Coccinia grandis* fruits. *Avicenna J. Phytomedicine.* 2017;7(4):295–307.
 12. Dias JS. Nutritional Quality and Effect on Disease Prevention of Vegetables. *Nutr Heal Dis. Our Challenges Now Forthcom. Time.* 2019;1–30.
 13. Dash SP, Dixit S and Sahoo S. Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta Indica* : An Important Medicinal Plant *Biochem Anal Biochem.* 2017;6(2):2–5.
 14. Cesa S. Phytochemical analyses and pharmacological screening of Neem oil. *South African J Bot.* 2019;120:331–337.
 15. Mattila PH. Contents of phytochemicals and antinutritional factors in commercial protein-rich plant products. *Food Qual Saf.* 2018;2:213–219.
 16. Park JH, Lee YJ, Kim YH and Yoon KS. Antioxidant and Antimicrobial Activities of Quinoa (*Chenopodium quinoa Willd .*) Seeds Cultivated in Korea. *Prev Nutr Food Sci.* 2017;22:195–202.
 17. Radovanović B, Mladenović J, Radovanović A, Pavlović R and Nikoli V. Phenolic Composition , Antioxidant , Antimicrobial and Cytotoxic Activites of *Allium porrum L .* (Serbia) Extracts. *Sci Educ Publ.* 2015;3(9):564–569.
 18. Lenková M, Bystrická J, Tóth T and Hrstková M, Evaluation and comparison of the content of total polyphenols and antioxidant activity of selected species of the genus *Allium* Vyhodnotenie a porovnanie obsahu celkových polyfenolov a antioxidačnej aktivity vo vybraných druhoch rodu *Allium.* *J Cent Eur Agric.* 2016;17(4):1119–1133.
 19. Kumari P, Verma RB, Rani N, Singh AK and Kumari A, Diversity in phytochemical composition of bitter gourd (*Momordica charantia L.*) genotypes based on principal component analysis. *Int J Chem Stud.* 2018;4:36–42.
 20. Jia S, Shen M, Zhang F and Xie J. Recent Advances in *Momordica charantia* : Functional Components and Biological Activities. *Int J Mol Sci.* 2017;18:1–25.
 21. Raina K, Kumar D, Agarwal R, States U and States U. Promise of bitter melon (*Momordica charantia*) bioactives in cancer prevention and therapy. *Semin Cancer Biol.* 2017;40(41): 116–129.
 22. Rahmani M, Hamel L, Dif MM, Moumen F and Rahmani H. Determination of antioxidant activity, phenolic quantification of four varieties of fenugreek *Trigonella foenum graecum L .* seed extract cultured in west Algeria Absorbance. *J Mater Environ Sci.* 2018;2508(6):1656–1661.
 23. Li G. Polyphenol Stilbenes from Fenugreek (*Trigonella foenum-graecum L .*) Seeds Improve Insulin Sensitivity and Mitochondrial Function in 3T3-L1 Adipocytes. *Oxid Med Cell Longev.* 2018;1–9.
 24. Nair SKP, Ganesan K, Sinaga M and Letha N. Preliminary phytochemical screening of different solvent extracts of leaves of *Echeveria elegans* rose, an engangered Mexican succulent herb. *J Glob Biosci.* 2016;5(1):3429–3432.
 25. Ghurde MU and Malode SN. Phytochemical screening and assessment of biomolecules compounds in *Scilla hyacinthina* (Roth) Macbr. bulb. *J Glob Biosci.* 2014;3(5):866–871.
 26. Takaidza S, Mtunzi F and Pillay M. Analysis of the phytochemical contents and antioxidant activities of crude extracts from *Tulbaghia* species. *J Tradit Chinese Med.* 2018;38(2):272–279.
 27. Owolabi OO, James DB, Sani I, Andongma BT, Fasanya OO and Kure B. Phytochemical analysis, antioxidant and anti-inflammatory potential of *FERETIA APODANTHERA* root bark extracts. *BMC Complement. Altern Med.* 2018;18(12):1–9.

28. Chandra S. Assessment of Total Phenolic and Flavonoid Content , Antioxidant Properties , and Yield of Aeroponically and Conventionally Grown Leafy Vegetables and Fruit Crops: A Comparative Study. Evidence-Based Complement. Altern Med. 2014;1-9.
29. Arya S, Baniya MK, Danekhu K, Kunwar P, Gurung R and Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants. 2019;8(96):1-12.
30. Sabu MC and Kuttan R. Antidiabetic and antioxidant activity of Terminalia belerica . Roxb. Indian J Exp Biol. 2009;47:270-275.
31. Munasinghe MAAK, Abeysena C, Yaddehige IS, Vidanapathirana T and Piyumal KPB. Blood Sugar Lowering Effect of Coccinia grandis (L .) J . Voigt: Path for a New Drug for Diabetes Mellitus. Exp Diabetes Res. 2011;1-4.