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Effect of different solvents on the chemical composition and anti-diabetic activity of *Acacia Arabica* and *Zizyphus Mauritiana*

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ABSTRACT

The current study was designed to investigate the effect of solvents on chemical composition and antidiabetic activity of *Zizyphus mauritiana* and *Acacia Arabica* extracts. Total five solvents were used for this purpose (100% methanol, 50% aqueous methanol, 100% ethanol, 50% aqueous ethanol and aqueous). The data obtained from the investigation was subjected to the statistical analysis by using analysis of variance technique. The present study revealed that maximum antioxidant activity was attributed to *Acacia arabica* ($96.53 \pm 0.46\%$) followed by *Zizyphus mauritiana* ($94.33 \pm 0.52\%$ by 50% aqueous ethanol extracts). Maximum total phenolic content of both *Zizyphus mauritiana* and *Acacia arabica* (670.83 ± 1.46 mg GAE/100g and 934.34 ± 0.89 mg GAE/100g) were shown by 50% aqueous ethanol extracts while maximum total flavonoid content (146.36 ± 0.81 mg QE/100 g, 172.52 ± 0.99 mg QE/100 g) was exhibited by 50% aqueous ethanol extract. The maximum (IC 50= 49.63 ± 0.12 $\mu\text{g/mL}$) antidiabetic activity was found in aqueous extract of *Acacia arabica* while in *Zizyphus mauritiana* the aqueous extract indicated excellent (IC 50= 46.90 ± 0.23 $\mu\text{g/mL}$) antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is metabolic disease which is commonly found now-a-days. Diabetes mellitus is hyperglycemic condition caused by defect in insulin production and insulin action or may be both. It creates negative impression because of its long-lived complication on the life of patient. The Food habits have been drastically changed over the last century; human life style escorts towards chronic diseases. The diseases that cause serious health problem, one of them is diabetes and some diseases related to diabetes like cardiovascular disease and in type I diabetes patient 10 time more chances for cardiovascular disease so diabetes is very harmful [1-3].

Utilization of plant based products in treatment of diabetes is gaining interests now a days. Many medicinal plants have been tested for antidiabetic activity [4]. The present investigation was conducted to estimate the antioxidant potentials and antidiabetic action of *Zizyphus mauritiana* and *Acacia Arabica* extracts.

MATERIALS AND METHODS

Chemicals and Regents

Alpha amylase, phosphate buffer (pH 6.9), starch solution DNSA (3,5-Dinitrosalicylic acid), methanol, ethanol, gallic acid, ascorbic acid, Folin-ciocalteu phenol reagent solution, quercetin, distilled water and all other chemicals and regents used in all over the experiment were purchased from the Merck (Darmstadt, Germany). All the chemicals and reagents used in experiments were of analytical grade.

Collection of Plant

Acacia arabica and *Zizyphus mauritiana* leaves were collected from botanical garden of University of Agriculture, Faisalabad. Plant leaves were dried under shade and after drying the leaves were washed to remove the dust particles and impurities from leaves. After that ground by using grinder and after grinding, samples were stored at room temperature for further analysis.

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Extract Preparation

The ground powder of the leaves was extracted by using different solvents (methanol, ethanol and aqueous solvent at controlled temperature by previously described method with minor modification [5]. Briefly, 20g of each sample powder was mixed with 200 mL of solvent (methanol, ethanol, 50% methanol, 50% ethanol and aqueous) and shake for 6 hours by using magnetic stirrer at room temperature. After shaking mixtures were filtered by using ordinary filter paper and after that sample mixtures were placed in water bath at 47°C to evaporate the solvents and filtrates were obtained. These filtrates were stored at 4°C for further analysis.

Determination of antioxidant activity by DPPH assay

The DPPH free radical scavenging assay was used for the determination of antioxidant activity which previously described [1].

Analysis of total phenolic content

The Folin-ciocalteu assay was used for the determination of phenolic contents by previously describe method with minor modification [6].

Estimation of total Flavonoid content

Total flavonoid contents were determined by aluminum chloride method described previously with minor modifications [7].

Determination of Antidiabetic activity

The antidiabetic activity of *Acacia arabica* and *Zizyphus Mauritian* was determined by alpha amylase inhibition assay described previously [6]. An aliquot, 500 μ L of each sample extract and mixed with 500 μ L of alpha amylase solution and incubated for 10 minutes. Then added 500 μ L of starch solution in it and again incubated for 10 minutes. After that 1 mL of DNSA solution was added in it boiled for 10 minutes by placing on water bath and after that cooled and added 10 mL of distilled water. The absorbance was measured at 540 nm by using Uv-Vis spectrophotometer and results were expressed in % inhibition by the following equation:

$$I (\%age) = \{(A_{blank} - A_{sample}) / A_{blank}\} \times 100$$

Statistical Analysis

All the experiments were done in triplicate and represented in mean and standard error. For statistical analysis one way ANOVA was used.

RESULTS AND DISCUSSIONS

Antioxidant activity

The present study showed that the antioxidant activity of *Zizyphus mauritiana* ranged from (94.33 \pm 0.52 to 87.4 \pm

0.52%). The maximum antioxidant activity (94.33 \pm 0.52%) was observed in 50% aqueous ethanol extract while in case of *Acacia arabica* the antioxidant activity ranged from (96.53 \pm 0.46 to 76.92 \pm 0.54%). The maximum antioxidant activity (96.53 \pm 0.46%) was found in 50% aqueous ethanol extract while ascorbic acid used as standard was found to be (88.48 \pm 0.09%). The detailed results of both plants are shown in (Table 1). The interesting fact of our study was that the 50% aqueous ethanol solvent was more useful for extraction of antioxidant compounds as compared to other solvents. The overall results showed indicated that ethanol solvent showed excellent antioxidant potential then methanol while minimum antioxidant potential shown by aqueous solvent. The results of antioxidant activity of corncob extract showed that if concentration of corncob extract increased then antioxidant activity gradually increased [8].

Total Phenolic contents

In current research work the range of total phenolic contents in *Zizyphus mauritiana* was (670.83 \pm 1.46 to 184.08 \pm 0.88 mg GAE/100 g). The maximum total phenolic contents (670.83 \pm 1.46 mg GAE/100 g) was present in 50% aqueous ethanol extract whether in *Acacia arabica* the range of phenolic contents was (934.34 \pm 0.89 to 183.06 \pm 0.4 mg GAE/100 g) while the highest phenolic contents (934.34 \pm 0.89 mg GAE/100 g) was shown by 50% aqueous ethanol extract. The results are discussed in detail in (Table 1). The results showed that the 50% ethanol extract was useful for the extraction of phenolic content from plants while overall results showed that the ethanol solvent was showed excellent extraction of phenolic contents from plants followed by methanol solvent while aqueous solvent extracted minimum phenolic contents from plants extracts. The phenolic contents of young leaves and barks of *Acacia arabica* are related to our results [9, 10].

Total flavonoid contents

In present work, total flavonoid contents of *Zizyphus mauritiana* were found in the range of (146.36 \pm 0.81 to 81.11 \pm 0.62 QE mg/100 g). The maximum total flavonoid contents (146.36 \pm 0.81 QE mg/100 g) was indicated by 50% aqueous ethanol solvent. *Acacia arabica* contained total flavonoid contents in the range of (172.52 \pm 0.99 to 88.73 \pm 0.41 QE mg/100 g) whether the maximum total flavonoid contents (172.52 \pm 0.99 QE mg/100 g) was shown by 50% aqueous ethanol solvent at. The detailed results are shown in (Table 1). The interesting effect of our research was that the 50% aqueous ethanol solvent was useful for extraction of flavonoid compounds from plants. The maximum flavonoids extracted by ethanol solvent followed by methanol solvent while minimum extraction of flavonoids was done by aqueous solvent. Our results were found similar to already existing research data [11].

Antidiabetic activity

In present study, the alpha amylase assay was used for the determination of antidiabetic activity of different solvents (aqueous, 100% ethanol, 50% aqueous ethanol, 100% methanol and 50% aqueous methanol) friction of *Zizyphus mauritiana* and *Acacia arabica*. The inhibition % of *Zizyphus mauritiana*

Table. 1: Antioxidant activity, total phenolic and flavonoid contents of different solvent fractions of *Zizyphus mauritiana* and *Acacia arabica*.

<i>Zizyphus mauritiana</i> and <i>Acacia arabica</i> solvent fractions and standard	Antioxidant activity (%)	Total phenolic content (mg GAE/100g)	Total flavonoid content (QE mg/100g)
<i>Zizyphus mauritiana</i>			
Aqueous	87.40 ± 0.52 ^c	184.08 ± 0.88 ^c	81.11 ± 0.62 ^a
100% Methanol	93.50 ± 0.5 ^a	478.30 ± 0.78 ^b	113.16 ± 0.47 ^c
50% aqueous Methanol	89.50 ± 0.97 ^b	476.24 ± 0.76 ^b	87.63 ± 0.55 ^d
100% ethanol	93.76 ± 0.49 ^a	452.30 ± 0.62 ^c	135.11 ± 0.67 ^b
50% aqueous ethanol	94.33 ± 0.52 ^a	670.83 ± 1.46 ^a	146.36 ± 0.81 ^a
<i>Acacia arabica</i>			
Aqueous	73.60 ± 0.52 ^e	183.06 ± 0.4 ^d	88.73 ± 0.41 ^d
100% Methanol	76.92 ± 0.54 ^d	802.26 ± 0.64 ^c	140.5 ± 1.32 ^c
50% aqueous Methanol	82.51 ± 0.45 ^c	892.61 ± 1.3 ^b	142.55 ± 0.5 ^c
100% ethanol	93.31 ± 0.3 ^b	899.80 ± 0.81 ^b	162.7 ± 0.43 ^b
50% aqueous ethanol	96.53 ± 0.46 ^a	934.34 ± 0.89 ^a	172.52 ± 0.99 ^a
Ascorbic acid	88.48 ± 0.09 ^{bc}	--	--

(--)= not detected

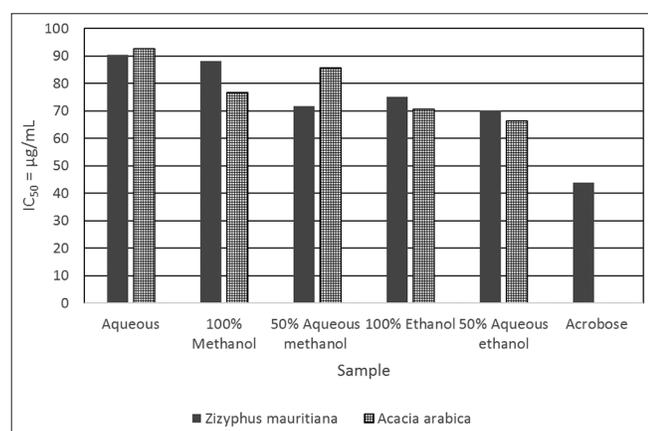


Figure 1: Inhibition (%) of α -amylase by different solvents of *Zizyphus mauritiana* and *Acacia arabica*

against alpha amylase enzyme lies in the range of $IC_{50} = 90.46 \pm 0.23 - 71.66 \pm 0.86 \mu\text{g/mL}$. The highest inhibition $IC_{50} = 90.46 \pm 0.23 \mu\text{g/mL}$ was shown by aqueous extract. While in case of *Acacia arabica* the inhibition % of alpha amylase enzyme activity lies in the range of $IC_{50} = 92.63 \pm 0.12 - 71.3 \pm 0.04 \mu\text{g/mL}$ by aqueous extract. The maximum inhibition $IC_{50} = 92.63 \pm 0.12 \mu\text{g/mL}$ was observed in aqueous extract while standard showed was found to be $IC_{50} = 31 \pm 0.06 \mu\text{g/mL}$. The detail results are shown in (Figure 1). The interesting fact of our research was that the aqueous solvent showed highest inhibition as compared to other solvents. Overall results solvent results showed that the aqueous extract showed excellent inhibition followed by methanol while minimum inhibition indicated by ethanol solvent.

CONCLUSION

In conclusion, our research work is report on antioxidant activity, total phenolic and flavonoid content as well as antidiabetic activity of different solvent extracts of *Zizyphus mauritiana* and *Acacia arabica*. The results of present research work showed that 50% aqueous methanol solvent indicated excellent antioxidant activity, total phenolic and flavonoid contents of both plants while in case of antidiabetic activity the aqueous solvent showed

highest inhibition of both plants. Moreover, the considerable antioxidant and antidiabetic activities of different solvents may assist in the formulation of herbal drugs for treatment of oxidative stress based disorders and diabetes.

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