

# Anti-diabetic and anti-lipidemic effect of *Albizia lebbek* seeds against HepG2 cells

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**Abstract:** *Albizia lebbek* has been a medicinally important plant for its pharmacological potential. This study aims to determine the *in vitro* antioxidant, anti-diabetic and anti-lipidemic potential of *A. lebbek* seeds. The seed extracts were prepared in petroleum ether, chloroform and methanol. Crude methanolic extract (ME ext) was subjected further to sequential fractionation in increasing polarity based solvents. Extracts and fractions were analyzed for their antioxidant, anti-diabetic and anti-lipidemic potentials using hepatic cell line, HepG2. Results showed that crude extracts of *A. lebbek* seeds specifically, ME ext are rich in polyphenols and flavonoids. ME ext has also shown highly significant antioxidant and alpha-amylase inhibition potential compared to petroleum ether and chloroform extracts. *In vitro* assays using different fractions of methanolic extract further highlighted the ethyl acetate and chloroform fractions exhibiting significant antioxidant and anti-diabetic potentials. Alpha-amylase inhibition coupled with enhanced glucose uptake of cells treated with ME ext and ethyl acetate fraction emphasized on significant anti-diabetic potential of the plant. Expression alteration of genes and reduced level of cholesterol suggested the lipid synthesis mediated anti-diabetic activity of the plant. It is therefore, concluded that *A. lebbek* seed has significant antioxidant, anti-diabetic and anti-lipidemic potentials.

**Keywords:** *Albizia lebbek*, diabetes mellitus, anti-diabetic, phytochemistry, lipid metabolism.

## INTRODUCTION

One of the major concerns of the 21st century is diabetes affecting millions of people worldwide (Moodley *et al.*, 2015b). Diabetes mellitus is a metabolic defect which is characterized by high glucose level in blood, abnormal level of lipids and proteins. As a result of these metabolic changes at least 50 percent of diabetic patients have one or more micro vascular and macro vascular complications by numerous mechanism including oxidative stress which leads to oxidative damage to lipids, carbohydrates and proteins (Moodley *et al.*, 2015a). Numerous therapies for treating diabetes are under investigation throughout the world (Visweswara Rao *et al.*, 2013). Conventional hypoglycaemic agents prove to be insufficient for glycemic control and their side effects often limit treatment choice (Kahn *et al.*, 2006). The most commonly prescribed oral hypoglycaemic agents for type II diabetes are metformin and sulfonylureas which are helpful for initial control of glucose level, however, they cannot manage a durable glycemic control which thereby results in the need for new treatment regimens (Inzucchi *et al.*, 2012). The use of thiazolidinediones has been limited due to their unwanted side effects such as obesity, edema and weakness of bones (Dormandy *et al.*, 2005).

Natural products have been used since thousands of years around the world for the treatment and prevention of human diseases. Various bioactive compounds showing potent biological activities have been isolated from different natural product sources (Atanasov *et al.*, 2021). Identification of medicinal plants with anti-diabetic activity are therefore, an important area of contemporary medical research. *Albizia lebbek* belongs to the family Mimosaceae, found in deciduous forests of Pakistan, India, Sri Lanka and Burma. It is an important herb tree in terms of a good source of pharmacologically active constituents and used traditionally since for a long time (Mohammad *et al.*, 2012). Ethno pharmacologically *A. lebbek* is used as an astringent and for boils. The plant is also used for treating cough, eye diseases, respiratory problems and abdominal tumors (Duke, 2008). Moreover, flowers of this tree have been reported to possess hepatoprotective activity (Al-Massarani *et al.*, 2017). Alkaloidal compounds isolated from *A. lebbek* leaves showed antibacterial and cytotoxic activity (Shenta & Al-Maliki, 2013; Ali *et al.*, 2018). New tri-O-glycoside flavonols from *A. lebbek* leaves were reported previously (El-Mousallamy, 1998). The bark of *A. lebbek* is a good source of flavonoids and possesses anti-diabetic potential (Ahmed *et al.*, 2014). The current study aims to evaluate the anti-diabetic potential of *A. lebbek* seed extract using different *in vitro* models and HepG2 cells.

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## MATERIALS AND METHODS

### *Plant material*

The plant material was collected and identified by Dr. Zaheer ud Din, Department of Botany Government College University, Lahore, Pakistan. The seeds were washed and ground to a coarse powder. The sequential extraction was performed on the 700g dried powder of seeds by Soxhlet apparatus using 3.5L of each solvent such as petroleum ether (PE ext), chloroform (CH ext) and methanol (ME ext). The extracts were further concentrated using Heidolph Laborota L-4002 rotary evaporator. Fractionation of 100g ME ext was performed by approximately 500ml of four different organic solvents with different polarities such as petroleum ether (PE fr), chloroform (CH fr), ethyl acetate (EA fr), n-butanol (N-BUT fr) and water fraction (W fr). The fractions were also concentrated using rotary evaporator.

### *Determination of phytochemicals*

#### *Determination of Total Flavonoid Content (TFC)*

TFC was performed according to the procedure reported previously (Chang *et al.*, 2002). To prepare the calibration curve, Quercetin was used in concentration range 1-20 µg/ml in methanol. A reaction mixture containing standard solution (0.2ml), 10% aluminium chloride (0.1 ml) and 1M potassium acetate (0.1ml) was prepared and volume was made upto 5 ml with distilled water. The reaction mixture was incubated at room temperature for 30 min and absorbance was taken at 415nm. Similarly, all the extracts of *A. lebbek* seeds (0.1mg/ml) were treated. The flavonoid content was expressed as quercetin equivalents using equation from calibration curve:  $Y = 0.0271X - 0.0302$   $R^2 = 0.9913$ .

#### *Determination of total polyphenol content (TPC)*

TPC of different extracts was estimated by previously reported procedure with slight modifications (Slinkard & Singleton, 1977). 20µg/ml methanolic extract was dissolved in methanol: Folin-Ciocalteu reagent (4:1)l followed by addition of 0.15% w/v Na<sub>2</sub>CO<sub>3</sub> (After incubating for 2 hours, absorbance was recorded at 760 nm. For calibration curve gallic acid was used as standard. The TPC was calculated from eq:  $Y = 0.0009X + 0.0147$   $R^2 = 0.9899$ .

#### *Determination of antioxidant activity*

Antioxidant activity of seed extracts and fractions of ME ext were evaluated by following *in vitro* assays.

#### *1,1-diphenyl-2-picryl-hydrazyl (DPPH) Assay*

Sample (1ml) of various concentrations were mixed in 0.1mM fresh methanolic solution of DPPH (1ml) followed by incubation in the dark for 10 minutes at 37°C. The optical density was measured at 517 nm. Butylated Hydroxytoluene (BHT) was used as the standard. The

following formula was used to evaluate activity (amboinicus Lour, 2010).

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

#### *Ferric reducing assay*

Ferric reducing assay was performed according to the protocol reported previously (Abdel-Hameed *et al.*, 2012). Each sample in concentration range of 100µg/ml was mixed with 0.2M sodium phosphate buffer pH 6.6 (2.5ml) and 1% w/w potassium ferricyanide (2.5ml) and incubated at 50°C for 20min. 10% w/v trichloroacetic acid (2.5ml) was added in this reaction mixture followed by 10 min centrifugation at 3000 rpm. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and 0.1% w/w ferric chloride (0.5ml). After incubation for 10 min, the absorbance against the blank was determined at 700 nm. Trolox was used as a standard.

#### *Phosphomolybdate antioxidant assay*

Each sample (1ml) was mixed with phosphomolybdate reagent (3ml) followed by incubation at 95°C for 90 min and then cooled at to room temperature. The optical density of this mixture was measured at 695nm. The results were expressed as the number of ascorbic acid equivalents (Abdel-Hameed *et al.*, 2012).

#### *Hydrogen peroxide scavenging capacity*

Different concentrations of extracts (100-1000 µg/ml) in methanol were mixed with hydrogen peroxide solution (0.6ml, 40mM pH 7.4) and incubated for 10 min. Absorbance at 230 nm was determined. Ascorbic acid was used as standard (Keser *et al.*, 2012). The activity was calculated by the following formula:

$$\% \text{ Scavenged } [H_2O_2] = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

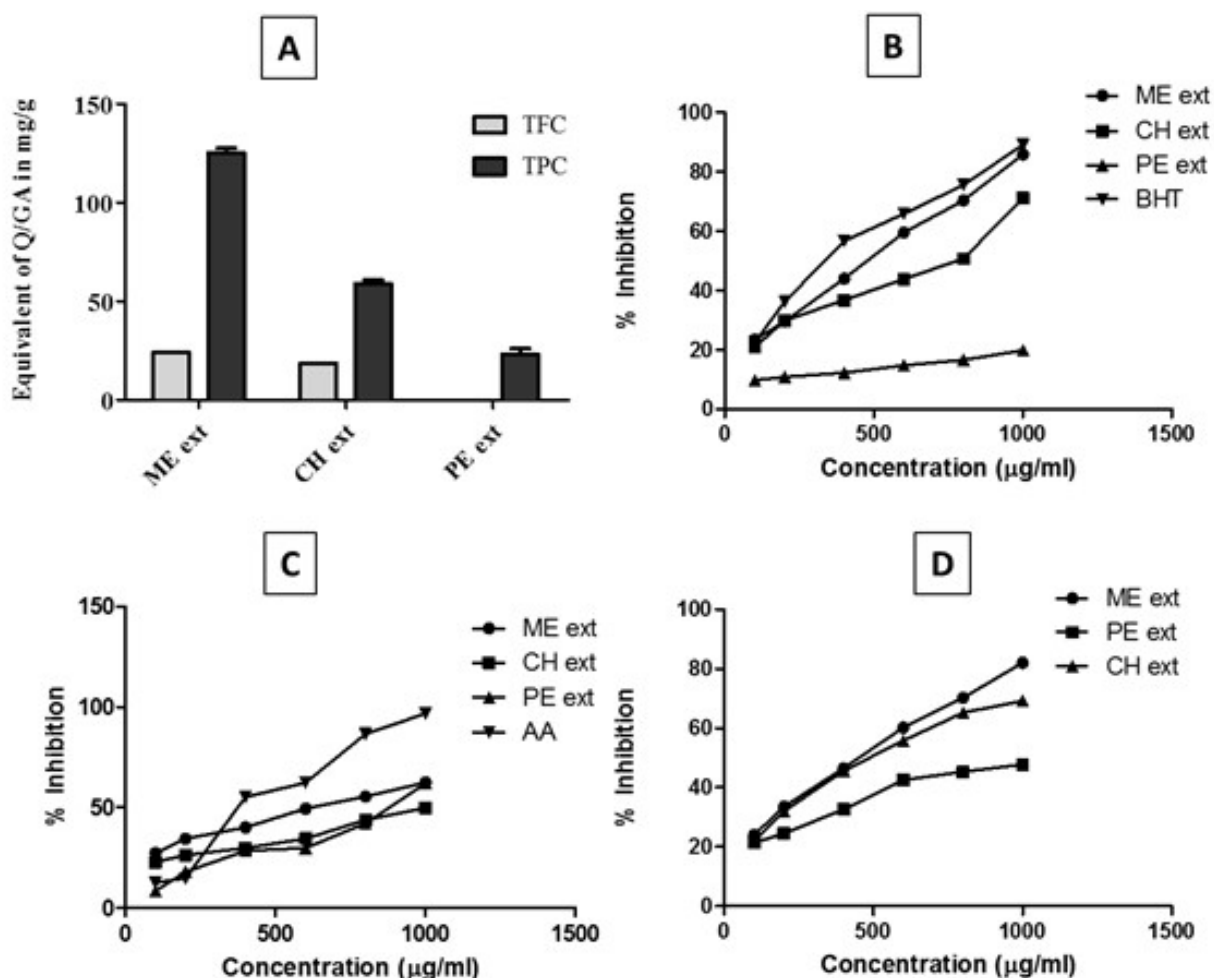
#### *Determination of in vitro anti-diabetic activity*

##### *Alpha-amylase inhibition assay*

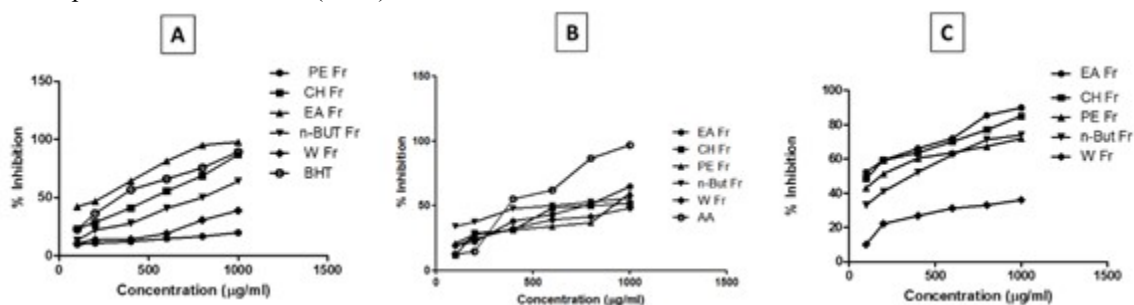
Each extract (0.5ml) and enzyme solution (0.5ml) 0.5 mg/ml, in 0.02M buffer pH 6.9 were mixed and incubated at room temperature for 10 min. To this mixture, 1% starch solution (0.5ml) 0.02M buffer pH 6.9 with 0.006M sodium chloride was added and then again incubated for 10 min. The reaction was stopped by dinitrosalicylic acid color reagent (1ml). The mixture was incubated in boiling water for 5 min, cooled to room temperature and then diluted by distilled water (10ml). Absorbance was taken at 540 nm (KWON *et al.*, 2008). Percentage inhibition was calculated by the following formula.

$$\% \text{ Inhibition} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100\%$$

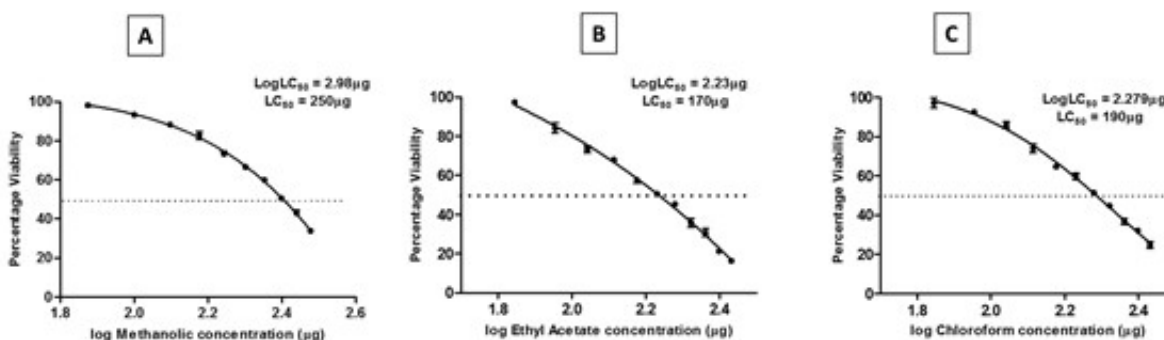
ME ext and its fractions which shows highest alpha amylase inhibition activity were evaluated for glucose uptake assay and anti-lipidemic effect by HepG2 cells.



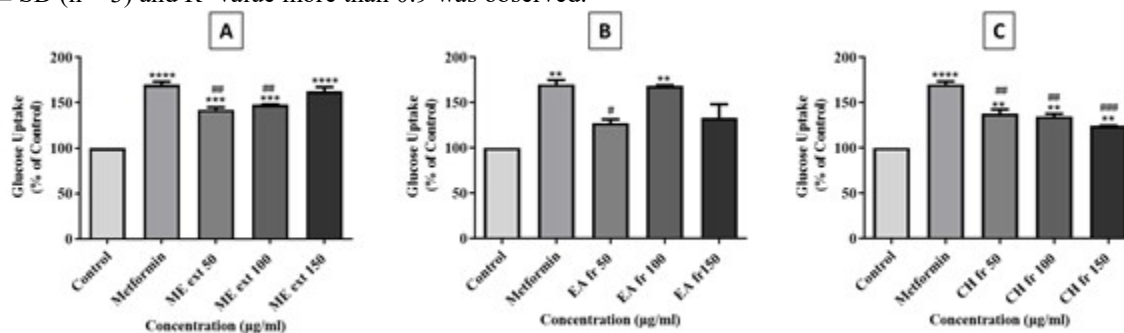
**Fig. 1:** Phytochemical, antioxidant and Alpha amylase inhibition analysis of extracts of *A. lebeck* seeds. ME ext (methanolic extract), CH ext (chloroform extract) and PE ext (petroleum ether extract) of *A. lebeck* seeds were analysed for (A) Total phenolic content equivalent (TPC) of GA (Gallic acid in mg/g) and total flavonoid content equivalent (TFC) of Q (Quercetin in mg/g) (B) Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay of ME ext, CH ext and PE ext of *A. lebeck* seeds at different concentration (100µg-1000µg/ml) using standard BHT (butylated hydroxyl toluene) (C) Hydrogen peroxide ( $H_2O_2$ ) reducing assay of ME ext, CH ext and PE ext of *A. lebeck* seeds at different concentration (100µg-1000µg/ml) using standard AA (Ascorbic acid) (D) Alpha amylase inhibition assay of ME ext, CH ext and PE ext of *A. lebeck* seeds at different concentration (100µg-1000µg/ml). The values are expressed as mean  $\pm$  SD (n = 3) and  $R^2$  value more than 0.9 was observed.



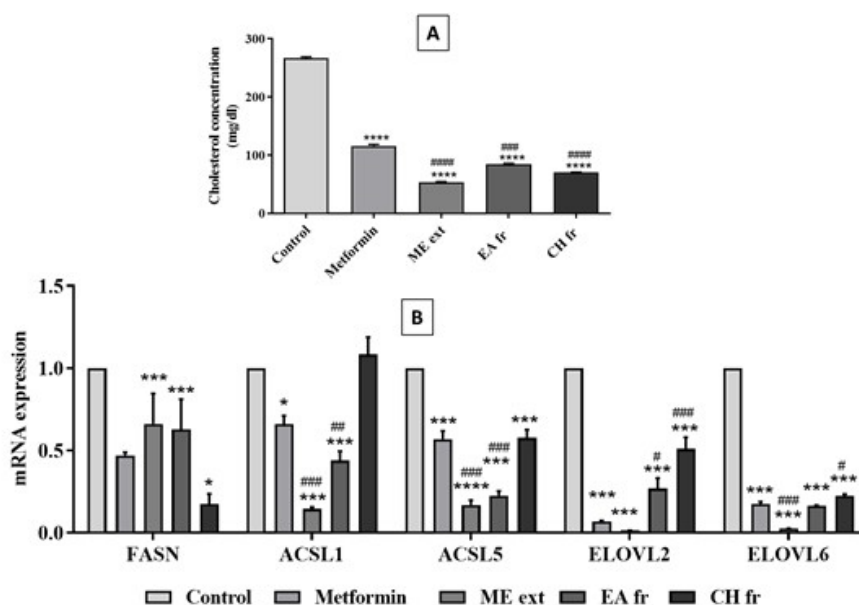
**Fig. 2:** Antioxidant and Alpha amylase inhibition analysis of ME extract of *A. lebeck* seeds and its fractions. ME extract and its PE fr (Petroleum ether fraction, CH fr (chloroform fraction), EA fr (ethyl acetate fraction), n-But fr (n-Butanol fraction) and W fr (water fraction) at different concentration (100µg-1000µg/ml). (A) DPPH scavenging activity assay (B)  $H_2O_2$  reducing assay (C) Alpha amylase inhibition assay The values are expressed as mean  $\pm$  SD (n = 3) and  $R^2$  value more than 0.9 was observed.



**Fig. 3:** MTT cell viability assay. HepG2 cells were subjected to a range of concentrations (2µg/ml-100µg/ml) of different samples of ME ext of *A. lebbek* seed for 72 hrs and analysed for cell viability. The values are expressed as mean ± SD (n = 3) and R<sup>2</sup> value more than 0.9 was observed.



**Fig. 4:** Glucose uptake assay. HepG2 cells were subjected to different concentrations of ME ext of *A. lebbek* seed and its fractions for 72 hrs followed by 2 hrs glucose treatment and assessment of glucose uptake. Metformin (0.1µg/ml) was kept as control. (A) ME ext (B) CH Fr and (C) EA Fr. Data is expressed as mean ± SD (n = 3) \* was used for comparison with control and # was used for comparison with metformin (\**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001 and \*\*\*\**p*<0.0001).



**Fig. 5:** Effect of ME ext of *A. lebbek* seed and its fractions on intracellular lipids and genes involved in lipid metabolism in HepG2 cells treated with IC<sub>50</sub> concentration of ME ext of *A. lebbek* seed and its fractions for 72 hrs compared to control without any treatment. (A) Quantification of intracellular cholesterol (B) Expression analysis of genes involve in lipid metabolism FASN, ACSL1, ACSL5, ELOVL2 and ELOVL6. The values are expressed as mean ± SD (n=3) and A *p*-value <0.05 was regarded as significant. \* was used for comparison with control and # was used for comparison with metformin (\**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001 and \*\*\*\**p*<0.0001).

**Table 1:** Antioxidant capacity of standard, seed extracts and ME extract fractions. BHT was kept as standard for DPPH inhibition assay whereas, ascorbic acid was used as standard for H<sub>2</sub>O<sub>2</sub> inhibition assay. R<sup>2</sup> value more than 0.9 was observed.

| Sample        | DPPH % Inhibition | IC <sub>50</sub> (µg/mL) | H <sub>2</sub> O <sub>2</sub> % Inhibition | IC <sub>50</sub> (µg/ml) | Reducing power activity | Total antioxidant capacity |
|---------------|-------------------|--------------------------|--|--------------------------|-------------------------|----------------------------|
| ME ext        | 85.97             | 486.28                   | 62.45                                      | 652.50                   | 14.654                  | 2.428                      |
| Ch ext        | 71.22             | 674.02                   | 49.82                                      | 1038.55                  | 10.456                  | 1.714                      |
| PE ext        | 19.78             | 3853.88                  | 59.93                                      | 869.86                   | 3.172                   | 0.64                       |
| PE fr         | 47.76             | 1227.72                  | 62.00                                      | 945.86                   | 5.765                   | 0.142                      |
| Ch fr         | 86.94             | 507.07                   | 52.05                                      | 820.30                   | 2.432                   | 1.928                      |
| EA fr         | 97.76             | 203.37                   | 65   | 721.82                   | 19.962                  | 3.214                      |
| n-But fr      | 64                | 771.30                   | 55.82                                      | 671.74                   | 9.222                   | 0.42                       |
| W fr          | 38.80             | 1463.57                  | 48.60                                      | 1020.60                  | 4.530                   | 0.071                      |
| BHT           | 89.24             | 406.32                   | -  | -                        | -                       | -                          |
| Ascorbic acid | --                | --                       | 96.86                                      | 469.26                   | --                      | --                         |

ME ext (methanolic extract), CH ext (chloroform extract), PE ext (petroleum ether extract), PE fr (Petroleum ether fraction), CH fr (chloroform fraction), EA fr (ethyl acetate fraction), n-But fr (n-Butanol fraction), W fr (water fraction) and BHT (butylated hydroxyl toluene).

### Cell culture

HepG2 cell line (Human Hepatocyte Carcinoma) was obtained from cell culture stock of School of Biological Sciences, University of the Punjab, Pakistan. The cells were cultured in DMEM (Dubecco's Modified Eagle Medium) prepared with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin under humidified controlled atmosphere at 37°C with 5% CO<sub>2</sub> in cell culture incubator. All the cell culture reagents were obtained from Gibco and Sigma.

### MTT Cell Cytotoxicity Assay

The cytotoxic effects was determined by the MTT assay on HepG2 cells as described previously (Perveen *et al.*, 2021). The cells were cultured in a 96-well plate at a density of 10,000 cells/well. After 24h, *A. lebbek* ME ext and its fractions in varying concentrations (0-500 µg) were subjected to the cells compared to control with equal volume of DMEM. After 72h, 0µl of MTT (3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) solution (5mg/ml) was added in each well followed by solubilization of formazan crystals with DMSO solution (100µl). The absorbance was determined at 570nm with ELISA plate reader for the experimental wells with respect to corresponding control wells.

### Glucose uptake assay

HepG2 cells were seeded in 96 well culture plate at a density of 5000 cells/well and allowed to reach 60% confluency. Cell were treated with various concentration of methanol extract and its fractions (50µg, 100µg, 150µg) for 72 h followed by 8mmol/L glucose exposure for 2 hrs. After 2 hrs, glucose concentration of media from each was estimated using a commercially available kit (Glucose liquicolor ®; HUMAN GmbH, Germany) according to manufacturer instructions. Briefly, Samples (1µl) were mixed separately with Glucose Liquicolor reagent (200µl) followed by incubation at 37°C for 5 min.

Optical density at 540 nm was recorded using Eliza plate reader. The glucose used by the cells was calculated by subtracting the cell-containing wells from the cell-free wells. Metformin was used as positive control. All the control and experimental groups were run in triplicates (Odeyemi & Dewar, 2020).

### Determination of anti-lipidemic activity

Anti-lipidemic activity was assessed through Gene expression regulation of de novo lipid synthesis pathway as reported previously (Perveen *et al.*, 2020). Trizol reagent (Thermofisher Scientific-15596018) method was used to isolate RNA from HepG2 cells treated with IC<sub>50</sub> dose for 72 hrs in independent experiments. Quality and quantity were ascertained by DeNovix DS-11 FX+ spectrophotometer. From purified RNA, cDNA was synthesized using Revert Aid First Strand cDNA synthesis kit (Thermofisher Scientific- K1622) for Real Time PCR. Expression of mRNA levels of lipid metabolism genes (FASN, ACSL1, ACSL5, ELOVL2, ELOVL6) was performed was analyzed using Thermo Fisher Syber Green master mix in Piko Real qPCR (Thermo Scientific) in triplicates. mRNA level of housekeeping gene GAPDH was used to normalize mRNA levels of targeted genes. List of primers used is provided in the table 1.

### STATISTICAL ANALYSIS

All determinations were carried out in triplicates and the results were mentioned as mean ± standard deviation. The statistical analysis was carried out using Microsoft Excel or Graph Pad Prism 5. The goodness of fit model was calculated for assays and R<sup>2</sup> value more than 0.8 was kept as significant. Half the maximum inhibitory concentration (IC<sub>50</sub>) was calculated by using linear regression on the dose-response curve. Student's t-test and one-way analysis of variance (ANOVA) with Tukey's as post hoc

test were applied for glucose uptake assay and gene expression analysis. A *p*-value <0.05 was regarded as significant.

## RESULTS

### *Determination of phytochemicals analysis has revealed that*

ME ext consists of the highest flavonoid content of 24±0.1529mg of Q/g equivalent compared to CH ext which shows 18.59±0.1210mg of Q/g equivalent. PE ext shows no flavonoid content. Highest polyphenol content was also present in ME ext about 125.5±2.57mg of G/g compared to CH extract containing 58.88±1.87mg of G/g whereas, PE ext exhibiting the lowest of about 23.11±2.9mg of G/g equivalent (fig. 1A).

### *Antioxidant effect of A. lebbek seed extracts and fractions*

The results have shown that with increasing the concentration of extracts the antioxidant effect was also increased (table 1). DPPH assay revealed that at the concentration of 1000µg/ml, ME ext showed the highest activity 85.97%, CH ext showed 71.22% and PE ext showed 19.78% (fig. 1B). Among fractions of ME ext, EA fr showed the highest activity (fig. 2A). Results of ferric reducing power showed that the EA fr of ME ext had the highest 19.962mg trolox/g followed by the ME ext 14.654 and CH ext 10.456mg trolox/g. Phosphor molybdenum method also showed that EA fr had a higher value of 3.214mg AA equivalent/g than the ME ext of 2.428mg AA equivalent/g and CH fr of 1.928mg AA equivalent/g (table 1). H<sub>2</sub>O<sub>2</sub> scavenging capacity of EA fr was highest 65% at the concentration of 1000µg/ml, then crude ME ext 62.45% and standard ascorbic acid shows 96.85% capacity (fig. 1C, fig. 2B).

### *Anti-diabetic effect of ME ext of A. lebbek seed and its fractions*

ME ext, CH ext and PE ext showed the best inhibitory activity with IC<sub>50</sub> of 473.47µg/ml, 549.25µg/ml and 976.28µg/ml respectively (fig. 1D). EA Fr, CH fr of ME ext showed maximum α-amylase inhibition about 89.93% and 85% respectively with an IC<sub>50</sub> value of 16.76µg/ml and 45.05µg/ml respectively. PE Fr, n-But fr and W fr of ME ext shows less inhibitory effect as compared to others at a concentration of 1000µg/ml about 71.92%, 74.04% and 36% with an IC<sub>50</sub> value of 394.78µg/ml, 189µg/ml and 1465.62µg/ml respectively (fig. 2C).

Cytotoxicity of ME ext and its two fractions which showed best alpha-amylase inhibition (EA fr, CH fr) against HepG2 cells was determined with LC<sub>50</sub> values using MTT assay. The LC<sub>50</sub> values for ME ext, EA fr and CH fr were 250µg/ml, 170µg/ml and 190µg/ml respectively (fig. 3). Glucose uptake assay was performed for three different concentrations (50,100 and 150µg/mL)

below the LC<sub>50</sub> for ME ext, EA fr and CH fr (fig. 4). The results indicated that EA fr showed significant utilization of glucose in HepG2 cells at 100µg/ml (168%) which is comparable with metformin (170%). Both ME ext showed a concentration-dependent increase in glucose uptake and a significant increase compared to untreated control (fig. 4).

### *Effect of ME ext of A. lebbek seed and its fractions on intracellular lipids and genes involved in lipid metabolism*

Evaluation of intracellular lipids has shown a significant decrease in cells treated with either of the ME ext, EA fr and CH fr of *A. lebbek* seed (fig. 5A). Significant down regulation of genes involved in lipid synthesis in treated cells compared to control has further strengthened the anti-lipidemic effect of *A. lebbek* seeds (fig. 5B). FASN relative expression was down regulated in all the groups. Its maximum down regulation was analyzed in chloroform group. ACSL-1, ACSL-5, ELOVL2 and ELOVL6 showed maximum downregulation of mRNA transcript in methanol group.

## DISCUSSION

The natural ability of phytochemicals to scavenge free radicals leading to their usage as antioxidants is increased due to their various pharmacological activities with minimal side effects (El Omari *et al.*, 2019). Free radicals contributes effectively in the development and progression of diabetes (Maritim *et al.*, 2003). As antioxidants, polyphenols plays a major role in the prevention of oxidative stress-related diseases that includes cancers, CVS diseases, neurodegenerative diseases, hyperglycaemia (Dhalaria *et al.*, 2020). Flavonoids are an important and most prevalent class of polyphenols, having the ability to reduce free radicals with redox potential by donating hydrogen atoms (Papuc *et al.*, 2017). Flavonoids having good antioxidant activity have been suggested to defend against the ruinous effects of diabetes by acting on alpha- glycosidase and dipeptidyl peptidase-4 (Sarian *et al.*, 2017). *A. lebbek*, known as "Siris" in Pakistan has been used as a traditional medicine. Previously a study has reported that root, stem and pod of *A. lebbek* are rich in polyphenols and flavonoids (Zia-Ul-Haq *et al.*, 2013). However, to date no study has documented the phytochemical profile, antioxidant and anti-diabetic potential of *A. lebbek* seeds.

The current study showed that ME ext of *A. lebbek* seeds is rich in polyphenols and flavonoids followed by CH ext and PE ext. Previously detected phytochemicals from various parts of *A. lebbek* has been reported for their various therapeutic potentials. A 3, 5-dihydroxy-4, 7-dimethoxy flavones were isolated from ethanolic extract of pods of *A. lebbek* which showed mild in vitro antibacterial activity (Rashid *et al.*, 2003). Flowers of *A.*

*lebbeck* showed significant pharmacological activities such as estrogenic, analgesic antipyretic and anti-inflammatory activity (Farag et al., 2013). A study has also reported in-vivo hepatoprotective activity of methanolic extract of *A. lebbeck* flowers and has also isolated terpenoidal compounds (Al-Massarani, 2017). Another study has reported that in alloxan-induced diabetic oxidative stress in rats, the *A. lebbeck* leaves extract had a good antioxidant effect (Resmi et al., 2006). Previously antioxidant studies through DPPH scavenging and H<sub>2</sub>O<sub>2</sub> scavenging assays on *A. lebbeck* revealed good antioxidant potential of its leaves and bark (Malla et al., 2014). While in this study, antioxidant assays has revealed highly significant antioxidant capacity of ME ext and its EA Fr.

Albiziahexoside, a white amorphous saponin compound was isolated from leaves of *A. lebbeck* previously (Ueda et al., 2003). It was reported that three flavonoids isolated from the bark of *A. lebbeck* i.e geraldone, isookanin and luteolin showed good alpha-amylase and alpha-glucosidase inhibition. Inhibition of these enzymes slows down the breakdown of starch in the gastrointestinal tract into the simpler saccharides, thus reducing the postprandial hyperglycaemia (Ahmed et al., 2014). In the current study, CH fr and EA fr showed excellent alpha-amylase inhibition followed by ME ext and CH ext. The results of glucose uptake assay in HepG2 cells showed that only EA fr showed significant utilization of glucose that was comparable to metformin. This indicates that EA fr could be similar to metformin by increasing the glucose utilized in the liver. The expected compounds for said activity of EA fr can be polyphenols and flavonoids. Anti-lipidemic effect was observed more significant by ME ext and CH fr as compared to EA fr.

## CONCLUSION

Based on the results of the current study, it is concluded that methanolic extract of *A. lebbeck* seeds and its ethyl acetate fraction are a good source of bioactive compounds with significant antioxidant, anti-diabetic and anti-lipidemic potentials. However, detailed studies are required for the isolation of pharmacologically active compounds and their therapeutic profiles towards medicinal use of *A. lebbeck*.

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