

DOI: <https://doi.org/10.56936/18290825-2025.19v.4-62>**PHYTOCOMPOUNDS AS ANTIDIABETIC AND HEPATOPROTECTIVE AGENTS: THE PROMISING POTENTIALS OF *ACACIA ARABICA* (LAM.) WILLD FLORAL METHANOLIC EXTRACT****ALSANOUSI N<sup>1,2</sup>**

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**ABSTRACT**

**Background:** The oxidative stress related diabetes mellitus is a significant metabolic disorder that creates serious complication and also affecting million people globally resulting prolonged medication leads liver toxicity and drug resistance. Hence, to reduce the side effects of the existing drugs, compounds of natural origine with nom known side effects could be a promising alternative.

**Materials and Methods:** The present study analysed the antidiabetic, antioxidant and hepatoprotective properties of the methanolic *Acacia arabica* flower extract by  $\alpha$  amylase activity inhibition assay, glucosidase activity inhibition assay, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay, 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid scavenging assay, nitric oxide scavenging activity, hepatoprotective activity on HepG2 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay etc.

**Results:** *A. arabica* flower extract (5 mg/ml) showed an ability to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes by 77% and 75% respectively. The antioxidant properties of the extract were after treatment and revealed to be 84% of free radical's inhibition, 89% of 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid scavenging inhibition and also 85% of nitric oxide scavenging activity at 5 mg/ml. The hepatoprotective effect was examined after treatment with *A. arabica* flower extract which revealed no toxicity to HepG2 cells.

**Conclusion:** *A. arabica* floral methanolic extract has found to have promising antidiabetic and hepatoprotective activities and the author recommend detailed phytochemical and in vivo investigations to develop it for its usage in the treatment of diabetes mellitus.

**KEYWORDS:** *A. arabica*, oxidative stress, drug toxicity,  $\alpha$ -amylase,  $\alpha$ -glucosidase**INTRODUCTION**

The diabetes mellitus epidemic and their complication create a major threat to public health owing to diabetes mellitus prevalence increase and became sixth most important chronic metabolic

disorder which cause severe injury and death [Zaharia et al., 2019; Kalmatov et al., 2024]. The report from International Diabetes Federation Atlas 10th edition 2021 says, 537 million population

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were affected by diabetic and it may extend up to 783 million adults by 2045 [Magliano and Boyko, 2021; Ogurtsova et al., 2021]. Also, 10% of total global health care budget was spent for diabetic related healthcare expenses [Soumya, Srilatha, 2011]. diabetes mellitus is a metabolic disorder characterized by enhanced blood glucose level due to defect in insulin production or insulin action or combination of both. The diabetes mellitus and their complications are mainly related to hyperglycemic condition which leads micro and macrovascular complications in both types (Type 1 and Type 2) [Garcia et al., 2018; Yun et al., 2021]. Amongst, type 2 is more prevalent among 90% of diabetic cases which is differentiated by insulin resistance and pancreatic beta cells damage [Arumugam et al., 2013; Saedi et al., 2016]. However, diabetes is mostly associated with several risk factors such as ethnicity, overweight, increasing age, previous family history and obesity.

The diabetes mellitus pathogenesis can be mediated through various mechanisms which comprises increased glucose absorption by carbohydrate digestion, reduced incretin hormones action, raised proteins glycation, reduced peripheral glucose uptake and insulin sensitivity in targeted tissues [Dirir et al., 2022]. Moreover, the report from World Health Organization says, cardiovascular and cerebrovascular diseases are the key factor for many diabetic deaths and also, renal diseases are responsible for another 10% of deaths due to diabetic [Sun et al., 2022]. Moreover, either single or multiple complications are persisted in diabetic patients and if the complication continues it can ruin the treatment processes [Chua et al., 2013]. The management of diabetes mellitus is mainly relied mostly on healthy lifestyle and sometimes, oral medications like  $\alpha$ -glucosidase inhibitors are generally used for the treatment of DM diabetes mellitus are mainly involved in hydrolysing carbohydrates and produced glucose which is absorbed by intestine. Therefore, the process can be hindered by the antidiabetic drugs leads prolonged time duration for digestion thereby reduction in glucose absorption. The long-term use of anti-diabetic drugs leads drug resistance and liver toxicity are encountered very often which is major concern [Bennett et al., 2011]. However, the type 2 diabetes mellitus associated with hyperlipidemia, hypertension and chronic hyperglycemia

are caused due to cellular redox balance disturbance which initiate the oxidative stress resulting serious complications [Tungmunnithum et al., 2018; Wang et al., 2019; Patel, Ghane, 2021; Hu et al., 2023]. Hence, the immediate need for potent antidiabetic and anti-oxidant for oxidative stress mediated diabetes mellitus.

For several decades, the plant-based biomolecules played a major role in pharma industry due their various biological activities including anti-microbial, anti-cancer, anti-inflammatory and anti-oxidant properties. Therefore, *Acacia arabica* from *Leguminosae* family is used in traditional medicine for longer time owing to their various medicinal values [Farzana and Tharique, 2014]. Moreover, the gum of the plant is widely used as dietary supplement in *Ayurvedic* medicine [Ansari et al., 2021]. Thus, our study explored the antidiabetic, antioxidant and hepatoprotective properties of methanolic *A. arabica* flower extract.

#### MATERIALS AND METHODS

**Methanolic crude extract preparation of *A. arabica* flower:** The *A. arabica* flower collected was fine powered after sun dried and 20g powder was added to fresh cellulose thimble which placed inner side of the Soxhlet apparatus as confirmed before [Harley et al., 2022]. The reaction continued for many hours after adding methanol and the crude extracts was obtained after completing the reaction. The solvent evaporated final product was used for all the studies.

**$\alpha$  - amylase activity inhibition assay:** *A. arabica* flower methanolic extract was explored for their  $\alpha$ -amylase activity inhibiting ability using  $\alpha$ -amylase activity inhibition assay as declared earlier [Alqahtani et al., 2019]. Briefly, the amylase enzyme was added to varying *A. arabica* flower methanolic extract concentrations (1 mg/ml, 2mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) and incubated at 25 °C for 10mins. Then, 1% starch solution prepared in 0.02 M sodium phosphate buffer (pH 6.9) was added to incubated mixtures and further incubated for 15 min followed by the addition of dinitrosalicylic acid. To stop the reaction, the above reaction mixtures were incubated for 5 min in a water bath and the final product was measured at 540nm to calculate  $\alpha$ -amylase activity inhibiting percentage after treatment.

**Glucosidase activity inhibition assay:** *A. arabica* flower methanolic extract capability to inhibit glucosidase activity was investigated through glucosidase activity inhibition assay as specified before [Elya *et al.*, 2008]. Shortly, the  $\alpha$ -glucosidase enzyme was permitted to react with various *A. arabica* flower methanolic extract concentrations (1 mg/ml, 2mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) for 10 mins. Then, the reaction was started after adding 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) and further incubated for 60 mins. Later, 0.1 M Na<sub>2</sub>CO<sub>3</sub> solution was added to stop the reaction and the end product was read at 400nm to compute percentage of glucosidase activity inhibition after treatment with *A. arabica* flower methanolic extract.

**DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:** The ability of *A. arabica* flower methanolic extract to scavenge the free radicals was examined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay as detailed before [Gayathri, Kumar, 2016]. In brief, in a dark situation, the *A. arabica* flower methanolic extract concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) was permitted to react with DPPH solution for 30 mins. Later, the end product was used for calculating percentage of radical scavenging activity after measuring at 517nm.

$$\text{Scavenging effect} = 100 \times \frac{\text{blank OD} - \text{sample OD}}{\text{blank OD}}$$

**ABTS (2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) scavenging assay**

*A. arabica* flower methanolic extract capability for scavenging activity of ABTS (2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) was examined through ABTS scavenging assay as cited earlier [Pacifico *et al.*, 2018]. Briefly, 7 mM ABTS and 2.45 mM potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) was used to prepare ABTS solution and left for 16 h in dark environment. Then, for the assay, the ABTS solution was diluted using 0.1 M sodium phosphate buffer (pH 7.4) to reach optical density  $0.750 \pm 0.025$  at 734 nm. The diluted ABTS solution was reacted with *A. arabica* flower methanolic extract concentrations (1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) for 6 mins and the end product measured at 734 nm was used for calculating percentage of ABTS scavenging activity.

$$\text{ABTS scavenging effect (\%)} = 100 \times \frac{\text{control OD} - \text{sample OD}}{\text{blank OD}}$$

**Nitric oxide scavenging activity**

To study the ability of *A. arabica* flower methanolic extract to scavenge nitric oxide, the nitric oxide scavenging activity assay was used as mentioned before [Alam *et al.*, 2013]. Shortly, 0.5 M of phosphate buffer was used for preparing 10 mM sodium nitroprusside that added to varying concentrations (1 mg/ml, 2mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml) of *A. arabica* flower methanolic extract and incubated for 60 min. The equal volume of Griess solution (1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid was added to above reaction mixture for pink colour product formation and read at 540 nm to calculate percentage of nitric oxide scavenging activity.

$$\text{Scavenging activity} = 100 \times \frac{\text{control OD} - \text{sample OD}}{\text{blank OD}}$$

**Hepatoprotective activity:** The hepatoprotective property of *A. arabica* flower methanolic crude extract was investigated on HepG2 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as mentioned earlier [Meiyazhagan *et al.*, 2015]. In short, *A. arabica* flower methanolic crude extract varying concentrations such as 1 mg/ml, 2mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml was added to wells containing dulbecco's modified eagles medium with HepG2 cells and incubated for 24 h. The treated and untreated wells received MTT solution for formazan product formation and it was dissolved after adding dimethyl sulfoxide followed by end product measurement at 570 nm for cell viability percentage calculation.

**Statistical analysis:** The mean and standard deviations were used statistical analysis and error bars calculation for all the experiments.

## RESULTS

**$\alpha$  - amylase activity inhibition assay:** *A. arabica* flower methanolic extract was investigated for inhibiting ability of  $\alpha$  amylase activity and the obtained result is displayed in figure 1A. As shown in figure, the calculated  $\alpha$  amylase activity inhibition percentage after treatment with 1-5mg/ml of *A. arabica* flower methanolic extract as 10%, 23%, 31%, 57% and 77% proved the antidiabetic potential of *A. arabica* flower methanolic extract, indicating its antidiabetic activity.

**Glucosidase activity inhibition assay:** The *A. ara-*

bica flower methanolic extract ability was examined for glucosidase activity inhibition and the calculated glucosidase activity inhibition percentage from glucosidase activity inhibition assay is accessible in figure 1B As shown in figure, the varying concentrations like 1-5mg/ml of *A. arabica* flower methanolic extract (1 - 5 mg/ml) showed 11%, 36%, 53%, 66% and 75% of glucosidase activity inhibition respectively.

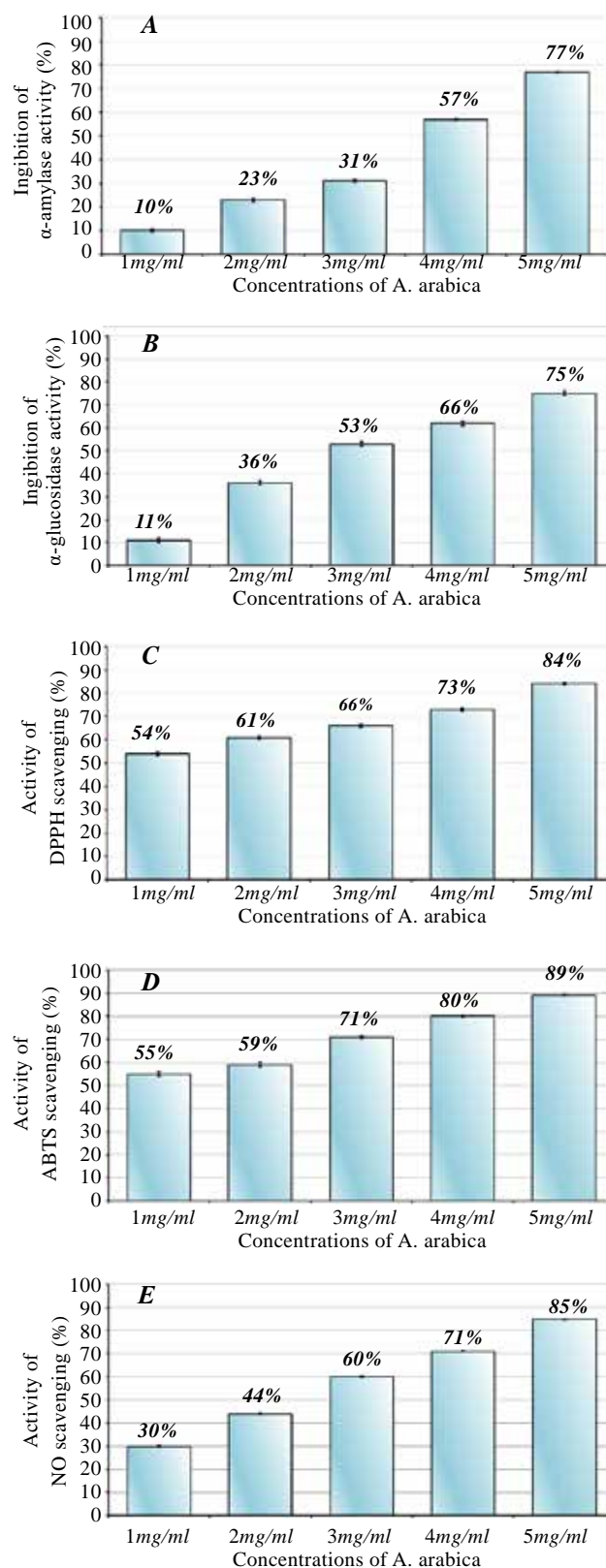
**DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay:** The DPPH free radical scavenging ability of *A. arabica* flower methanolic extract was investigated and the DPPH free radical scavenging activity quantified is presented in figure 1C As shown in figure, the graph represents the quantified radical scavenging activity which was calculated after varying concentrations such as 1-5mg/ml of *A. arabica* flower methanolic extract treatment that exhibited 54%, 61%, 66%, 73% and 84% of scavenging activity accordingly.

**ABTS scavenging assay:** ABTS scavenging activity of *A. arabica* flower methanolic extract was studied after treatment with 1-5mg/ml of methanolic extract and the scavenging activity calculated after treatment is mentioned in figure 1D The figure exhibited 55%, 59%, 71%, 80% and 89% of scavenging activity after treatment with 1-5mg/ml of *A. arabica* flower methanolic extract.

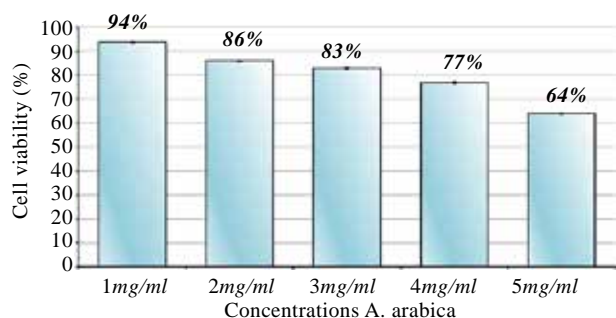
**Nitric oxide scavenging activity:** The nitric oxide scavenging activity of *A. arabica* flower methanolic extract was examined and the predicted nitric oxide scavenging activity percentage after treatment is displayed in figure 1E The figure exposed the 30%, 44%, 60%, 71% and 85% of nitric oxide scavenging activity after 1-5mg/ml of *A. arabica* flower methanolic extract treatment which proved the antioxidant property of the methanolic extract.

**Hepatoprotective activity:** *A. arabica* flower methanolic extract impact on HepG2 (hepatic cells) was monitored through MTT assay and the attained cell viability percentage is displayed in figure 2 The figure represented the hepatoprotective ability of *A. arabica* flower methanolic extract after treatment with 1-5mg/ml of concentration shows cell viability as 94%, 86%, 83%, 77% and 64% which proved the hepatoprotective property of *A. arabica*.

## DISCUSSION



**FIGURE 1:** Methanol extract of *A. arabica* flowers after treatment demonstrated potential capability. (A) inhibition of  $\alpha$ -amylase enzyme activity, (B) inhibition of glucosidase activity, (C) inhibition of free radical scavenging activity, (D) inhibition of nitric oxide scavenging activity, (E) inhibition of ABTS binding percentage.



**FIGURE 2.** Hepatoprotective cell viability after treatment with methanol extract of *A. arabica* flowers.

diabetes mellitus as well as its associated problems are affecting million people globally that creates an enormous threat to public in many countries including developed and developing countries resultant high mortality rate. However, the long term anti-diabetic drugs usage leads drug resistance and drug induced liver toxicity. Thus, to limit the drug resistance and toxicity, plant-based biomolecules which having antidiabetic and other pharmaceutical properties are gaining huge response in drug development process. Therefore, during the search of novel of biomolecules the whole plants namely leave, stem, root, flower, bark and fruit were investigated to identify the pharmacological product. Hence, our study examined the methanolic extract of *A. arabica* flower antidiabetic, antioxidant and hepatoprotective properties. Accordingly, *A. arabica* flower methanolic extract was studied for their inhibiting ability of  $\alpha$ -Glucosidase and  $\alpha$ -amylase enzymes that are primarily involved carbohydrate hydrolysis which produce the glucose and intestinal absorption thereby elevated blood glucose level in blood resulting diabetic condition. Hence, our study examined the *A. arabica* flower methanolic extract showed excellent inhibition of  $\alpha$ -Glucosidase and  $\alpha$ -amylase enzymes action which may result in late or reduced intestinal glucose absorption directed the reduction in blood glucose level. In support of this, the ethanolic extract of *A. arabica* bark was investigated for its antidiabetic properties via *in vitro* and *in vivo* studies using high fat-fed rats and showed 0.04-5 mg/ml extract was significantly amplified the insulin secretion. Later, the insulin secretion impact was again confirmed with membrane depolarization, increased intracellular  $\text{Ca}^{2+}$  level due to the existence of tannin, anthraquinone and flavonoids and this naturally present phytochemicals contribute

the promising antidiabetic activity of *A. arabica* ethanolic extract [Ansari et al., 2023]. Sameway, another group studied the herb formulation of *A. arabica* along with other extract was evaluated for their antioxidant properties using *A. arabica* and showed highest antidiabetic activity in hot water extract [Patwekar et al., 2022]. Similarly, hot water extracted *A. arabica* bark extract was examined for *in vitro* and *in vivo* insulin secretion and signal transduction and also the mechanism of action involved glucose diffusion, membrane depolarization and enhanced glucose tolerance due to the presence of kaempferol and quercetin which proved the *A. arabica* as potential agent for diabetic conditions [Ansari et al., 2021]. Another study revealed the chloroform extracted *A. arabica* bark extract studied for antidiabetic activity in alloxan induced diabetic albino rats which revealed the decreased glucose level, reverse cholesterol and triglycerides which proved the antidiabetic property of *A. arabica* bark extract [Patil et al., 2011; Rajvaidhya et al., 2012].

The diabetes mellitus was developed due to multiple risk factors which includes oxidative stress and metabolic disorders. Chiefly, the most important risk factor was oxidative stress for diabetes mellitus development leads severe difficulties that are associated with hyperglycaemia resulting inequity of oxidative stress thereby inflammation, cell survival and glucose homeostasis. Also, the oxidative stress may stimulate through various molecular and biochemical mechanisms leads high level free radicals' production and reduced the activity of antioxidant enzymes [Dave, Kalia, 2007; Rajendran et al., 2011]. Therefore, in the diabetes mellitus development, antioxidant is an important defence mechanism which acts against toxic cells by eliminating free radicals from the body [Nishikawa et al., 2000]. Thus, our study investigated the antioxidant property of methanolic *A. arabica* flower extract by various *in vitro* biochemical reactions and found the *A. arabica* flower extract promising antioxidant activity. In support of this, a methanolic fraction called AN-2 obtained from *A. arabica* flower was examined for antioxidant activities *in vitro* by deoxyribose, lipid peroxidation tests and complexing power and found the promising activities through direct free radical scavenging and hydrogen or electron

donating property [Kumar et al., 2024]. A recent reported the showed the extensive study on *Acacia* sp antioxidant property through *invitro* using DPPH and revealed the potent antioxidant activity [Amoussa et al., 2020]. Further, methanolic *A. arabica* flower extract confirmed the hepatoprotective effect on HepG2 cells to study the liver toxicity. Totally, the present study revealed the *A. arabica* flower methanolic extract effectively inhibited anti diabetic enzymes and also had the promising antioxidant property with hepatoprotective property on HepG2 cells.

### CONCLUSION

The study emphasized the methanolic *A. arabica* flower extract ability in inhibiting diabetes mellitus associated antidiabetic enzymes like  $\alpha$ -amylase and  $\alpha$ -glucosidase action and also, explored the potent antioxidant property by inhibiting free radicals. The liver toxicity of methanolic *A. arabica* flower extract showed the no toxic effect. These properties created the *A. arabica* flower extract was potent candidate for diabetes mellitus and their related complications in healthcare applications.

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