In vivo hypoglycemic, antihyperglycemic and antidyslipidemic effects of the solvent fractions of Hagenia abyssinica leaves in mice

Zemene Demelash Kifle a, Alem Endeshaw Woldeyohanin c, Faisel Dula Sema b, Simachew Gidey Debeb b, Asmamaw Emagn Kasahun c, Chilot Abiyu Demeke c, Yaschilal Muche Belayneh d, *

a Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
b Department of Clinical Pharmacy, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
c Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
d Department of Pharmacy, College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia

ARTICLE INFO

Keywords:
Antihyperglycemic
Hagenia abyssinica
Leaves
And streptozotocin

ABSTRACT

Background: Hagenia abyssinica leaves have been used traditionally for the management of different diseases including diabetes mellitus (DM) although the antidiabetic effect of different solvent fractions of hydromethanol H. abyssinica leaf extract has not been scientifically studied. Thus, this study was conducted to investigate the in vivo hypoglycemic, antihyperglycemic and antidyslipidemic effects of the solvent fractions of Hagenia abyssinica leaf extract.

Methods: The antidiabetic effect of the solvent fractions was evaluated in normal, oral glucose loaded and streptozotocin-induced diabetic mice. Hypoglycemic, antihyperglycemic, antidyslipidemic activities and effect on body weight change were evaluated after administration of three different doses of the solvent fractions (100, 200, and 400 mg/kg). One-way ANOVA followed by Tukey’s post hoc test was used for data analysis, and p < 0.05 was considered as statistically significant.

Results: The crude hydromethanol extract of H. abyssinica leaves did not show any sign of toxicity at the dose of 2000 mg/kg in mice. In normoglycemic mice, both aqueous and ethyl acetate fractions of H. abyssinica leaves showed significant (P < 0.05) hypoglycemic activity. In oral glucose loaded mice, the two doses of the aqueous fraction, 200 mg/kg (p < 0.05) and 400 mg/kg (p < 0.001), showed a significant antihyperglycemic effect at 60 and 120 minute post-oral glucose loading while the ethyl acetate fraction showed significant antihyperglycemic effect at 60 (P < 0.05 for 200 mg/kg and P < 0.001 for 400 mg/kg) and 120 min (P < 0.01 for 400 mg/kg) post-oral glucose loading. In single dose-treated diabetic mice, all doses of the solvent fractions caused a significant (P < 0.05) reduction in blood glucose level except 100 mg/kg of the aqueous and chloroform fractions. Additionally, repeated daily treatment with the aqueous fraction significantly reduced hyperglycemia, body weight loss, and improved dyslipidemia of diabetic mice.

Conclusion: This study has revealed that the solvent fractions of H. abyssinica leaves possess in vivo blood-glucose-lowering activities on normal, oral glucose loaded, and streptozotocin-induced diabetic mice. Additionally, the aqueous fraction prevented diabetic body weight loss and dyslipidemia in mice after repeated daily dose administration.

1. Background

Diabetes mellitus is a group of metabolic diseases characterized by glycosuria, negative nitrogen balance, hyperglycemia, and sometimes ketonemia resulting from defects in insulin action, insulin secretion, or both. A chronic hyperglycemic condition is linked to long-term damage and dysfunction of different organs such as eyes, heart and blood vessels, kidneys, and nerves [1,2].

Approximately 70–90% of the population in both developing and developed countries use complementary and alternative medicines for diabetes treatment. The majority of the population use traditional herbal medicines to treat various diseases including diabetes mellitus. Hagenia abyssinica is a species of flowering plant in the family Fabaceae commonly known as Abyssinian bean or Abyssinian oak. It is a large, deciduous, thorny tree native to Ethiopia, Eritrea, Djibouti, and Somalia. The plant has been used in traditional medicine for the management of different diseases including diabetes mellitus, malaria, and fever among others [3].

E-mail address: yaschilal.muche19@gmail.com (Y.M. Belayneh).

https://doi.org/10.1016/j.metop.2021.100139
Received 17 August 2021; Received in revised form 11 October 2021; Accepted 12 October 2021
Available online 14 October 2021
2589-9368/© 2021 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
their primary healthcare needs as they are comparatively safer and inexpensive [3]. Despite several clinical investigations ongoing, there are still numerous unmet needs of the public. Thus, further investigations are required for more effective and safer antihyperglycemic agents from herbal medicine. Currently, various plant species including *Ajuga remotan* [4], *Satvia rebaudianum* [5], *Calpurina aurea* [6], *Datatura stramonium* [7], *Asyga integrifolia* [8], *Aloe pulecherrima* [9], *Bersama abyssinica* [10], *Pentas schimperiana* [11], *Moringa stenopetala* [12], *Otostegia integrifolia* [13], and *Caylusea Abyssinica* [14] have been investigated and their antidiabetic effect was confirmed using animal models.

*Hagenia abyssinica* is the sole species of the genus *Hagenia* which belongs to the family *Rosaceae* [15]. *H. abyssinica* is found in Ethiopia, Kenya, Tanzania, Uganda, Sudan, Congo, Malawi, Burundi, and Rwanda [16]. The leaf part of *H. abyssinica* is traditionally used to treat different ailments in Ethiopia [17,18]. Specifically, it has been used for the treatment of DM in Ethiopia. Ethnobotanical surveys which were carried out in Ethiopia reported that the leaf of the plant is taken orally to treat hypoglycemic and anti-amylose activities of the leaves on blood glucose has not been scientifically studied.

Pharmacological investigations showed that plants that belong to the *Rosaceae* family have insulinomimetic and antidiabetic activities [22, 23]. *H. abyssinica* is one of the plant species in the family, *Rosaceae* [15], suggesting it may have an anti-diabetic property. Additionally, a previous study revealed that the crude extract and solvent fractions of *H. abyssinica* leaves have in vitro α-amylase and α-glucosidase inhibitory and antioxidant activities [24]. Similarly, the repeated doses of hydro methanole crude extract of *H. abyssinica* leaves showed significant in vivo antidiabetic activity in streptozotocin-induced diabetic mice [25].

The antidiabetic activity of medicinal plants is due to the presence of phenolic compounds (anthraquinones, C-glycosylated anthrones, 2-hydrox-3-methyl-anthraquinone, physcion), flavonoids, terpenoids, alkaloids, glycosides, steroid, peptides, lipids, and other constituents [26–28]. Fortunately, phytochemical screening of the hydro methanol leaf extract of *H. abyssinica* in a previous study revealed the presence of saponins, tannins, terpenoids, phenols, flavonoids, and steroids [29] which are known to have antidiabetic activity. Accordingly, this study was conducted to investigate the in vivo hypoglycemic, anti-hyperglycemic, and antisyndromic effects of the solvent fractions of the 80% hydro methanol extract of *Hagenia abyssinica* leaves.

2. Methods

2.1. Plant materials

The fresh leaves of *H. abyssinica* were collected from Kosoye which is 15 km far from Gondar town (located in Amhara region, northwest Ethiopia) in March 2019. The botanical identification and authentication of the plant material were performed by a botanist Mr. Abiyu Enyew, and a voucher specimen (002ZZDK/2019) was deposited in the Herbarium of Biology Department, University of Gondar.

2.2. Experimental animals

Healthy Swiss albino mice (weighing 20–28 g and 6–10 weeks of age) were purchased from Ethiopian Public Health Institute (EPHI), Addis Ababa. The animals were kept in polypropylene cages under standard conditions (12 h light & dark cycle, at room temperature) and were allowed free access to a pellet diet and water ad libitum. Animals were acclimatized to the laboratory conditions for 2 weeks before initiating the experiment. All procedures complied with The Guide for the Care and Use of Laboratory Animals, and the study was approved by the research and ethics committee of Wollo University with a Reference number of WU Phar/116/11.

2.3. Preparation of plant crude extract

The preparation of plant crude extract was carried out according to the methods described by Kifle ZD et al. [30]. The leaves of the plant were thoroughly washed with distilled water to remove dirt and then dried under shade at room temperature with optimal ventilation. The dried leaves were ground into coarse powder using an electrical mill. Then, the coarsely powdered leaves were macerated in 80% methanol for 72 h and then the extract was filtered by using Whatman filter paper No. 1. The marc was re-macerated two times with fresh hydro methanol, each for 72 h, and the filtrates obtained from the successive macerations were concentrated under reduced pressure using a rotary evaporator (Hamato, Japan) followed by a hot air oven (Medit-Medizin Technik, Germany) set at 40 °C. The semi-dried residues were then frozen in a refrigerator overnight and then dried using a lyophilizer (Labfreez, China) to completely remove the aqueous residue. The dried leaf extract was kept in a desiccator until used for the experiment.

2.4. Fractionation of the crude hydro methanol extract

Solvent fractionation of the crude leaf extract was carried out according to the methods described by Kifle ZD et al. [30], using water, ethyl acetate, and chloroform. First, 30 g of the crude 80% methanol extract was suspended in 400 ml of warm water, and the suspension was shaken in a separatory funnel by adding an appropriate amount of chloroform. Then, separation was done based on the principle of liquid-liquid extraction. The addition of the extractive solvent proceeded till the solution becomes clear. The same procedure was used to obtain an ethyl acetate fraction. The chloroform and ethyl acetate fractions were concentrated in a rotary evaporator. Finally, the aforementioned fractions were stored in a refrigerator. On the other hand, the aqueous fraction was concentrated in a lyophilizer and kept in a desiccator until used for the experiment.

2.5. Acute toxicity study

An acute oral toxicity test was carried out for the crude extract of *H. abyssinica* leaves based on the limit test recommendations of the Organization for Economic Cooperation and Development (OECD) No 425 Guideline. On the first day of the test, one female Swiss albino mouse fasted for 4 h was given 2 g/kg of the extract orally, and the mouse was observed strictly for physical or behavioral changes for one day. Since the sign of toxicity was not observed in the first mouse, other four female mice were recruited on the second day and fasted for 4 h. Then, the mice were given orally a single dose of 2 g/kg of the crude extract, and they were observed strictly in the same manner. The observation was continued for a total of 2 weeks for any sign of toxicity [30,31].

2.6. Grouping and dosing of animals

In all in vivo experimental studies (normoglycemic, oral glucose loaded, and streptozotocin-induced diabetic mice models) male mice were used [32–34]. In all cases, mice were assigned randomly into different groups of 6 mice each (n=6).

In the normoglycemic and oral glucose loaded mice models, there were a negative control group (groups I) which received distilled water (DW); a positive control group (group II) which received glibenclamide 5 mg/kg (GLC 5 mg/kg); test groups (group III to group V) which received 100 mg/kg, 200 mg/kg and 400 mg/kg of the Aqueous fraction (AQF), respectively. Likewise, test groups (group VI to group VIII) received 100 mg/kg, 200 mg/kg, and 400 mg/kg of the Ethyl acetate fraction (EAF), respectively.

In the single dose-treated diabetic animal model, there were a negative control group (groups I) that received the vehicle, distilled water (DW); a positive control group (group II) which received the
standard drug, Glibenclamide 5 mg/kg (GLC 5 mg/kg); and nine test
groups (group III-XI) among which group III, IV and V were treated with
100 mg/kg, 200 mg/kg and 400 mg/kg aqueous fraction (AQF), respectively; group VI, VII and VIII were treated with 100 mg/kg, 200
mg/kg and 400 mg/kg of Ethyl acetate fraction (EAF); group IX, X, and XI
were treated with 100 mg/kg, 200 mg/kg and 400 mg/kg of Chloro-
form fraction (CF), respectively.
In the repeated daily dose-treated diabetic mouse model, the animals
were randomly divided into six groups (five groups of diabetic mice and
1 group of normal mice). Group I (diabetic mice) was used as diabetic
control (DC) which was treated with distilled water (DW), Group II
(positive control) received a standard drug, glibenclamide 5 mg/kg
(GLC 5 mg/kg), Group III-V (test groups) received different doses of
aqueous fraction (100 mg/kg, 200 mg/kg and 400 mg/kg AQF), and
Group VI (non-diabetic mice) was used as normal control (NC) that
received distilled water (DW) [14,35].
Plant extract doses to be administered were determined based on the
result of the acute toxicity study. The volume of administration was 1
ml/100 g of body weight of the mouse [31]. The middle dose was
one-tenth of the limit dose, the higher dose was twice the middle dose,
and the lower dose was calculated as half of the middle dose. Gliben-
clamide was selected as a standard drug for the study based on earlier
studies [14]. The study was conducted using the oral route of admin-
istration because the plant leaves are traditionally used by people via the
oral route [19-21].

2.7. Evaluation of the effect of solvent fractions on blood glucose level of
normoglycemic mice
To evaluate the effect of the solvent fractions on blood glucose level
of normoglycemic mice, we followed the methods of Kifle ZD et al. [30].
Healthy normoglycemic mice were fasted overnight for 14 h but with
free access to water. Fasted mice were randomly divided into five
different groups (6 mice per group) and treated as described above.
Using aseptic conditions, blood samples were collected from the tips of
the tail of each mouse to determine blood glucose level (BGL) just before
treatment (at 0 h) as a baseline, and then at 1-, 2-, 4-, and 6-h post-
treatment.

2.8. Evaluation of the effect of solvent fractions on oral glucose tolerance
test (OGTT)
To evaluate the effect of solvent fractions on oral glucose tolerance
test, we followed the methods of Kifle ZD et al. [30]. Mice were grouped
as described above and the baseline blood glucose level was measured
just before administration of treatments. Then, 2 g/kg of glucose solu-
tion was administered orally to each mouse 30 min after extract
administration. Blood sugar level was measured for each animal at 30,
60, and 120 min following glucose administration.

2.9. Induction of experimental diabetes
In this experimental study, diabetes was induced using streptozoto-
cin (STZ). The drug was dissolved in 0.1 M citrate buffer (pH=4.5). The
solution was then administered intraperitoneally at 150 mg/kg dose to
mice which were fasted overnight for 14 h before administration. Six
hours after the administration of STZ, animals were allowed to drink 5%
glucose solution for the next 24 h. Seventy-two hours later, animals were
screened for diabetes. Mice that showed fasting blood glucose levels>
200 mg/dl were included in the study as diabetic mice [36-38].

2.10. Antidiabetic activity of a single dose of the solvent fractions in STZ-
induced diabetic mice
To assess the antidiabetic activity of a single dose of the solvent
fractions in STZ-induced diabetic mice, we followed the methods of Kifle

Z.D. Kifle et al. [30]. Following overnight fasting for 14 h, STZ-induced dia-
abetic mice were assigned randomly into 5 groups. Then, distilled water,
glibenclamide, and solvent fractions were administered to each group as
described above. Blood glucose level was measured just before treat-
ment (at 0 h) as a baseline, and then at 2, 4, 6, and 8-h post-treatment.

2.11. Antidiabetic activity and effect on body weight of repeated dose of
the solvent fraction in STZ-induced diabetic mice
To evaluate the antidiabetic activity and effect on body weight of
repeated dose of the solvent fraction in STZ-induced diabetic mice, we
followed the methods of Kifle ZD et al. [30]. In the STZ-induced diabetic
mouse model, the mice were randomly grouped into six groups. Then, the
distilled water, glibenclamide, and each solvent fraction were admin-
istered once daily for 14 days. After overnight fasting (14 h), fasting
blood glucose level and body weight of mice were measured just before
starting treatment (day 0) and then at the 7th and 14th day of treatment
[12,39].

2.12. Effect of the solvent fraction on serum lipid level of diabetic mice
To assess the effect of the solvent fraction on serum lipid level of
diabetic mice, we followed the methods of Kifle ZD et al. [30]. On day
15, using a sterile tube blood samples were collected through cardiac
puncture under halothane anesthesia from the overnight fasted (14 h)
diabetic mice. The blood samples were kept at room temperature for
about 2 h and then centrifuged. Then, the supernatant was immediately
separated from the pellet to prepare serum samples to determine the
serum level of total cholesterol, triglyceride, low-density lipoprotein,
and high-density lipoprotein.

2.13. Statistical analysis
Statistical analysis was performed by using a statistical package for
social sciences (SPSS) version 24 software. Between and within-group
analyses were carried out using one-way ANOVA, followed by Tukey’s
multiple comparison tests. P values < 0.05 were considered statistically
significant.

3. Result
3.1. The percentage yield of plant material extraction
A total of 153 (14.6% w/w) grams of dried hydromethanol crude
extract of H. abyssinica leaf was harvested at the end of the extraction
process. The yields of the fractions were 47.8% w/w (73.1 g), 29.8% w/
w (45.6 g), and 17.5% w/w (26.8 g), for the aqueous, ethyl acetate, and
chloroform fractions, respectively.

3.2. Acute toxicity test
The leaf crude extract of H. abyssinica did not show any sign of
toxicity at the dose of 2 g/kg in mice. Thus, the median lethal dose
(LD50) of the extract is greater than 2 g/kg.

3.3. Hypoglycemic activity of the solvent fractions in normoglycemic mice
After administering the solvent fractions and glibenclamide to the
mice, the BGL was measured just before treatment (at 0 h) as a baseline,
and then at 1-, 2-, 4-, and 6-h post-treatment. Based on the findings
designated in Table 1, there was a significant reduction in BGL at the 1st
hour post administration of the standard drug (P < 0.001) and the higher
doses of the solvent fractions (AQF 400 mg/kg, P < 0.05 and EAF 400
mg/kg, P < 0.05) when compared to the negative control. At 2 h, AQF
400 mg/kg (P < 0.05), EAF 200 mg/kg (P < 0.05), EAF 400 mg/kg
(P < 0.01), and GLC 5 mg/kg (P < 0.001) showed significant BGL
Table 1
Hypoglycemic activity of the solvent fractions in normoglycemic mice.

| Group            | Blood glucose level (mg/dl) | 0 h   | 1 h   | 2 h   | 4 h   | 6 h   |
|------------------|-----------------------------|-------|-------|-------|-------|-------|
| DW 10 mg/kg      |                             | 117.00| 118.83| 115.17| 117.83| 114.67|
| GLC 5 mg/kg      |                             | 112.83| 81.17 | 64.83 | 59.00 | 52.33 |
| AQF 100 mg/kg    |                             | 107.83| 108.67| 99.67 | 96.50 | 87.00 |
| EAF 400 mg/kg    |                             | 116.00| 99.33 | 92.83 | 83.17 | 79.33 |
| EAF 200 mg/kg    |                             | 114.67| 110.83| 105.50| 97.50 | 90.83 |
| EAF 100 mg/kg    |                             | 114.50| 115.17| 93.00 | 86.17 | 81.17 |
| DW 10 mg/kg      |                             | 110.17| 98.83 | 89.17 | 81.50 | 75.67 |

Each value represents mean ± SEM; n = 6 for each treatment. *compared to the negative control, and † compared to baseline blood glucose level, *compared to GLCS. **<0.05, ***<0.01, and ****<0.001. AQF = aqueous fraction, EAF = ethyl acetate fraction, DW = distilled water, and GLC = glibenclamide.

reduction compared to the negative control. A statistical significant BGL reduction was observed at 4 h post-administration of AQF 100 mg/kg (P<0.05), AQF 200 mg/kg (P<0.01), AQF 400 mg/kg (P<0.001), EAF 200 mg/kg (P<0.001), EAF 400 mg/kg (P<0.001) and GLC 5 mg/kg (P<0.001) when compared to the negative control. Likewise, at 6 h, AQF 100 mg/kg (P<0.01), AQF 200 mg/kg (P<0.001), AQF 400 mg/kg (P<0.001), EAF 100 mg/kg (P<0.05), EAF 200 mg/kg (P<0.001), EAF 400 mg/kg (P<0.001), and GLC 5 mg/kg (P<0.001) showed significant BGL reduction compared to the negative control group. The largest dose of both solvent fractions (400 mg/kg) showed the fastest hypoglycemic activity post-treatment, but the lowest dose of the fractions showed delayed action (Table 1).

3.4. Antihyperglycemic activity of the solvent fractions on oral glucose loaded mice

Following oral glucose loading (2 g/kg) to control and test groups, the BGL was raised to the maximum level at 30 min. At 30 min post-oral glucose loading, only the standard drug (GLC, 5 mg/kg) treated group showed a significant (p<0.001) glucose tolerance compared to the negative control group. The two doses of the aqueous fraction, AQF 200 mg/kg (P<0.05) and AQF 400 mg/kg (p<0.001), showed a significant antihyperglycemic effect at 60 and 120 min post-oral glucose loading. Similarly, the ethyl acetate fraction showed a significant antihyperglycemic effect at 60 min (P<0.05 for EAQ 200 mg/kg and P<0.001 for EAQ 400 mg/kg) and at 120 min (P<0.01 for EAQ 400 mg/kg) post-oral glucose loading. Likewise, GLC 5 mg/kg showed a significant (p<0.001) reduction of BGL at 60- and 120-min post-administration when compared to the negative control group (Fig. 1, Table 2).

3.5. Antihyperglycemic activity of the single dose of the solvent fractions of Hagenia abyssinica leaves on STZ-induced diabetic mice

The effects of H. abyssinica solvent fractions on BGL of STZ-induced diabetic mice are shown in Table 3. Between and within-group analyses were performed to see BGL differences across the various groups and time points, respectively. The between-group analysis indicated no significant disparity in baseline fasting BGL across all groups. Likewise, there was no significant disparity in BGL at all time points when groups treated with plant extract were compared to each other.

The standard drug (GLC 5 mg/kg) produced a significant BGL reduction at the 4th (p<0.05), 6th (p<0.01), and 8th (p<0.001) hours compared to the negative control. Similarly, significant reduction in BGL was observed with AQF200 at the 6th (p<0.05), and 8th (p<0.001)
Table 2

Effect of the solvent fractions on blood glucose level of oral glucose loaded mice.

| Group          | Blood glucose level (mg/dl) | 0hr  | 2hr  | 4hr  | 6hr  | 8hr  |
|----------------|----------------------------|------|------|------|------|------|
|                | mg/kg                      |      |      |      |      |      |
| GLC 5 mg/kg    | 93.3 ± 14.93 ± 70.83 ± 61.50 |      |      |      |      |      |
| AQP 100        | 84.17 ± 183.67 ± 213.81 ± 65.87 |      |      |      |      |      |
| AQP 200        | 89.23 ± 174.17 ± 123.00 ± 79.50 |      |      |      |      |      |
| mg/kg          | 4.01 ± 8.64 ± 5.33 ± 8.34 |      |      |      |      |      |
| mg/kg          | 5.01 ± 10.65 ± 5.33 ± 7.54 |      |      |      |      |      |
| mg/kg          | 4.36 ± 5.33 ± 5.33 ± 11.36 |      |      |      |      |      |
| mg/kg          | 86.67 ± 170.50 ± 76.8 ± 86.67 |      |      |      |      |      |
| mg/kg          | 11.25 ± 16.54 ± 5.33 ± 11.25 |      |      |      |      |      |
| EAF 100        | 82.17 ± 181.00 ± 139.83 ± 86.64 |      |      |      |      |      |
| mg/kg          | 2.78 ± 12.65 ± 5.33 | 5.67 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 87.33 ± 177.67 ± 126.67 ± 85.83 | 5.67 | 5.33 | 11.36 | 5.33 | 5.33 |
| EAF 200        | 83.83 ± 166.67 ± 8.64 | 11.01 | 11.01 | 7.77 | 11.01 | 11.01 |
| mg/kg          | 1.91 ± 5.33 | 5.33 | 5.33 | 11.36 | 5.33 | 5.33 |

Each value represents mean ± SEM; n = 6 for each treatment. *compared to the negative control, (p < 0.05); †compared to baseline blood glucose level, (p < 0.01); ‡p < 0.001; and βp < 0.001. AQP = aqueous fraction, EAF = ethyl acetate fraction, DW = distilled water, and GLC = glibenclamide.

Table 3

Anthihyperglycemic effect of the single dose of the solvent fractions on STZ-induced diabetic mice.

| Group          | Blood glucose level (mg/dl) | 0hr  | 2hr  | 4hr  | 6hr  | 8hr  |
|----------------|----------------------------|------|------|------|------|------|
|                | mg/kg                      |      |      |      |      |      |
| GLC 5 mg/kg    | 320.33 ± 318.00 ± 262.67 ± 327.33 ± 322.67 |      |      |      |      |      |
| AQP 100        | 343.83 ± 268.00 ± 205.00 ± 180.33 ± 160.00 |      |      |      |      |      |
| AQP 200        | 290.67 ± 292.33 ± 282.17 ± 268.83 ± 262.67 |      |      |      |      |      |
| mg/kg          | 6.94 ± 5.54 ± 6.54 ± 9.34 | 8.65 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 11.25 ± 16.54 ± 5.33 ± 11.25 | 8.65 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 2.78 ± 12.65 ± 5.33 | 5.67 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 250.17 ± 255.33 ± 245.50 ± 231.17 ± 233.18 | 81.30 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 8.87 ± 13.54 ± 14.25 | 9.58 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 307.83 ± 287.17 ± 273.50 ± 268.00 ± 262.67 | 81.30 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 10.24 ± 16.54 ± 14.56 | 15.21 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 325.00 ± 280.33 ± 267.50 ± 254.83 ± 238.17 | 81.30 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 8.87 ± 13.54 ± 14.25 | 9.58 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 250.17 ± 255.33 ± 245.50 ± 231.17 ± 233.18 | 81.30 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 8.87 ± 13.54 ± 14.25 | 9.58 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 325.00 ± 280.33 ± 267.50 ± 254.83 ± 238.17 | 81.30 | 5.33 | 11.36 | 5.33 | 5.33 |
| mg/kg          | 8.87 ± 13.54 ± 14.25 | 9.58 | 5.33 | 11.36 | 5.33 | 5.33 |

Each value represents mean ± SEM; n = 6 for each treatment. *compared to the negative control, (p < 0.05); †compared to baseline blood glucose level. (p < 0.01); ‡p < 0.001, and βp < 0.001. AQP = aqueous fraction, EAF = ethyl acetate fraction, DW = distilled water, and GLC = glibenclamide.

Table 4

Antihyperglycemic activity of repeated daily doses of the aqueous fraction in diabetic mice

| Group          | Baseline | 7th day | 14th day | % reduction |
|----------------|----------|---------|----------|-------------|
|                | Fasting blood glucose level (mg/dl) | 320.33 | 324.83 | 327.17 | 5.67 | -1.40 | -2.13 |
|                | mg/kg    | 16.52 | 16.25 | 16.52 | 10.29 | 10.29 | 20.21 |
| AQP 100        | 309.67 | 257.67 | 246.50 | 16.79 | 20.39 |
| mg/kg          | 21.25 | 8.52 | 9.54 | 14.25 | 14.25 | 28.92 | 31.64 |
| AQP 200        | 250.33 | 231.00 | 222.17 | 30.75 | 28.92 | 40.58 | 44.10 |
| mg/kg          | 14.59 | 14.25 | 14.25 | 14.25 | 14.25 | 28.92 | 31.64 |
| AQP 400        | 298.17 | 177.17 | 166.67 | 40.58 | 44.10 |
| mg/kg          | 10.26 | 10.26 | 10.26 | 10.26 | 10.26 | 28.92 | 31.64 |
| NC             | 91.34 | 89.98 | 90.78 | 1.49 | 0.61 |

Each value represents mean ± SEM; n = 6 for each group. *compared to the diabetic control, †compared to the normal control, and β compared to baseline blood glucose level. (p < 0.05), †(p < 0.01), and ‡p < 0.001. AQP = aqueous fraction, GLC = glibenclamide, DC = Diabetic control, NC = Normal control.
aqueous fraction significantly reduced serum level of total cholesterol while significantly increasing the high-density lipoprotein-cholesterol (P < 0.01) (Table 6).

Results are expressed in mean ± S.E.M, n = 6; compared to the diabetic control, *compared to the normal control; 1p < 0.05, 2p < 0.01, and 3p < 0.001; AQF = aqueous fraction, GLC = glibenclamide, DC = diabetic control; NC = normal control.

3.8. Effect of repeated daily doses of the aqueous fraction on serum lipid level of diabetic mice

The two doses of the aqueous fraction (AQF100 mg/kg, P < 0.05 and AQF 200 mg/kg, P < 0.01) significantly reduced LDL-cholesterol after daily treatment for 14 days. Additionally, AQF 400 mg/kg of the aqueous fraction significantly reduced serum level of total cholesterol (P < 0.001), triglyceride (P < 0.01), very low-density lipoprotein-cholesterol (P < 0.05), and low-density lipoprotein-cholesterol (P < 0.001) while significantly increasing the high-density lipoprotein-cholesterol (P < 0.01) (Table 6).

Table 5

| Group     | Before induction of DM (g) | Baseline (g) | 7th day of treatment (g) | 14th day of treatment (g) |
|-----------|---------------------------|--------------|-------------------------|--------------------------|
| DC        | 26.55 ± 0.68              | 27.07 ± 0.67 | 25.58 ± 1.04           | 24.08 ± 0.94             |
| GLC 5 mg/kg | 27.77 ± 1.02              | 26.70 ± 0.57 | 28.97 ± 0.60           | 29.46 ± 0.67             |
| AQF 100 mg/kg | 29.63 ± 0.67              | 28.15 ± 0.57 | 28.44 ± 0.54           | 29.18 ± 0.34             |
| AQF 200 mg/kg | 28.89 ± 0.57              | 27.39 ± 0.57 | 28.39 ± 0.67           | 29.35 ± 0.67             |
| AQF 400 mg/kg | 31.88 ± 0.77              | 29.12 ± 0.84 | 31.51 ± 0.33           | 31.94 ± 1.21             |
| NC        | 27.01 ± 1.21              | 26.94 ± 0.37 | 26.67 ± 0.47           | 28.93 ± 0.62             |

The result presented as mean ± SEM; n = 6 for each group. *compared to the diabetic control, **compared to the normal control, and # compared to baseline bodyweight. 1p < 0.05, 2p < 0.01, and 3p < 0.001; AQF = aqueous fraction, GLC = glibenclamide, DC = diabetic control, NC = normal control.

4. Discussion

In the acute oral toxicity study of the 80% methanolic crude leaf extract of *H. abyssinica*, there was no mortality or any signs of behavioral changes or toxicity observed after oral administration of the extract at the dose level of 2000 mg/kg in mice. This indicates that the plant has a good safety profile.

Evaluation of the antidiabetic effect of *H. abyssinica* was done on streptozotocin-induced diabetic mice, which is similar to human DM [40,41]. N-methyl nitro carbamoyl-o-glucosamine popularly known as streptozotocin is a well-known diabetogenic agent for evaluating the antidiabetic activities of medicinal plants in different animal models [42,43]. This compound is a potent methylating agent for DNA and acts as a nitric oxide donor in pancreatic cells. Pancreatic cells are particularly sensitive to damage by nitric oxide (via inhibition of aconitase activity) and free radicals because of their low levels of free radical scavenging enzymes [44]. Basically, STZ induces diabetes by destroying the insulin-secreting pancreatic β-cells, resulting in a decreased insulin secretion. In accordance, findings from the current investigation showed that STZ administration (i.p) at 150 mg/kg effectively induced diabetes mellitus in physiologically normal mice as reflected by hyperglycemia. Repeated daily dose administration of all doses of the aqueous fraction showed a significant (P < 0.001) antihyperglycemic effect at the 7th and 14th day of treatment. The largest % reduction in blood glucose level (40.58% on the 7th day and 44.10% on the 14th day) was induced by 400 mg/kg of the aqueous fraction. In the single-dose STZ-induced diabetic mice model, the solvent fractions of *H. abyssinica* revealed significant glucose-lowering activity when compared to the negative control.
control group. This finding is in agreement with previous similar studies [2,9,37]. Previous findings revealed that GLC possesses hypoglycemic activity in normal mice through stimulation of pancreatic β-cells to release insulin [45]. It has also been stated that the leaf solvent fraction of H. abyssinica contains terpenes, steroidal compounds, flavonoids, phenolic compounds alkaloids, tannins, and saponins [46], and these phytoconstituents were reported to stimulate insulin release from pancreatic β-cells [47]. Thus, the antihyperglycemic effect of the solvent fraction of H. abyssinica may be due to the availability of any of these secondary metabolites that act synergistically or individually to either mimic insulin action or stimulate insulin secretion.

The oral glucose tolerance test model was employed to evaluate the effect of solvent fractions on glucose metabolism [48–50]. It is reported that a high level of glucose in blood leads to insulin release, and insulin reduces the blood glucose level to normal level in two to 3 h [45,49]. Following glucose loading to all groups of mice, the BGL was increased to the maximum level at 30 minute. The two doses of the aqueous fraction, AQF 200 mg/kg (p<0.05) and AQF 400 mg/kg (p<0.001), showed a significant antihyperglycemic effect at 60 and 120 min post-oral glucose loading. Similarly, the ethyl acetate fraction showed a significant antihyperglycemic effect at 60 and 120 min post-oral glucose loading. These findings showed that a high level of glucose in blood leads to insulin release, and insulin reduces the blood glucose level to normal level in two to 3 h [45,49].

Following glucose loading to all groups of mice, the BGL was increased to the maximum level at 30 minute. The two doses of the aqueous fraction, AQF 200 mg/kg (p<0.05) and AQF 400 mg/kg (p<0.001), showed a significant antihyperglycemic effect at 60 and 120 min post-oral glucose loading. Similarly, the ethyl acetate fraction showed a significant antihyperglycemic effect at 60 min (P<0.05 for EAQ 200 mg/kg and P<0.001 for EAQ 400 mg/kg) and at 120 min (P<0.01 for EAQ 400 mg/kg) post-oral glucose loading. These findings showed that the improvement in glucose tolerance by the solvent fractions may be because of insulin-sensitizing effect of H. abyssinica possibly through peroxisome proliferator-activated receptor-gamma activation or simulation of β-cells of the pancreas to release insulin leading to increased glucose utilization by peripheral tissues [50,51]. The mechanism involved in decreased postprandial hyperglycemia is inhibition of enzymes like α-amylase and α-glycosidase in the gastrointestinal system which avoids postprandial hyperglycemia [52,53]. In a previous study, the crude extract of H. abyssinica leaves showed significant in vitro α-glycosidase and α-amylase inhibitory activities [24]. The α-glucosidase and α-amylase inhibitors control BGL particularly postprandial BGL by delaying the gastric emptying time and the absorption of fructose and glucose in the gastrointestinal tract [54]. Furthermore, decreasing the rate of starch metabolism may endorse weight loss through reduced accessibility of carbohydrate-derived calories [55]. The other possible mechanism of antidiabetic activity of H. abyssinica on the oral glucose tolerance test may be secondary to the availability of phytoconstituents like flavonoids [56], because flavonoids are known to regenerate glycogenesis in the liver, and/or increasing glucose utilization by the body [67]. Additionally, phytochemicals are known to induce regeneration of the damaged beta cells and inhibit oxidative stress in beta cells of experimental diabetic rats [68].

This study didn’t identify the exact molecular mechanism of antidiabetic activity of the solvent fractions as limitation. Additionally, this study didn’t isolate and identify the exact secondary metabolites responsible for the antidiabetic activity.

5. Conclusions

The findings of the present study confirmed that the solvent fractions of Hagenia abyssinica leaves possess antidiabetic activity in normoglycemic, oral glucose loaded and STZ-induced diabetic mice. Additionally, the solvent fractions induced improvement in diabetes associated body weight change and serum lipid profile. Thus, this study validates the traditional use of Hagenia abyssinica in the management of DM.

CRediT authorship contribution statement

Zemene Demelash Kifle: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Roles/Writing – original draft, Writing – review & editing.
Alem Endeshaw Woldeyohanin: Formal analysis, Funding acquisition, Investigation.
Faisel Dula Sema: Methodology, Project administration, Resources.
Simachew Gidey Deben: Software, Supervision, Validation, Visualization, Writing – original draft, Roles/ Writing – original draft, Writing – review & editing.
Asmamaw Emagn Kasahun: Software, Supervision, Validation, Visualization, Writing – original draft, Roles/ Writing – original draft, Writing – review & editing.
Chilot Abiyu Demeke: Methodology, Project administration, Resources.
Yaschilal Muche Belayneh: Conceptualization, Data curation.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgment

The authors would like to thank Wollo University for allowing us to use the laboratory facility for the experimental works of the study.

List of abbreviations

BGL Blood Glucose Level
DNSA 3, 5-Dinitrosalicylic Acid
DM Diabetes Mellitus
DPPH 2, 2-Diphenyl-1-Picrylhydrazyl
OECD Organization for Economic Cooperation and Development
OGTT Oral Glucose Tolerance Test
STZ Streptozotocin

Declarations

Ethics Approval: Ethical clearance was obtained from the research and ethics committee of the department of pharmacy, Wollo University with a
Reference number of WU Phar/116/11 to conduct the experiment.

Availability of data and materials

The data used to support the findings of this study are included in the article.

Funding

No funding was received.

References

[1] Holman N, Young B, Gaddy R. Current prevalence of Type 1 and Type 2 diabetes in adults and children in the UK. Diabet Med: a journal of the British Diabetic Association 2015;32(9):1119–20.
[2] Kille ZD, Enyew EF. Evaluation of in vivo anti diabetic, in vitro α-amylase inhibitory, and in vitro antioxidant activity of leaves crude extract and solvent fractions of bersama abyssinica frezen (melanthaceae). Journal of Evidence-Based Integrative Medicine 2020;25. 2515690X20935827.
[3] Robinson MM, Zhang X. Traditional medicines: global situation, issues and challenges, vol. 3. The world medicines situation; 2011.
[4] Assels F, Seifi D, Makonnen E. Antihyperglycemic and antihyperlipidemic effects of ethanol extract of Ajuga remota Benth (Harmeguesa) leaves in streptozotocin induced diabetic rats. African Journal of Pharmacy and Pharmacology 2017;11(2):17–24.
[5] Bekele T, Hymeke A, Tadesse M, Makonnen Y. Antidiabetic activity and phytochemical screening of crude extracts of Stevia rebaudiana Bertoni and Ajuga remota Benth grown in Ethiopia on alloxan-induced diabetic mice. Department of Pharmaceutical Chemistry, School of Pharmacy, Addis Ababa University April, 2008.
[6] Belayneh YM, Birru EM. Antidiabetic activities of hydroethanolic Leaf Extract of Calpurnia aurea (Ait.) Benth. Subspecies aurea (Fabaceae) in mice. Evidence-Based Complementary and Alternative Medicine2020;2020.
[7] Melaku BC, Amare GG. Evaluation of antidiabetic and antioxidant potential of hydroethanolic seed extract of Datura stramonium linn (solancaceae). J Exp Pharmacol 2020;12:181.
[8] Elene M, Abdelwahab M, Belay A, Yaze TS. Evaluation of antidiabetic activity of Ajuga integrifolia (Lamiaceae) Root Extract and Solvent Fractions in Mice. Evidence-Based Complementary and Alternative Medicine2020;2020.
[9] Amare GG, Mehari BG, Belayneh YM. Evaluation of antidiabetic activity of the Leaf Latex of Aloe pulcherrima Gilbert and Sebsebe (Aloaceae). Evidence-Based Complementary and Alternative Medicine2020;2020.
[10] Kille ZD, Anteneh DA, Amaife SA. Hypoglycemic, anti-hyperglycemic and anti-hyperlipidemic effects of bersama abyssinica frezen (melanthaceae) leaves’ solvent fractions in normoglycemic and streptozotocin-induced diabetic mice. J Exp Pharmacol 2020;12:385.
[11] Dinka T, Tadesse S, Ares K. Antidiabetic activity of the leafextracts of Pentas schimperiana subsp. schimperiana (A. Rich) Vatke on alloxaninduced diabetic mice. Ethiop Pharm J 2010;28:22–6.
[12] Toma A, Makonnen E, Mekonnen Y, Debella A, Adisakwattana S. Antidiabetic activities of aqueous ethanolic and n-butanol fraction of Moringa stenopetala leaves in streptozotocin-induced diabetic rats. BMC Complimentary Alternative Med 2015;15(1):19.
[13] Tamiru W, Engidawork E, Asres K. Evaluation of the hypoglycemic and antihyperlipidemic activity of aqueous extract of Justicia Schimperiana leaves in normal and streptozotocin induced diabetic mice. Int J Pharmaceut Sci Res 2016;7(2):107–13.
[14] Baquer NZ, Kumar P, Taha A, Kale R, Cowisk S, McLean P. Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissue. J Biomed Biotech 2011;2011:368–96.
[15] Belayneh YM, Birru EM, Ambakar D. Evaluation of hypoglycemic, antihyperglycemic and antihyperlipidemic activities of 80% methanolic seed extract of Calpurnia aurea (Ait.) Benth.(Fabaceae) in mice. J Exp Pharmacol 2019;11:73.
[16] Belayneh YM, Birru EM. Antidiabetic Activities of Hydroethanolic Leaf Extract of Calpurnia aurea (Ait.) Benth. Subspecies aurea (Fabaceae) in mice, 9. Evidence-Based Complementary and Alternative Medicine2018;2018.
[17] Sharma S, Choudhary M, Bhardwaj S, Choudhary N, Rana AC. Hypoglycemic potential of alcoholic root extract of Cassia occidentalis Linn. in streptozotocin induced diabetes in albino mice. Bull Fac Pharm Cairo Univ 2014;52(2):211–7.
[18] Adisa RA, Choudhary M, Choudhary NS. Hypoglycemic activity of Buchholzia coriaceae (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. Exp Toxicol Pathol 2011;63(7–8):619–25.
[19] Hammes WW, Emori YK, Ayalew Getahun K, Kahaliiw W. Antidiabetic and antihyperlipidemic activities of the leaf latex extract of Aloe malagacantha baker (Aloaceae) in streptozotocin-induced diabetic model. Evidence-Based Complementary and Alternative Medicine2019; 2019.
[20] Dimo T, Rakotontsinina SV, Tan PV, Azay J, Dongo E, Kamtoching P, et al. Effect of Scruccaria hixarea (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. J Ethnopharmacol 2007;110(3):434–8.
[21] Oliveira HC, dos Santos MP, Grigulo R, Lima JC, et al. Antidiabetic activity of Vatairea macrocarpa extract in rats. J Ethnopharmacol 2008;115(3):515–9.
[22] Spinosa RA. The dual role of nitric oxide in islet β-cells. Physiology 1999;14(2): 49–54.
[23] Sabir M, Ziyyat A, Mekhfi H, Tahiri A, Legssyer A. Medicinal plants with potential antidiabetic activity. A review of ten years of herbal medicine research (1990–2000). Int J Diabetes Metab 2006;14(1):1.
[24] Patel D, Prasad S, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of tropical biomedicine 2012;2(4):520.
[25] Kille ZD, Debeg S, Belayneh YM. Vito α-amylase e α-Glucosidase Inhibitory and antioxidant activities of the Crude Extract and Solvent Fractions of Hagenia abyssinica Leaves. BioMed Research International2021; 2021.
[26] Bekele T, Awas T, Wilkin P, Weber O, Bachman S, et al. Four new species of Aloe (Aloaceae) from Ethiopia, with notes on the ethics of describing new taxa from foreign countries. Kew Bull 2011;66(1):111–21.
[27] Grover J, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol 2008;118(1):105.
[28] Vuksan V, Sievenpiper JL. Herbal remedies in the management of diabetes: lessons learned from the study of ginseng. Nutr Metab Cardiovasc Dis 2005;15(3):149–60.
[29] Wolde S, Bizayehu B, Hallemarian T, Tirhaha K. Phytochemical analysis and Antimicrobial activity of HAGENIA ABBYSSINICA. Indian J Pharm Pharmacol 2016; 3(3):127–34.
[30] Toma A, Anderson J, Armstrong A, Gastineau D, Hiddinga H, Jahangir A, et al. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. Lab Anim 2011;45(3):131–40.
[31] Kifle ZD, Yousef JS, Amaife SA. Evaluation of in vitro and in vivo anti diabetic, anti hyperlipidemic and anti-oxidant activity of flower crude extract and solvent fractions of hagenia abyssinica (rosaceae). J Exp Pharmacol 2020;12:151.
[32] Zayed Y, Taha M, Kasim M, Taha S. Pomegranate peel extract: synergistic effect on streptozotocin-diabetic rats. J Exp Pharmacol 2018;18(1):85.
[33] Demirem E, Zorlu A, Yulan Y, Kilic I, Arslan A, et al. Hypoglycemic activity of aqueous extract of Justicia Schimperiana leaves in normal and streptozotocin induced diabetic mice. Int J Pharmaceut Sci Res 2016;7(2):107–10.
streptozotocin-induced diabetic rats. European Journal of Integrative Medicine 2015;7(3):263–73.

51. Chávez-Silva F, Cerón-Romero I, Arias-Durán I, Navarrete-Vázquez G, Almanza-Pérez J, Román-Ramos R, et al. Antidiabetic effect of Achillea millefolium through multitarget interactions: α-glucosidases inhibition, insulin sensitization and insulin secretagogue activities. J Ethnopharmacol 2018;212:1–7.

52. Tekulu GH, Araya EM, Mengreha HG. In vitro α-amylase inhibitory effect of TLC isolates of Aloe megalacantha baker and Aloe monticola Reynolds. BMC Compl Alternative Med 2019;19(1):1–7.

53. Aloulou A, Hamden K, Eloussi D, Ali MB, Hargui K, Jaouadi B, et al. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. BMC Compl Alternative Med 2012;12(1):1–9.

54. He K, Shi J-C, Mao X-M. Safety and efficacy of acarbose in the treatment of diabetes in Chinese patients. Therapeut Clin Risk Manag 2014;10:505.

55. Celleno L, Tolaini MV, D’Amore A, Perricone NV, Preuss HG. A dietary supplement containing standardized Phaseolus vulgaris extract influences body composition of overweight men and women. Int J Med Sci 2007;4(1):45.

56. Beshir K, Shibeshi W, Ejigu A, Engidawork E. In-vivo wound healing activity of 70% ethanol leaf extract of Beciumgrandiflorum Lam.(Lamiaceae) in mice. Ethiopian PharmaceuticaJournal 2016;32:117–30.

57. Jadhav R, Puchchakayala G. Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. Group 2012;1:100g.

58. Ramkumar KM, Vanitha P, Uma C, Suganya N, Bhakkiyalakshmi E, Sujatha J. Antidiabetic activity of alcoholic stem extract of Gymnema montanum in streptozotocin-induced diabetic rats. Food Chem Toxicol 2011;49(12):3390–4.

59. Tanko AG J, Mohammed ADTA, Musa KY. Hypoglycaemic effects of the methanolic extract of aerial part of Chrysanthemum indicum. J Nat Prod Plant Resour 2011;1:1–7.

60. Abou-Seif MA, Youssef A-A. Evaluation of some biochemical changes in diabetic patients. Clin Chim Acta 2004;346(2):161–70.

61. Elsani A, Ahmed A. Patterns of lipid changes among type 2 diabetes patients in Sudan. EMIJ-Eastern Mediterranean Health Journal 2008;14(2):314–24. 2008.

62. Ikonen E. Mechanisms for cellular cholesterol transport: defects and human disease. Physiol Rev 2006;86(4):1237–61.

63. Sharma B, Salunke R, Balamajumder C, Daniel S, Roy P. Anti-diabetic potential of alkaloid rich fraction from Capparis decidua on diabetic mice. J Ethnopharmacol 2010;127(2):457–62.

64. Oliver-Bever B. Medicinal plants in tropical West Africa. Cambridge university press; 1986.

65. Ivoor M, Paya M, Villar A. A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol 1989;27(3):243–75.

66. Kameswara Rao BGR, Kesavulu MM, Appa Rao C. Herbal medicine: in the management of diabetes mellitus. Manphar Vaidhya Patrika 1997;1(4):33–5.

67. Sezik E, Aslan M, Yenilada E, Ito S. Hypoglycemic activity of Gentiana olivieri and isolation of the active constituent through bioassay-directed fractionation techniques. J Ethnopharmacol 2007;103(3):333–8.

68. Alexandru V, Balan M, Gaspar A, Coroiu V. Antioxidant activity, phenolics and flavonoid content of some selected Romanian medicinal plants. Planta Med 2007;73(9):P.261.