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Study of Antioxidant and Anti Hyperglycemia of some Active Chemicals Compounds Isolated for Iraqi Arnebia hispidissima medicinal plant

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ABSTRACT --*Arnebia hispidissima is a medicinal plant belonging to family boraginaceae, which has Anti-inflammatory, Antibacterial, wound-healing and the Antifungal properties. General extracts were prepared from Iraqi Arnebia hispidissima . They are hot aqueous , cold aqueous and ethylacetate extracts. Moreover, preliminary qualitative tests were carried out for all extracts prepared, qualitative analysis the phytochemical arnebia hispidissima extracts showed the presence of glycosides, terpenoids, saponin, alkaloids, phenols, carbohydrates, flavonoids and tannins, than had estimated total quantitative of phenol and flavonoids, where the highest yield of phenol (0.0175mg/ml) in the cold aqueous extract .While the results showed the highest yield of flavonoids(0.1037mg/ml) in the hot aqueous extract And in light of testing antioxidant effectiveness by inhibiting the DPPH scavenging activity, Reducing power and Fe3+ Reducing, it showed that the studied plant exhibits an antioxidant activity with different levels in all plant extracts. The highest DPPH scavenging activity , Fe3+ Reducing and Reducing power activity were found in hot aqueous extract of A. hispidissima at a concentration of (100µg/ml) with a (91.22%) inhibition ratio. the highest ferric reducing power (75.11mg/ml) at concentration(5 mg/ml) and highest chelating activity of ferrous ion at Concentration (5mg/ml) was (79.15mg/ml) Moreover, study of hypoglycemic action in blood glucose levels in alloxan induced diabetic mice to know activity these extracts in decreasing blood glucose levels in these mice . the results indicated that all extracts showed good decreasing in glucose level. Hot aqueous extract from A. hispidissima showed high activity to decrease blood glucose than others extract ,where significant decreasing (P<0.05) was found at second and sixth hrs. and high significant decreasing (P<0.05) at twenty four hrs. The present study concluded that A. hispidissima extracts have antioxidant and antihyperglycemic activity as it lower blood-glucose level in diabetic mice.*

Key words-- *Antioxidant , Hypoglycemic, Medicinal plant , Arnebia hispidissima, DPPH*

I. INTRODUCTION

Medicinal plant is defined as a plant that contains one or more of its chemical substances, in low or high concentration, which has the ability to treat a particular disease or at least it reduces the symptoms of infection. And it has their values in the substances present in various plant tissues with specific physiological action in human body and are still widely used as good sources for the present modern drugs.1,2 carbohydrates , alkaloids ,flavonoids, tannins, phenols ,glycosides, terpenoids, Steroids and saponin is phytochemical compounds which can

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be extracted from plants as pure compounds or as extracts have responsible for their properties medical and used to treat many diseases.3,4,5

Arnebia hispidissima is among the medicinal plants that classified under the family Boraginaceae, and Arnebia important genus of the Boraginaceae family comprises 25 species. The major phytochemicals existing in Arnebia species are Pyrrolizidine alkaloids, triterpene derivatives flavonoids and phenolic acids, which have widespread pharmacological properties including anti-inflammatory, antimicrobial, wound healing and anti-tumorous activity.The root of A. hispidissima and flowers has been described as a source of a number of active chemical compounds namely naphthoquinones, vitexin, apigenin, cyanidin, kaempferol, which possess antibiotic and anticancerous properties. Moreover, the organic extracts of A. hispidissima exhibited antibacterial and antitumor activity which were attributable to the active chemical compounds. 6-13

Human body is continuously exposed to different types of agents that results in the production of reactive species called as free radicals (ROS/RNS) which by the transfer of their free unpaired electron causes the oxidation of cellular machinery. In order to encounter the deleterious effects of such species, body has got endogenous antioxidant systems or exogenous antioxidants from diet. Any imbalance between the RS and antioxidants leads to produce a condition known as “oxidative stress” that results in the development of pathological condition among which one is diabetes is a hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes.14-17

II. Material and Method

2.1.Collection of Plant Material:

Fresh plant of A. hispidissima (family-Boraginaceae) was collected as whole plant in (1st March -1st April 2019) from Thi-qar , Southern of Iraq . The plant was classified in department of Biology, college of Sciences, University of Thi-Qar , Iraq. Then the dry whole plant were crushed all by a mill, and kept in dark glass containers until day of use.

2.1.1.Preparation of cold aqueous extract: 50gm. Powder of the whole plant from A. hispidissima was macerated with distilled water (250ml) with stirrer by a magnetic bar for 24hours at room temperature and filtered by using filter paper .Then filtrate was concentrated using a rotary evaporator.18

2.1.2.Preparation of hot aqueous extract: 50gm.Powder of the plant whole from A. hispidissima was refluxed with distilled water (250 ml) for 24h at room temperature and filtered by using filter paper. The clear filtrate was concentrated using a rotary evaporator19.

2.1.3. Ethyl Acetate extract preparation of A. hispidissima: Powder (50 gm) of the whole A. hispidissima plant was put in thumble in Soxhlet then it was extracted by 250ml ethyl acetate solvent for 24 h, than the solvent removed under vacuum by rotary evaporator 20.

2.2.Preliminary qualitative and analysis of A. hispidissima extracts .

Qualitative analysis was performed for all extracts prepared by using the following tests:

2.2.1. Carbohydrates test

Carbohydrates were tested using Molish's reagent21.

2.2.2.Saponin test

Saponin were tested using aqueous Mercuric Chloride (5%) Test22

2.2.3. Phenols test

Phenols were tested using Ferric Chloride (1%) Test23

2.2.4. Flavonoids test

Flavonoids were tested using (5N)Alcoholic KOH24

2.2.5.Amino Acids and Peptides test

Using 1% ninhydrin Test 25

2.2.6.Tannins test

1 % FeCl3 test26

2.2.7. Steroids Test

(2 %) perchloric acid test27

2.2.8.Alkaloids Test

Wagner Test28

2.2.9. Glycosides Test22

2.2.10.Triterpenes Test29

2.3. Determination of Total Phenol and Total Flavonoid Content from A. hispidissima extracts:

The total phenolic content of analyzed plants was applied by the Folin-Ciocalteu method30. And aluminum chloride colorimetric method was used for determination of total flavonoids according to the Chang method31.

2.4.Evaluation of Antioxidant Activity from A. hispidissima extracts:

2.4.1. DPPH radical Scavenging Assay:

(1ml) of the extract solution was mixed with 2 mL of a freshly prepared (0.004w/v) DPPH radical methanol solution. The reaction volume was incubated for 30 min in the dark at 25°C. The absorbance was recorded with spectrophotometer UV at 517 nm. Ascorbic acid and BHA were used as a positive control using a positive control blank. The low absorbance of the reaction mixture indicated the highest radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following formula:

% inhibition of DPPH =[(Ac-As) / Ac] x 100

where Ac: is the absorbance of the control (DPPH + methanol) and As: the absorbance in the presence of the DPPH + extract32.

2.4.2. Ferric reducing antioxidant power assay:

Reducing the power of extracts was assessed as per the established method of Oyaizu (1986), five fraction different concentrations of each extract were mixed with 2.5ml potassium ferric cyanide (1%) and phosphate buffer (pH 6.6), was added. The reaction mixture was incubated at 50 °C for 20 min. Trichloroacetic acid 1% (2.5) was added to the reaction mixture and centrifuged for 10 min at 2000rpm. After centrifugation, the upper layer of the reaction mixture (5 mL) was added in the mixture of distilled water, (1.0 mL) and FeCl3 (0.1%), incubated the mixture for 20 min and finally, the absorbance was determined at 700 nm in a UV spectrophotometer. The inhibition percentage was calculated by reducing power activity as follows33.

Inhibition (%) = [(Ac - As)/Ac] × 100.

2.4.3.Fe3+ reducing assay:

Fraction was assessed as per the established method of Welch & Decker(1990), five different concentrations of each extracts (1-5 mg\ml) were mixed with0.4ml Ferric chloride (2mM) and 0.4ml 8- hydroxy quinolone (5mM). The reaction mixture was incubated at room temperature for 10 min and finally the absorbance was determined at 562 nm in a UV spectrophotometer. Link ability was also estimated Ferric ion of EDTA compound in the same way for comparison. The control sample was prepared by the way itself, except for the extracts. The inhibition percentage was calculated follows34. Inhibition (%) = [(Ac - As)/Ac] × 100.

2.5.Study of anti-hyperglycemia activity from A. hispidissima extracts:

Diabetes was induced in mice by a single injection of aqueous alloxan monohydrate (150mg/kg. B.W) after being dissolved in normal slain After injections they had free access to food and water and received a 5% glucose solution for drinking at night to counter the shock of blood sugar. After 72hours of injection, animals with the serum glucose level more than 200 mg/dl (diabetic) were selected for the study35.

2.5.1. Experimental Design:

Diabetic animals were divided into Four groups, each with five mice. .

Group1: (Control normal treated with distilled water)

Group2:(control diabetic treated with distilled water)

Group3:(treated with 250 mg/kg B.W) cold aqueous extract)

Group 4:(treated with (250 mg/kg B.W hot aqueous extract)

Group5:(treated with (250 mg/kg B.W) ethyl Acetate extract) of A. hispidissima one dose by orally for one day. Blood samples were collected from the tip of the tail at times (0 (as fasting), 2,4,6 and 24 hours). After administration to determine the blood glucose level was measured via electronic glucometer.

Statistical analysis:

Data were analyzed, using Special Package for Social Science (SPSS) version26 The descriptive data were given as means ± S.D. Least Significant Difference (LSD(.The accepted level of significances were ($P < 0.05$).

III. Results and Discussion

Percentages of A. hispidissima extracts were calculated , as illustrated in table (1)

Table(1). Percentages of A. hispidissima extracts

No.	Extract type	Yield (%)	Physical state
1	Hot aqueous	21.34%	Sticky , Brown
2	Ethyl acetate	14.6%	Sticky, Dark brown
3	cold aqueous	12.04%	Sticky, Dark brown

Results obtained showed that the hot aqueous extract of A. hispidissima highest percentage than others extract, where contained 21.34% metabolites compounds crud.

Qualitative Analysis of A. hispidissima extracts

The phytochemical analysis of A. hispidissima extracts plant showed the presence of major metabolites of saponins, alkaloids, tannins, glycosides, Carbohydrates, Phenols, Flavonoids and Steroids. Table (2) indicates qualitative analysis results of hot aqueous, cold aqueous and ethyl acetate extract of A. hispidissima. The number of positive signs indicates the intensity of the reactions that reflects the quantity of secondary metabolism active compounds present in the extract.

Table (2). Qualitative analysis of A. hispidissima extracts

Regents	Extracts			Results
	Hot aqueous	cold aqueous	Ethyl acetate	
Molish	++	++	++	Carbohydrates are present
Benedict	+	+	+	Glycosides are present
1% Ninhydrin	++	++	–	Free amine groups and peptides are present
1%Ferric chloride	+	+	++	Phenols are present
5%$HgCl_2$(aqueous)	+	++	+	Saponin is present
Alcoholic KOH	+	++	++	Flavonoids ar present
(2%)Perchloric acid	+	+	+	Steroids are present
Salkoviski	+	+	+	Triterpenoids are present
wagner	++	+	+	Alkaloids are present
(1%)Ferric Chloride	+	++	+	Tannins are present

+ positive test - negative test ++ quantitative presence

The preliminary phytochemical analysis of aqueous extract and ethyl acetate extract of study plant (Table 2) showed the presence of a high number of phytochemicals. The presence of major metabolites of saponins, alkaloids, tannins, glycosides, carbohydrates, Phenols, Flavonoids and Steroids. The number of positive signs indicates the intensity of the reactions that reflects the quantity of phytogroup present in the extract13,36.

Quantification of phenol and Flavonoid of A. hispidissima extracts

The results of the quantitative determination of total phenols and flavonoids are as presented in Table 3. The results revealed that the highest total phenol content was found in Cold aqueous extract. While the highest total flavonoids values in hot aqueous extract (0.1037mg/ml) (Hot aqueous > Ethyl acetate> cold aqueous) (Table3).

Table (3). Results of quantification of phenol and Flavonoid of A. hispidissima extracts

Extracts	cold aqueous	Hot aqueous	Ethyl acetate
The quantity of Equivalent phenol(mg/ml)	0.0175	0.0168	0.0109
The quantity of Equivalent Flavonoid (mg/ml)	0.0653	0.1037	0.0935

Phenols and Flavonoid are secondary plant metabolites that are present in plants and plant products. Many phenols and flavonoids compounds, exhibit strong antioxidant activity, antidiabetic and several other diseases37. Therefore, the amounts of total phenol, flavonoids in A. hispidissima extracts were measured in this study. Based on the results indicated in the tables(3), It is showed that Cold aqueous extract contains the highest amount of phenol (0.0175mg/ml) due to the high polarity of most extracted compounds, but some of these compounds are less polar and can, therefore, be extracted by ethyl acetate. On the other hand, the highest value of flavonoids found in hot aqueous extract. The variation of phenolic and flavonoid compounds content in the extracts depends on the influence of the difference in the polarity of the organic solvent of the extracted phytochemical compound38.

Inhibition of DPPH Radical (%) by A. hispidissima extracts

The results of the free radical scavenging activities of A. hispidissima extracts in Table(4) and Fig.1. The results showed that DPPH (2, 2-diphenyl-1- picrylhydrazyl) scavenging activities increased with increased concentration , As shown the superiority of the hot aqueous extract of A. hispidissima at a concentration of 100µg/ml with a 91.22% inhibition ratio, compared to other extracts of A. hispidissima as compared to standard ascorbic acid 83.57% and BHA 70.96% , at a concentration of 100 µg/ml.

Table(4). Effect of A. hispidissima extracts with different concentrations of DPPH free radical scavenging

Extract type of *A.hispidissima*	% RSA (radical scavenging activity) concentrations(µg/ml)				
	20 µg/ml	40 µg/ml	60µg/ml	80 µg/ml	100 µg/ml
Hot aqueous	40.35	57.89	59.64	77.19	91.22
Cold aqueous	56.23	73.68	84.00	85.90	87.72
Ethyl acetate	42.10	49.14	57.89	64.92	75.46
Ascorbic acid	51.70	59.53	64.35	73.68	83.57
BHA	43.56	52.56	60.78	68.21	70.96

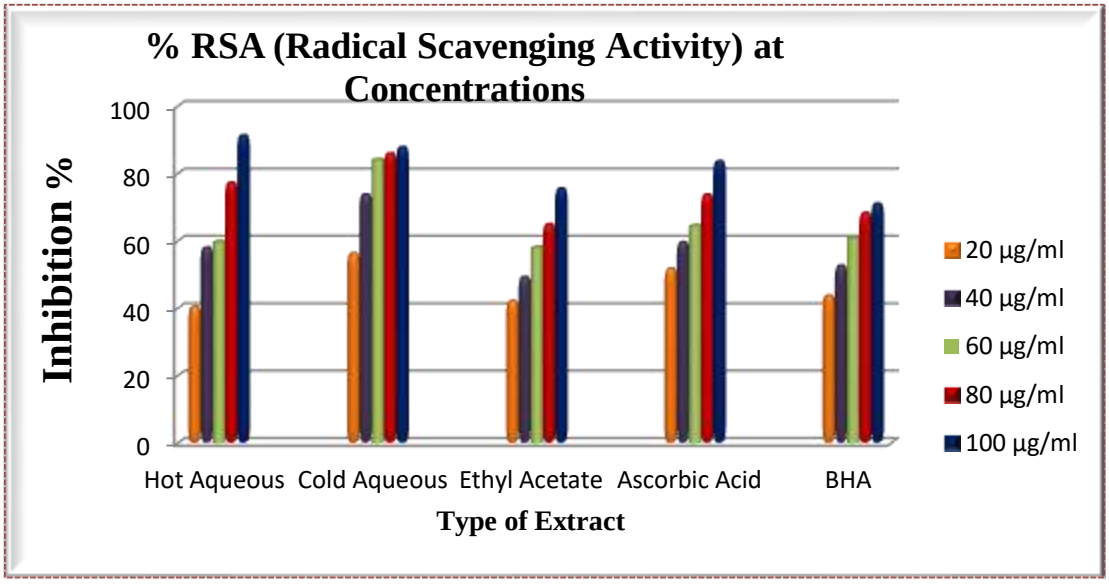

Fig.1: DPPH radical scavenging activity of A. hispidissima extracts with different concentration when compared to the standard (ascorbic acid, BHA).

Ferric Reducing Antioxidant Power of A. hispidissima extracts:

The results of ferric reducing antioxidant power of A. hispidissima extracts are presented in Table5 and Fig.2. Ferric reducing antioxidant potentials of hot aqueous extract was exhibited the highest ferric reducing power compared to the natural antioxidant ascorbic acid, it is clear that the superiority of the hot aqueous extract over the rest of the extracts, followed by the cold aqueous extract > Ethyl acetate Compared to ascorbic acid at the highest concentration (50mg/ml) .

Table 5: The results of Reducing Power

Extract type of *A. hispidissima*	**% Reducing Power at concentrations (mg/ml)**				
	10mg/ml	20mg/ml	30mg/ml	40mg/ml	50mg/ml
Hot aqueous	65.50	66.29	71.20	73.00	75.11
Cold aqueous	64.00	667.51	69.63	71.30	73.32
Ethyl acetate	60.89	63.25	66.52	67.35	69.64
Ascorbic acid	76.27	78.81	82.63	85.01	88.25

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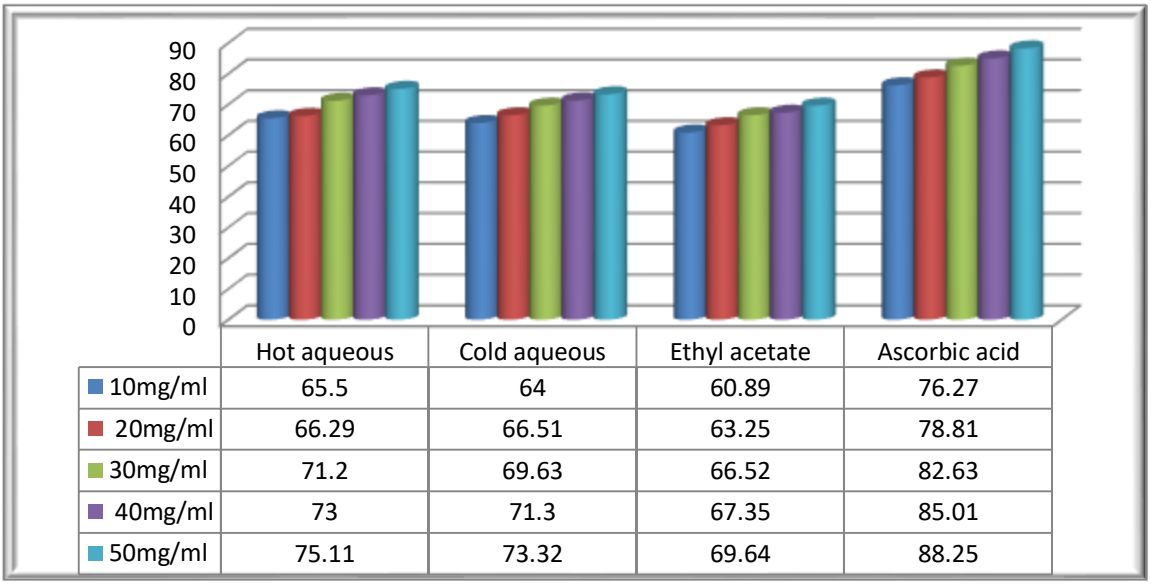

	Hot aqueous	Cold aqueous	Ethyl acetate	Ascorbic acid
10mg/ml	65.5	64	60.89	76.27
20mg/ml	66.29	66.51	63.25	78.81
30mg/ml	71.2	69.63	66.52	82.63
40mg/ml	73	71.3	67.35	85.01
50mg/ml	75.11	73.32	69.64	88.25

Fig.2: The effect reducing Power A. hispidissima extracts at different concentrations (mg/ml).

Fe 3+ Reducing of A. hispidissima extracts:

The results of the blog in a table(6) and Fig.3 showed the susceptibility of A. hispidissima plant extracts to bind ferrous ion in comparison with ethylene diamine-tetra acid acetic (EDTA). The extracts showed link ability Ferrous ion, the susceptibility to hot aqueous extract is highest chelating activity of ferrous ion at concentration (5mg/ml) than other extracts. This result indicates the study plant extracts. It possesses part of the effectiveness of the metal ion binding.

Table (6). Effect of A. hispidissima extracts on Fe 3+ Reducing.

Extract type of *A. hispidissima*	**% Fe $^{3+}$ Reducing at concentrations (mg/ml)**				
	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml
Hot aqueous	69.55	71.23	74.54	78.46	79.15
Cold aqueous	65.40	67.77	69.81	71.41	77.26
Ethyl acetate	52.72	57.63	60.39	64.93	72.16
EDTA	79.14	81.97	83.14	84.83	86.67

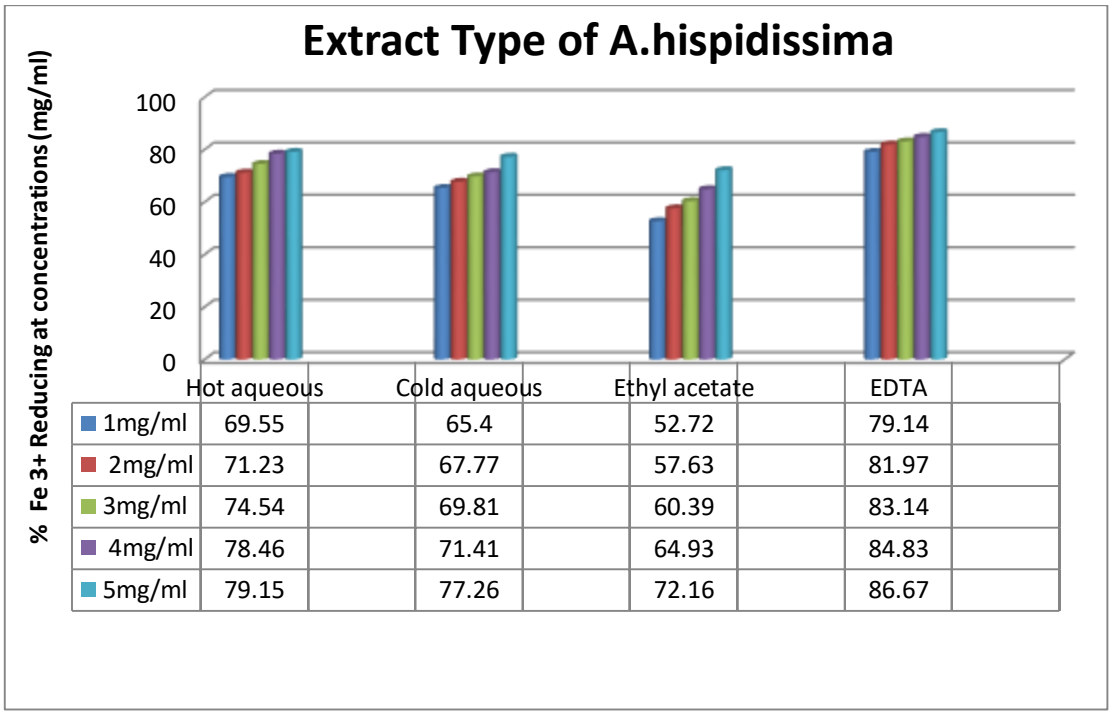

Fig.3: Fe 3+ Reducing of A. hispidissima extracts at different concentrations (mg/ml).

The active chemical compounds extracted from medicinal plants play such as phenolic compounds, flavonoids, tannins, saponin and terpenoids an important role in limiting the production of the active oxygen species that are mainly in the oxidation of sinter, and therefore the reducing ability of the compounds to chelate minerals such as Fe3+ a measurable antioxidant effect. These compounds their antioxidant activity is based on their redox properties through breaking the free radical chain by donating a hydrogen atom or preventing peroxide formation. Based on our results of qualitative analysis of all extracts from A. hispidissima , Which showed the presence of active chemical families(phenolic compounds, flavonoids, tannins, saponin, glycoside and terpenes in the extracts of the study plant it can be concluded that extracts of A. hispidissima irrespective of the mode of preparation, can inhibit oxidative processes leading us to conclude that the extract has substantial antioxidant activity in vitro. This could be assigned to the high content of the phenolic compounds in these extracts. Various studies highlighted the correlation between the phenolic content and the antioxidant capacity of plant extracts indicating that extracts with the highest polyphenol, phenolic substances such as flavonoids, phenolic acids, and tannins contents show higher antioxidant activity well known that contribute directly to the antioxidant capacity of plants, Also, phenols are organic compounds that contain a hydroxyl group bound directly to the aromatic ring, and the H-atom of the hydroxyl group can trap peroxyl radicals, preventing other compounds to be oxidized. Also, studies on flavonoids derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases. So comparable with the findings in the literature for other extracts of plant products our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity of A. hispidissima extracts39-48

The effect from A. hispidissima extracts in hyperglycemia mice:

experiments revealed that A. hispidissima extracts has significant hyperglycemic activity. Effects of oral administration of A. hispidissima extracts hyperglycemia mice compared with metformin mice group are presented in Table7.

Table 7: Effect of A. hispidissima extracts on level blood glucose conc. in diabetic mice

Extracts Dose (mg/kg.BW)	No.	Blood glucose conc. (mg/100ml)				
		0 hrs.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Normal control	5	87.6 ± 5.57	86.80 ± 6.40	85.00 ± 4.83	85.80 ± 4.83	87.00 ± 5.47
Diabetic control	5	286.80 ± 10.68	290.10 ± 10.43	291.80 ± 10.62	295.10 ± 10.16	299.60 ± 9.16
(250mg/kg.BW) hot aqueous	5	266.60 ± 32.5 [a]	238. 20 ± 24.0 [b]	216.60 ± 34.1 [b]	193.20 ± 31.7 [c]	176.80 ± 20.7 [d]
(250mg/kg.BW)Ethyl acetate	5	260.60±19.68 [a]	229.40±16.65 [b]	181.80±11.82 [c]	176.20±19.91 [c]	186.60±17.26 [c]
(250mg/kg.BW)cold aqueous	5	331.0 ± 33.20 [a]	305.20 ± 26.5 [b]	286.60 ± 26.0 [b]	265.60 ± 24.4 [c]	248.40 ± 19.6 [c]
(100mg/kg.BW)Metformin	5	304.40± 32.1 [a]	272.2± 37.9 [b]	248.20 ± 28.4 [c]	230.40 ± 24.0 [c]	202.20 ± 24.0 [d]

-Each value represents mean □ S.D values with non-identical superscript (a , b or c..etc.) were considered significantly differences ($P \leq 0.05$).

-No: Number of mice.

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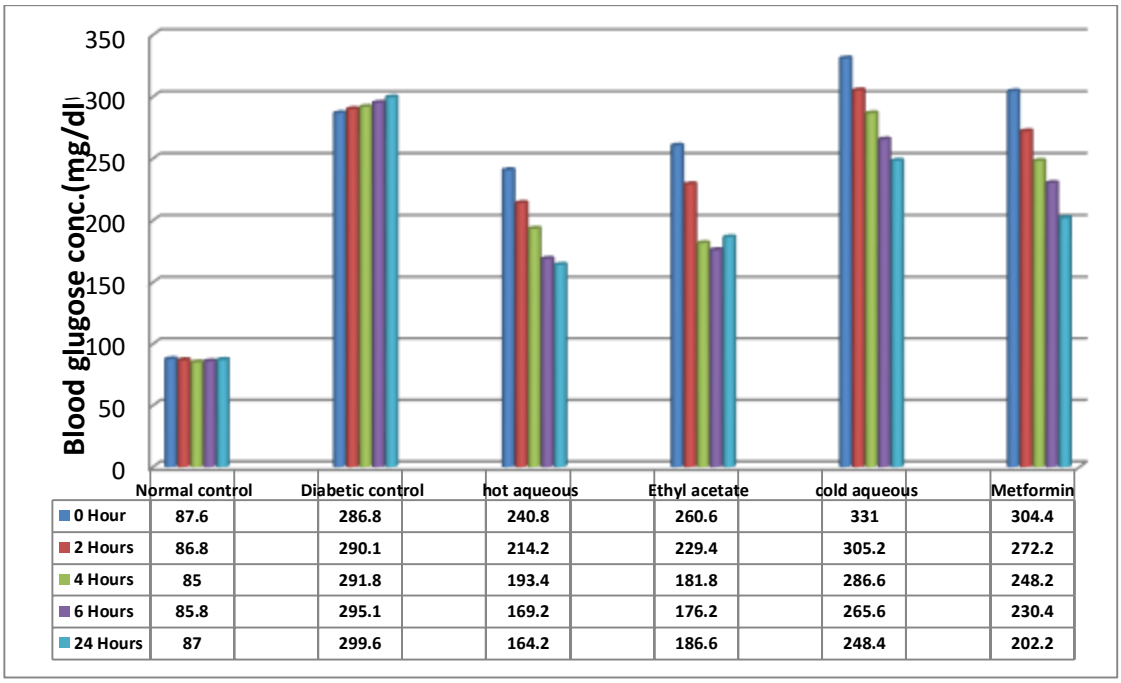

	Normal control	Diabetic control	hot aqueous	Ethyl acetate	cold aqueous	Metformin
0 Hour	87.6	286.8	240.8	260.6	331	304.4
2 Hours	86.8	290.1	214.2	229.4	305.2	272.2
4 Hours	85	291.8	193.4	181.8	286.6	248.2
6 Hours	85.8	295.1	169.2	176.2	265.6	230.4
24 Hours	87	299.6	164.2	186.6	248.4	202.2

Fig.4: Effect of A. hispidissima extracts on level blood glucose conc. in diabetic mice

The results show hot aqueous extract isolated from A. hispidissima effect of on blood glucose levels in hyperglycemic mice at different times after oral administration. Find out that the extract significantly reduces glucose, at 2hrs and 4hrs($P < 0.05$) and a significant decrease occurred in 6hrs and 24 hours ($p<0.05$) compared with(100mg/kg.BW)Metformin. While Oral administration of (250mg/kg.BW)Ethyl acetate extract of A. hispidissima acted on reduced blood glucose levels after 2hrs of oral administration in hyperglycemic mice and a significant decrease after 6hrs($P < 0.05$). As well as Oral administration of (250mg/kg.BW)cold aqueous extract of A. hispidissima acted on reduced blood glucose levels after 2hrs and a significant decrease after 6h and 24h ($p<0.05$) compared with(100mg/kg.BW)Metformin. Table(7). From these, a significant decreasing was recorded at 2hrs and 4hours($P<0.05$) , because of metabolism of active compounds begins after 2hrs from oral administration of extract.49

Preliminary phytochemical analysis of A. hispidissima extracts revealed the presence of flavonoids, alkaloids, glycosides, terpenoids, saponins and polyphenolic compounds. Flavonoids and saponins containing plants were known to exhibit antidiabetic activity.50 Hence, the great significant decreasing effect on blood glucose concentrations in hyperglycemic mice because of the presence of alkaloids, glycosides, flavonoids, and terpenoids may have a more because these compounds induce tissues body to absorb glucose from the blood then increase of consumption of these tissues to glucose51. The presence of flavonoids and sterol in the A. hispidissima extracts, may be responsible for such active.

IV. Conclusion

Based on the aforementioned results, the Arnebia hispidissima extracts have the presence of most of the phytochemicals (terpenoids, flavonoids, saponins, tannins, polyphenols, alkaloids, and glycosides). The hot aqueous extract has the highest extraction percentage. Also, the highest yield of phenol in the cold aqueous extract,

While the highest yield of flavonoids in the hot aqueous extract. We concluded that A. hispidissima extracts have antioxidant and were the highest hot aqueous extract activity at a concentration of 100µg/ml, Also and antihyperglycemic activity as it lowers the blood glucose level in diabetic mice. However, the molecule(s) responsible for such an effect requires further investigation in general.

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