Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 488-491
Received: 13-03-2017
Accepted: 14-04-2017

Nafessa Saeed Khalaf Allah
Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan

Ikram Mohamed Eltayeb
Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan

Amna El Hassan Hamad
Department of Pharmacology, Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan

Correspondence
Ikram Mohamed Eltayeb
Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan

Phytochemical screening and hypolipidemic activity of extracts from seeds and leaves of *Vigna unguiculata* growing in Sudan

Nafessa Saeed Khalaf Allah, Ikram Mohamed Eltayeb and Amna El Hassan Hamad

Abstract
Vigna unguiculata belonging to the family Fabaceae is widely grown in Africa. In Sudan there are a number of varieties which are commonly as food. The plant is traditionally used for treating of many diseases such as stomatitis, corneal ulcers, colic diseases, epilepsy, chest pain and sexually transmitted diseases. This study represents the first attempt to investigate the lipid profile effect of *V. unguiculata* leaf and seed extracts. Thirty-five Wistar rats divided into seven groups were used in the experimental study. Five rats in each group as follows; normal control group fed on normal diet and hypercholesterolemic groups fed with high cholesterol diet. The hypercholesterolemic groups were further divided into six groups with five rats in each group. The first group (control group), received hypercholesterolemic diet only; second group treated with Atorvastatin, standard drug (0.18 mg/Kg). Third and fourth group received ethanolic extract of *V. unguiculata* seeds of 250 mg/Kg and 500 mg/Kg respectively. Fifth and sixth groups received ethanolic extract of *V.uiculata* leaves 250 mg/Kg and 500 mg/Kg respectively. Blood samples were taken from the Retro-orbital venous plexus after four weeks of the experiment from all group after 24 hours fasting. The lipid profile parameters, TC, TGs, LDL, HDL, were measured. The results revealed that, seed extract posses the more hypolipidemic activity than leaf extracts through the reduction of total cholesterol (TC) and low density lipoprotein (LDL).

Keywords: Phytochemical, Screening, Hypolipidemic, *Vigna*, *unguiculata*, Seed, Leaf, Extracts.

1. Introduction
Non communicable diseases (NCDs), also known as chronic diseases are the leading causes of death and disease burden worldwide. One of the most important and account for most NCDs deaths are cardiovascular diseases (CVDs) which are group of disorders of the heart and blood vessels [1, 2]. The most common reason for these is a build-up of fatty deposits on the inner walls of the heart and blood vessels [3]. The dyslipidemia, a deposition of fats is defined as a lipid metabolism failure characterized by an elevation of total cholesterol (TC), total triglycerides (TGS) low density lipoprotein (LDL) and high density lipoprotein (HDL); or a combination of these abnormalities [1, 3].
In developing countries the heart diseases are treated by medicinal plants as a cheapest and safest alternative traditional medicine [4]. *Vigna unguiculata* is one of medicinal plant traditionally used for its and cardioprotective activities [5].
Vigna unguiculata, commonly known as cowpea belongs to the family Fabaceae is an annual herbaceous, originally from West Africa and is cultivated for its edible seeds or as fodder [6]. It is one of the most important tropical dual-purpose legumes, the vegetables leaves and grain seeds are used as dietary food whereas the flowers are used as a fodder [7]. The plant has ethnomedicinal importance to treat stomatitis, corneal ulcers, colic diseases, epilepsy, chest pain and sexually transmitted diseases [8, 9]. It has antioxidant, antihyperglycemic, antinociceptive, antihilmentic, antibacterial and cardioprotective. Many secondary metabolites such as flavonoids and saponins were reported to be found in the plant [8, 9]. The results of phytochemical screening of the leaf extracts and assessment of their hypolipidimic activity are reported in the present paper.

2. Materials and Methods
I. Plant Material Collection and Preparation
Seeds and leaves of *Vigna unguiculata* were collected in the month of February, 2016 from the Khartoum central Local Market, and were identified and authenticated at the Medicinal and Aromatic Plants Research Institute (MAPRI).

The voucher specimens were deposited at Pharmacognosy Department, Faculty of pharmacy, University of Medical Science and Technology (UMST).Fresh leaves were picked, washed, and then air dried over night at room temperature. Seeds of the plant were sorted and cleaned to remove foreign materials. Both leaves and seeds were ground to a fine powder using an electrical grinder, packed and then stored in clean containers.

II. Phytochemical Screening Test

The phytochemical constituents of the plant were detected using standard procedures as described by Trease and Evans (2002) [10] and Sofowora, (1993) [11]. The extract was prepared by reflex of 20 grams of powdered plant material with 250 ml 70% ethanol for two hours, the extract was then filtered and used for phytochemical screening.

Detection of Flavonoids

Two ml of the extract were mixed with diluted NaOH to produce yellow coloration. Disappearance of the color upon addition of dil. HCl indicates the presence of flavonoids.

Detection of Sterols

Two ml of the extract were evaporated to dryness, the residue was then dissolved in two ml of chloroform and transferred to clean dry test tube, two ml of acetic anhydride were added followed by addition of conc. H_2SO_4 carefully to the wall of the tube. Color development from violet to blue or green indicates presence of a steroidal moiety.

Detection of Terpenoids (Salkowski Test)

Two ml of the extract were mixed with two ml of chloroform. Three ml of conc. H_2SO_4 were added carefully to form a layer, formation of a reddish brown color at the interface indicates the presence of terpenoids.

Detection of Alkaloids

Two ml of the extract were acidified with 1% HCl, few drops of Mayer's reagent were added, appearance of turbidity indicates the presence of alkaloids.

Detection of Saponins

Frothing Test

One gram of the powdered drug was extracted by boiling with ten ml of distilled water for ten minutes and was filtered. The filtrate was shacked vigorously in a test tube for thirty seconds and was allowed to stand for thirty minutes. Result was observed.

Ether Test

One gram of the powdered drug was extracted by boiling with twenty ml. of methanol, under reflux, for 10-15 minutes and was filtered. The filtrate was cooled and 5-10 ml of ether was added. Result was observed.

Detection of Tannins

One gram of the powdered drug was extracted by boiling with 20ml. of distilled water for 10 minutes and was filtered.

Ferric Chloride Test

One drop of ferric chloride was added to two ml of the extract, in a test tube. Result was observed.

Gelatin Test

Few drops of 1% gelatin solution containing NaCl were added. Result was observed.

Detection of Cardiac Glycosides

Two grams of the powdered drug was extracted with 25 ml 70% ethanol on water bath for 15-30minutes, and then filtered. The filtrate was diluted with the same volume of distilled H2O; 1-2 ml of strong lead acetate was added to precipitate resins, tannins and pigments. The filtrate was extracted with 15 ml (5 X 3) chloroform. The extract was divided into two portions (I and II),

Extract I (Keller Killiani test)

- Extract I was evaporated to dryness (rotary evaporator)
- Extract I was dissolved in 2 ml of 3.5% FeCl3 in glacial acetic acid, transferred to a clean dry test tube.
- 2 ml conc. H_2SO_4 was poured on the wall of the test tube. Then the result was observed.

Extract II (Kedd's test)

Extract II was evaporated to dryness, then dissolved in few drops of alcohol, 3,5dinitrobenzoic acid (Kedd's A) was added followed by NaOH (Kedd's B), then the color was noticed.

Detection of Reducing Sugars

Two ml of the extract was heated with equal volumes of Fehling solution A and B. Appearance precipitate indicates the presence of reducing sugars.

III. Assay of Hypolipidemic Activity

The Hypolipidemic was carried out according to the method described by Yang, *et al*., (2010) [12]. Thirty-five Wistar rats were used in the experimental study. They were divided into seven groups, five rats in each group as follows; normal control group fed on normal diet and hypercholesterolemic groups were fed with high cholesterol diet containing 1% cholesterol, 10% saturated animal fat, they were chow for four weeks. The hypercholesterolemic groups were further divided into six groups, five rats in each group. The first group, control group received hypercholesterolemic diet only; second group treated with Atorvastatin as standard drug (0.18 mg/Kg). Third and fourth group treated with ethanolic extract of *V. unguiculata* seeds of 250 mg/Kg and 500 mg/Kg respectively. Fifth and sixth groups treated with ethanolic extract of *V. unguiculata* leaves of 250 mg/Kg and 500 mg/Kg respectively. After four weeks the blood samples were taken from the Retro-orbital venous plexus of all tested groups after 24 hours fasting. The lipid profile parameters, TC; TGs; LDL; HDL; were measured [12].

IV. Statistical Analysis

Values were expressed as Mean and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) to compare the different groups and then determine the significance (p-value) of data.

3. Results and Discussion

The phytochemical screening results of *V. unguiculata* seeds and leaves are shown in table 1. The results revealed the presence of flavonoids, alkaloids, tannins, and reducing sugar in both seeds and leaves parts of the plant. Sterols and terpenoids are only shown in leaves part. The results are compatible with the chemical of Fabaceae Family [13] and *Vigna* Genus [14].

Table 1: Phytochemical Screening Results of *V. unguiculata* Seeds and Leaves Extracts

Secondary Metabolite Test	Result	
	Seeds	Leaves
Flavonoids	+	+
Sterols	-	+
Terpenoids	-	+
Alkaloids	++	++
Saponins	+	++
Taninns	++	++
Cardiac glycosides	-	-
Reducing sugars	+	+

The results of lipid profile effects of *V. unguiculata* seeds and leaves extracts are shown in table 2, 3 and figure 1.

In compare to hypercholesterolemic control group, a dose of 250 mg/kg and 500 mg/kg of *V. unguiculata* seed extract given daily for a period of four weeks resulted in 27 and 22.6 mg/L reduction in serum TC (Total cholesterol), TGs was reduced by 26.2 and 23.8 mg/L. LDL which plays a major role in atherogenesis was reduced by 12.1 and 13 mg/L respectively. HDL levels was elevated by 4 and 6.7 mg/L at the dose of 250 and 500mg/kg respectively. These results reveal that, the crude ethanolic extract of *V. unguiculata* seed induced a decrease in TC, TGs and LDL, which is considered the "bad lipoprotein" addition to an increase in serum HDL "the good lipoprotein".

A dose of 250 mg/kg and 500 mg/kg of *V. unguiculata* leaves extract resulted in (18.8 and 20.1) mg/L, (1.2 and 7.6) mg/L and (11.8 and 8.7) mg/L reduction of TC, TGs and LDL; and increase of (17.2 and 15)) mg/L in HDL respectively. From these results it appeared that the lipid profile effect of *V. unguiculata* extracts was found to be dose independent.

The Atorvastatin standard drug showed 9.4 and 0.5 mg/L reduction in TGs and LDL respectively, TC and HDL were found to be increase by 2.8 and 26. This result was compatible with the mechanism action of the drug (Atorvastatin standard) [15]. From the results it appeared that, the extracts of *V. unguiculata* seeds and leaves decrease the total cholesterol, triglycerides and bad lipoprotein parameters in the serum and increase the good lipoprotein. The Atorvastatin drug decrease the triglycerides and bad lipoprotein parameters in the serum, but it increased the total cholesterol in the serum which consider the negative aspects for the Atorvastatin drug.

From the results it appeared that the extracts of *V. unguiculata* seeds and leaves posses significance hypolipidemic activity than the Atorvastatin. Addition to the seeds extract was found to posses hypolipidemic activity more significance than the leave extract.

The results confirm the cardioprotective properties of *V. unguiculata* seeds and leaves, since dyslipidemia is considered as a major factors contributing to the development of cardiovascular atherosclerotic disease.

The primary phytochemical screening of *V. unguiculata* seeds and leaves showed the present of flavonoids which posses cardioprotective effect against cardiovascular disease due to their antioxidants properties of LDL, the main lipoprotein involved in the formation of atherosclerotic plaque and that results in reduction of its plasma level [15]. Also the presence of tannins, the major antioxidants agents are mainly correlated to their lipid profile effect.

Table 2: Lipid profile effects of *V. unguiculata* seeds and leaves extracts

Group	Hematological Parameter			
	TC mean ±SD	TGS mean ±SD	LDL mean ±SD	HDL mean ±SD
Hypercholesterolemic (negative control)	66.4±9.45	62.6±37.213	19.44±17.266	15.9±6.79
Atorvastatin (0.18 mg/kg) (positive control)	69.2±15.881	53.2±13.424	18.98±8.826	41.96±18.708
seeds extract (250mg/kg)	39.4±7.861	36.4±3.362	7.36±3.314	19.94±2.903
Seeds extract (500mg/kg)	43.8±5.586	38.8±7.95	6.44±1.135	22.64±5.747
Leaves extract (250mg/kg)	47.66±22.427	61.4±29.871	7.66±3.207	33.16±11.462
Leaves extract (500mg/kg)	46.3±23.107	55±10.909	10.78±4.85	30.98±3.393
Normal Control Group	57.6 ± 11.781	47.8±23.102	17.26±9.935	28.18±3.588

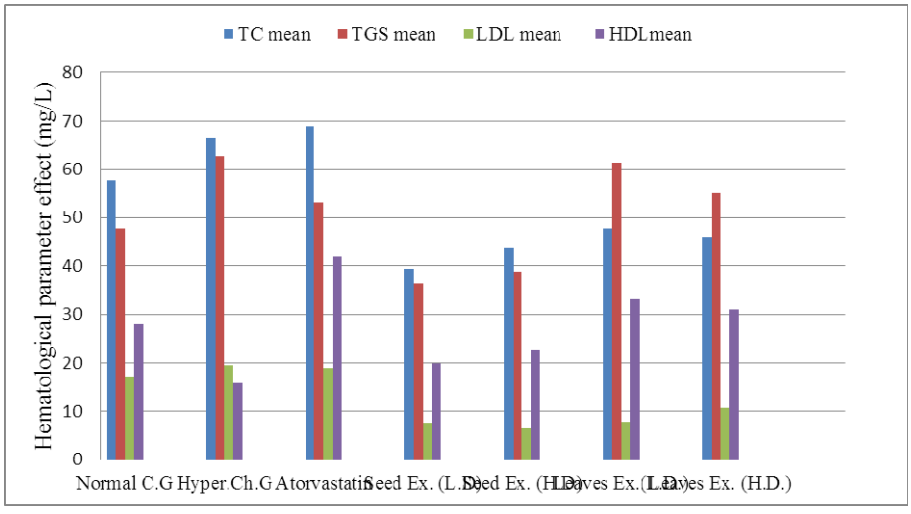

Figure 1: Lipid profile effects of *V. unguiculata* seeds and leaves extracts

Table 3: Statistical Analysis Results

Comparing Groups		Parameters Mean Difference			
		TC	TGS	LDL	HDL
Seeds extract 2 50 mg	Seeds extract 500 mg	-4.4	-2.4	0.92	-2.7
	Leaves extract 250 mg	-8.26	-25	-0.3	-13.220*
	Leaves extract 500 mg	-6.9	-18.6	-3.42	-11.04
	Atorvastatin 0.18 mg/kg	-29.800*	-16.8	-11.620*	-22.020*
	Cholesterol diet	-17	-26.2	-12.080*	-8.96
Seeds extract 500 mg	Seeds extract 250 mg	4.4	2.4	-0.92	2.7
	Leaves extract 250 mg	-3.86	-22.6	-1.22	-10.52
	Leaves extract 500 mg	-2.5	-16.2	-4.34	-8.34
	Atorvastatin 0.18 mg/kg	-25.400*	-14.4	-12.540*	-19.320*
	Cholesterol diet	-12.6	-23.8	-13.000*	-6.26
Leves extract 250 mg	Seeds extract 250 mg	8.26	25	0.3	10.52
	Seeds extract 500 mg	3.86	22.6	1.22	13.220*
	Leaves extract 500 mg	1.36	6.4	-3.12	2.18
	Atorvastatin 0.18 mg/kg	-21.540*	8.2	-11.320*	-8.8
	Cholesterol diet	-8.74	-1.2	-11.780*	4.26
Leaves extract 500 mg	Seeds extract 250 mg	6.9	18.6	3.42	11.04
	Seeds extract 500 mg	2.5	16.2	4.34	8.34
	Leaves extract 250 mg	-1.36	-6.4	3.12	-2.18
	Atorvastatin 0.18 mg/kg	-22.900*	1.8	-8.2	-10.98
	Cholesterol diet	-10.1	-7.6	-8.66	2.08
Atorvastatin 0.18 mg/kg	Seeds extract 250 mg	29.800*	16.8	11.620*	22.020*
	Seeds extract 500 mg	25.400*	14.4	12.540*	19.320*
	Leaves extract 250 mg	21.540*	-8.2	11.320*	8.8
	Leaves extract 500 mg	22.900*	-1.8	8.2	10.98
	Cholesterol diet	12.8	-9.4	-0.46	13.060*
Cholesterol diet	Seeds extract 250 mg	17	26.2	13.000*	8.96
	Seeds extract 500 mg	12.6	23.8	12.080*	6.26
	Leaves extract 250 mg	8.74	1.2	11.780*	-4.26
	Leaves extract 500 mg	10.1	7.6	8.66	-2.08
	Atorvastatin 0.18 mg/kg	-12.8	9.4	0.46	-13.060*

* Significant at $P < 0.05$

4. Conclusion

The study concludes that, *Vigna unguiculata* seeds and leaves extracts possess significance hypolipidemic effect compared to Atorvastatin standard drug. Addition to seed extract was found to have highly hypolipidemic effect than leaves extract.

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