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Original Research Article

Antihyperlipidemic Properties of Powder Fractions and Extracts of Diospyros Mespiliformis HOCHST Fruits

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Abstract: Hyperlipidemia is one of the major risk factors for the development of cardiovascular disease. Many bioactive compounds extracted from plants, particularly polyphenols, have demonstrated a beneficial effect on health. Obtaining these molecules requires extraction, conventional extraction techniques uses solvents whose residue affects these compounds, the environment and the health of consumers. Faced with these problems some techniques, a new axis has been developed it is the dry extraction (controlled sieving-differential spray). Diospyros mesiliformis is poorly studied regarding the bioactive compounds of its fruits. The aim of this study is to find out the optimal conditions for the dry extraction of bioactive compounds compare to solvent extraction and to study antihyperlipidemic activity of the fruit. Granulometric classes ranging from 50 µm to 315 µm were studied. Hydroethanolic and ethanolic extracts of Diospiros mesiliformis were analyzed for total phenolic, total flavonoid, and total tannin contents. For study of antihyperlipidemiac activity, hyperlipidemia was induced by feeding animals with high fat diet daily and orally ad libitum. Ten groups of five animals in each received normal saline, high fat diet, high fat diet plus D. mesiliformis powder (600 mg/kg) and extracts (600 mg/kg) body weight was given in both models and Atorvastatin (10mg/kg) body weight was given in both models respectively for 30 days. At the end of the study, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), atherogenic index (AI) of rats were evaluated and were compared with the rats treated with Atorvastatin (10mg/kg) body weight. The particle size and the extracts had high influence on the total phenolic compounds, total flavonoid and tannin content as well as on the antihyperlipidemic activity. Diospyros mespiliformis can be safely used in the treatment of mild to moderate cases of hyperlipidemia considering its easy availability, cost effectiveness, and other beneficial effects.

Keywords: Diospyros mespiliformis, dry extraction, solvent extraction, lipids, phenolic compounds.

INTRODUCTION

Hyperlipidemia is a modifiable risk factor of an important killer disease "cardiovascular diseases". It is now established that it represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. Its prevalence is growing not only in developed countries but also in developing countries [1]. Hyperlipidemia is a condition which characterized by abnormal elevation of lipid such as (triglyceride and cholesterol) and lipoproteins such as (LDL, VLDL) levels in the blood [2]. Treatment of hyperlipidemia reduces cardiovascular events [3]. The diet enriched with saturated fats and cholesterol, contribute to the elevated lipid levels in our population as well as in many other developed countries around the world [4]. The pharmacological therapy for abnormal lipids is effective but is costly and associated with side-effects leading to patient incompliance [5]. Therefore, alternative therapies particularly, medicinal plants, legumes and fruits based are being explored, they have demonstrated a beneficial effect on health [6, 7]. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. The phytochemical investigation of a plant may involve following steps: authentication and extraction of the plant material, separation and isolation of the constituents of interest, characterization of the isolated compounds and quantitative evaluation [8]. Obtaining of these bioactive molecules requires extraction. Extraction is one of the oldest known chemical operations and represents the key for the recovery and purification of active compounds from plant materials [9]. Many techniques for the extraction of bioactive compounds in medicinal plants exist. These techniques are broadly classified as conventional and non-conventional techniques. The conventional techniques include soxhlet extraction, maceration, and hydro-distillation and solvent- solvent extraction [10]. Due to the attendant

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limitations of the conventional techniques some of which include; lengthy extraction time, need for costly and high purity solvents, evaporation of the large amount of solvent, poor extraction selectivity and thermal decomposition of thermolabile compounds [11], more promising extraction techniques referred to as the nonconventional techniques were developed which include ultrasound assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction [12]. Recent dry method refers to drying and grinding process and extraction, it is combination of drying, grinding, and controlled sieving processes [13]. Becker *et al.* [14], reported that the sieving process separates plant powders by granulometric differentiation through sieves of decreasing mesh, leading to selective distribution of bioactive molecules in the different granulometric fractions.

This research work is aimed to examine the influence of powder fractions obtained by extraction techniques using grinding and sieving, and solvent extracts on total phenolic content, total flavonoid, total tannin contents and antihyperlipidemic activity of *D. mespiliformis* fruit.

MATERIALS AND METHODS

Plant material

The dried fruits of *Diospyros mespiliformis* was collected during the month of February, 2017, in Kaélé, Far-North region of Cameroon, and were transported at the laboratory of Biophysics, Food Biochemistry and Nutrition of Ngaoundere University.

Preparation of ethanolic and hydroethanolic extracts of Diospyros mespiliformis fruits

The collected fruit pulp was finely ground into powder form by using electrical grinder. The seeds were separated from the pulp using a mesh. The powder was stored in a clean air-tight plastic container at room temperature until use. About 100 g of the powder was weighed into 1000ml of ethanol (98 %) for ethanolic extracts and 100 g of the powder was weighed into 1000ml of ethanol (50 %) in the ratio 1/10 (m / v) for hydroalcoholic extracts for 24 hours with occasional shaking. The ethanol soluble residue was filtered off and concentrated under vacuum at room temperature using a rotary evaporator to yield extract according to conventional procedure. The obtained product after evaporation was frozen in a freezer and then lyophilized using a lyophilizer.

Production of powder fractions

About 1.5 kg of *D. mespiliformis* fruits were slowly ground (50 g plant for each batch) with the Ultra Centrifugal Mill ZM 200 (Retsch France), operating by impact and shearing effects. Grinding was performed at room temperature (approximately 20 °C) using a 1 mm sieve with trapezoid holes. The grinding time was fixed to 2 min. Indeed, [15], showed that concentrations of polyphenols, flavonoids and the antioxidant properties decrease when the grinding time increases. One speed was used for milling: 6000 rpm. After grinding, plant powders were sieved with the vibratory sieve shaker Analysette 3 Spartan (Fritsch, Idar-Oberstein, Germany) at 0.5 mm vibration amplitude for 10 min. 20 mm diameter sieves (Fritsch) were employed so as to obtain the following granulometric fractions: <50µm; 50–180µm; 180–315µm; >315µm. Powders were then stored under vacuum in sealed bags at 10 °C.

Total phenolics content

Preparation of extracts

Extraction of polyphenols from *D. mespiliformis* fruits were carried out according to the method of Kim et al. [16], with some modifications. 5 g ethanolic, hydroalcoholic extracts of dried fruits and 2 g of powdered dried fruits were macerated during 24 h under stirring, in 25 mL methanol/water 70/30 (v/v). Then, the methanol/water extract was centrifuged at 20,000 rpm for 20 min. The supernatant layer was filtered, brought to 15mL and stored at 4 °C before analysis.

Total phenolic compounds were determined according the protocol used by Wafa et al [17]. 20 μ L of samples was mixed with 500 μ L of Folin-Ciocalteu reagent (1 N) and 400 μ L of sodium carbonate (Na₂CO₃, 20%). Samples and blank were thoroughly mixed and vortexed. After 30 min of incubation at room temperature, the absorbance was measured at 760 nm. Total phenolic content was expressed in terms of equivalent amounts of gallic acid (0,2 g/L) per 100 g of dry matter (GAE/100g DW). Absorbance measurements were carried out with UV–visible spectrophotometer.

Total flavonoids content

Total flavonoid compounds were dosed by a colorimetric assay described by Dewanto et al. [18]. 0.1mL of sample were added to a volumetric flask containing 2.4mL of distilled water and 0.1mL of sodium nitrite (Na₂NO₂, 5 %) were added to the flask. After 6 min, 0.3mL of aluminum chloride (AlCl₃, 10 %) were added. After 5 min, 1mL of sodium hydroxide (NaOH, 1 M) were added to the mixture. At this level the mixture was diluted with 2.5 mL of distilled water, then the vortexed samples were kept at room temperature. The absorbance was directly measured at 510 nm. For use as calibration curve, diluted solutions of catechin (0,1 g/L) were used and total flavonoids were expressed as mg of catechin equivalent per 100g of dry matter (mg CE/100 g DM). As for Total phenolic compounds, absorbance measurements were performed with UV–visible spectrophotometer.

Total tannins content

Total tannins compounds were dosed by a colorimetric assay described by Sun et al [19]. 0.05 μ L of sample were added to a volumetric flask containing 3mL of vanillin (4 %), 1.5mL of sulfuric acid concentrate were added to the flask. After vortexed samples were incubate 30min at room temperature, the absorbance was measured at 500 nm. The absorbance was directly measured at 510 nm. For use as calibration curve, diluted solutions of catechin (0,1 g/L) were used and total tannins were expressed as mg of catechin equivalent per 100g of dry matter (mg CE/100 g DM). As for Total phenolic compounds, absorbance measurements were performed with UV–visible spectrophotometer.

Animals

Healthy Wistar albino male rats weighing between 200g to 300g were obtained from the animal house of National School of Agro-Industrial Sciences of the University of Ngaoundere in Cameroon. The animals were kept in well-ventilated cages maintained under standard lab conditions (12/12 h light/dark cycle; 25±1°C, 35 to 60% relative humidity) in the animal house of biochemistry and nutrition laboratory. The rats had access to food and allowed free access to clean fresh water in bottles ad libitum. The animals were left to acclimatize to laboratory conditions for at least two weeks before the start of the experiment.

Experimental design

Preparation of High Fat Diet for inducing Hyperlipidemia.

The High Fat Diet induced hyperlipidemia (HFD) was formulated with 300 g of egg yolk, 2 g of cholesterol, 250 g of coconut oil and 50 g of soya oil, as described by Hamlat *et al.* [20], with some modifications. Table 1 shows the different formulations.

Constituents		Normal Diet	High Fat Diet
Proteins	Fish powder	200	140
Glucids	Corn ash	590	300
	Sucrose	50	50
Lipids	Coconut oil	0	250
	Yellow egg	0	300
	Soybean oil	50	50
Other	Cellulose	50	0
	Minerals	50	50
	Vitamins	10	10
Total		1000	1150

Table -1: Diff	erent formula	tions for rats	s (g / k	g of feed)

Animal study

For the study, the animals were weighed, recorded, numbered, and randomly divided into 10 groups of five animals each. Treatment was done at the same time as induction for 30 days between 07:00 and 10:00 h Group I rats were treated as the normal control received normal diet + distilled water. Groups II rats served as negative control received High Fat Diet + distilled water. Groups III rats served as positive control received High Fat Diet + atorvastatine (10mg/kg of body weight). Group IV animals were treated orally with *D. mespiliformis* powder fraction <50 µm at a dose of 600mg/kg of body weight in 5ml of sterile water. Groups V animals were treated orally with *D. mespiliformis* powder fraction 180-315 µm at a dose of 600mg/kg of body weight. Groups VII animals were treated orally with *D. mespiliformis* powder fraction > 315 µm at a dose of 600mg/kg of body weight. Groups VII animals were treated orally with *D. mespiliformis* powder fraction > 315 µm at a dose of 600mg/kg of body weight. Groups VIII animals were treated orally with *D. mespiliformis* powder at a dose of 600mg/kg of body weight. Groups VIII animals were treated orally with *D. mespiliformis* powder at a dose of 600mg/kg of body weight. Groups IX animals were treated orally with *D. mespiliformis* unsieved powder at a dose of 600mg/kg of body weight. Groups IX animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups IX animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups X animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups X animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups X animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups X animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight. Groups X animals were treated orally with *D. mespiliformis* of 600mg/kg of body weight.

On 30nd day, after overnight fasting, animals were weighed, anesthetized and dissected; the blood was collected from the neck. The serum was obtained by centrifugation of blood at 1000 rpm, for 10 minutes. The liver, kidney, stomach, heart and testis tissues of each rat were removed and weighed immediately and the ratio of organ to body weight calculated.

Biochemical Parameters

Levels of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were evaluated using enzymatic kits (HUMAN kits) according to procedure described by Richmond and Glick et al. [21, 22]. Low Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) were measured by using Friedewald's formula. VLDL cholesterol (mg/dl) = TG ÷ 5 LDL cholesterol (mg/dl) = TC – (HDL + VLDL).

Determination of atherogenic index (AI)

Atherogenic index is an important measure to elucidate the anti-atherogenic index potential of a plant. Al was calculated as: AI = (TC-HDL- C)/HDL-C [23].

STATISTICAL ANALYSIS

Results were expressed as mean + S.D and all statistical comparisons were made by means of one-way ANOVA test followed analysis and P – Values less than or equal to 0.05 were considered significant. Duncan's multiple ranking test was used to rank the averages. All of these analyzes were done using the Stat Graphics Plus 5.0 software and plot curves using the Excel 2010 software.

RESULTS AND DISCUSSION

The first part of this study was to determine influence of particle size of dry powder fruits, and solvent extraction (ethanol and hydroethanol) on total phenolics, flavonoids, tannins.

Results are presented in Table 2; the solvent extraction concentrates more polyphenol compounds than powder fractions. the powder fractions concentrate more polyphenol compounds is fraction < 50 μ m after follow by 50-180 μ m fraction.

Sample	Total flavonoids (mg	Total tannins (mg CE/g	Total phenolics (mg			
-	CE/g DW)	DW)	GAE/g DW)			
< 50 µm	41.46 ± 2.32 ^d	62.80 ± 2.26 ^f	152.89 ± 1.86 ^e			
100 -180 μm	30.55± 4.08°	42.73 ± 3.98 ^d	138.34 ± 1.86°			
180 -315 μm	27.88 ± 4.25 ^b	35.25 ± 2.07°	148.55 ± 1.87 ^d			
≥ 315 µm	31.65 ± 4.27°	29.25 ± 2.08ª	115.23 ± 1.89 ^b			
Powder	25.79 ± 4.30ª	32.21 ± 4.19 ^b	90.20 ± 2.56ª			
Hydroethanol extracts	43.54 ± 2.18 ^e	44.73 ± 4.25 ^e	160.00 ± 1.86 ^f			
Ethanol extracts	56.90 ± 9.05^{f}	76.42 ± 3.319	204.27 ± 1.879			

Table -	2. Total	nhenolics	flavonoids and	tannins of	Diospyros	mesniliformis fruits
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DW: dry weight; CE: catechin equivalents; GAE: gallic acid equivalents

Values in the same column with different superscripts (a to g) are significantly different (P<0.05).

Total phenolic content increased when granulometry decreased from 315 μ m to 50 μ m. The ethanol extract showed a highest total phenolic content 204.27 mg GAE/100 g DW, which is superior than total phenolic in powder and hydroethanol extract. With powder optimal parameter to obtain the highest total phenolic was the < 50 μ m granulometry which given 152.89 mgGAE/100gDW. Total flavonoids like the polyphenols, are distributed according to their particle sizes which in this study are particularly concentrated in the fraction < 50 μ m and 50 -180 μ m. Our results are in accordance with results of [24], who found the highest content of flavonoids in green tea leaves in the 100-180 μ m fraction. And total tannin content followed the same tendency as total phenolics and total flavonoids.

Effect of solvents extracts and powder of *Diospiros mespiliformis* fruits on food intake, body weight gain/loss, organs weight, food efficiency and lipid profile high-fat diet induced

Results related to effect of *D. mespiliformis* on body weight and food intake of albino rats in different experimental groups have been provided in Table 3. After 30 days of treatment only Negative control group showed significant increasing of body weight and test groups lost. The body weight gain is due to high fat diet used to induce hyperlipidemia in the rats. High fat diet increase energy intake and energy storage [25]. The loss of body weight may be due to the ability of *D. mespiliformis* to reduce the fat absorption and lipogenic enzymes and increase the fat excretion [26]. In the present investigation, *D. mespiliformis* reduced the body weight gain in different groups with solvents extracts as well as with powder fractions. This may be due to the polyphenolics, flavonoids and tannins compounds, in *D. mespiliformis* fruits which may reduce fluid absorption and facilitate weight loss [27].

Parameters	Body weight	Food intake
Groups	gain/loss (g)	(g)
Negative control	13.14 ± 2.85 ^d	200.01 ± 4.80 ^a
Normal control	0.49 ± 2.94°	198.89 ± 4.85 ^a
Positive control	-4.02 ± 2.95 ^{bc}	194.02 ± 4.84 ^a
< 50µm	-14.23 ± 2.89ª	190.41 ± 4.82ª
50-180µm	-8.77 ± 2.95 ^{ab}	189.82 ± 4.80 ^a
180-315µm	-3.58 ± 2.88 ^{bc}	193.40 ± 4.86 ^a
> 315 µm	-1.05 ± 2.95 ^{bc}	196.27 ± 4.83 ^a
Unsieved powder	-1.0 ± 2.92 ^{bc}	190.42 ± 4.82 ^a
Hydroathanolic extract	-8.40 ± 2.94 ^{ab}	196.60 ± 4.81ª
Ethanolic extract	-8.76 ± 2.90 ^{ab}	196.79 ± 4.85 ^a

Tableau-3: Food intake and body weight gain/loss of rats

Values in the same column with different superscripts (a to d) are significantly different (P<0.05).

Organ-to-body weight ratios of the rats after administration of the *D. mespiliformis* extracts and powder fractions are presented in Table 4. Decrease of organ-to-body weight ratios indicating a hyper functioning of that organ; increase in the weight may be due to it degenerative changes or loss of tissue of that particular organ [28]. The organ-to-body weight ratios of liver, heart, kidney, stomach and testis of all test groups were significantly upper to those of negative control group and no different to those of normal and positive control.

-					
Organs	Heart	Liver	Kidney	Stomach	Testis
Groups			-		
Negative control	0.43 ± 0.004e	4.38 ± 0.007e	$0,84 \pm 0,003^{i}$	0.92 ± 0.003 ^f	1.04 ± 0.060b
Positive control	0.30 ±0.008bc	3.25 ± 0.003 ^{bcd}	$0,52 \pm 0,006^{bc}$	0.62 ± 0.004^{ab}	1.10 ± 0.006 ^{bc}
Normal control	0.29 ± 0.004 ^b	3.35 ±0.003 ^{bcd}	0,50 ± 0,002ª	0.58 ± 0.000 ^a	1.14 ± 0.006 ^{bc}
< 50µm	0.30 ± 0.019^{bc}	3.58 ± 0.054 ^{cd}	$0,52 \pm 0,003^{abc}$	0.64 ± 0.040^{bc}	1.04 ± 0.040 ^b
50-180µm	0.30 ± 0.002^{b}	3.14 ± 0.019 ^{bc}	$0,55 \pm 0,005^{bc}$	0.67 ± 0.002^{bc}	0.88 ± 0.009 ^a
180-315µm	0.31 ± 0.004 ^{cd}	3.05 ± 0.013 ^b	$0,57 \pm 0,020^{cd}$	0.69 ± 0.030^{bc}	1.10 ± 0.009 ^{bc}
> 315 µm	0.30± 0.011b	2.42 ± 0.024 ^a	0,60 ± 0,025 ^{de}	0.67 ± 0.012℃	0.84 ± 0.004 ^a
Unsieved powder	0.36 ± 0.003^{d}	3.21 ± 0.050 ^{bcd}	0,71 ± 0,006 ^{gh}	0.81 ± 0.014 ^e	1.05 ± 0.047⁵
Hydroethanolic	0.24 ± 0.005 ^a	3.55 ± 0.575 ^{cd}	0,72 ± 0,046 ^h	0.74 ± 0.042 ^d	1.18 ± 0.112°
extract					
Ethanolic extract	0.32 ± 0.003°	3.60 ± 0.263 ^d	$0,67 \pm 0,003^{fg}$	0.73 ± 0.000^{d}	1.11 ± 0.100 ^{bc}

Table-4: Organ-to-body weight ratios in rats after oral administration of extracts and powder fractions of *D. mespiliformis* fruits

Values in the same column with different superscripts (a to g) are significantly different (P<0.05).

The results of the effect of solvents extracts and powder fractions of *D. mespiliformis* fruits on lipid profile of rats are summarized in Table 5. It was seen that, there was a significant increase in all the lipid parameters (p < 0.05), except HDL- C, following administration of high fat diet. It is well known that diet plays an important role in the control of cholesterol homeostasis. The consumption of cholesterol-enriched diet is regarded as an important factor in the development of cardiovascular diseases as it leads to the development of hyperlipidemia, atherosclerosis and abnormal lipid oxidation/metabolism [29, 30]. The hypolipidemic activity of the groups taken extracts or powder fractions of *D. mespiliformis* was found to be slightly less efficacious than that of the standard drug but compared to the negative control group, their total cholesterol, triglyceride, LDL- C and VLDL- C are significantly low and their HDL-c higher. Hypolipidemic properties of *D. mespiliformis* may be explain by their polyphenols, in fact, polyphenols induce metabolic hypolipidemic effect mainly by their ability to reduce cholesterol acyltransferase and HMG-CoA reductase activities [3].

Parameters	TC	HDL- C	TG	LDL- C	VLDL- C	
Groups						
Negative control	173.93 ± 3.20 ^e	20.9 ± 0.31ª	145.79 ± 7.67d	125.17 ± 3.20g	29.16 ± 1.54 ^d	
Positive control	101.47 ± 1.77 ^{bcd}	47.65 ± 1.34 ^f	72.55 ± 1.51ª	39.31 ± 0.73 ^b	14.51 ± 0.30ª	
Normal control	82.22 ± 0.32ª	41.42 ± 1.90 ^e	78.84 ± 9.53 ^{ab}	25.03 ± 0.31ª	15.75 ± 1.91 ^{ab}	
< 50 µm	94.99 ± 1.34 ^b	25.01 ± 0.31b	66.0± 0.07ª	56.79 ± 1.01°	13.2 ± 0.01ª	
50-180µm	101.87 ± 0.95 ^{bcd}	27.86 ± 0.62 ^{bcd}	74.44 ± 6.18 ^a	59.13 ± 0.92 ^{cd}	14.89 ± 1.23ª	
180- 315 µm	107.95 ± 3.19 ^{cd}	27.86 ± 1.36 ^{bcd}	93.16 ± 6.13 ^{bc}	61.46 ± 0.61 ^{de}	18.63 ± 1.23 ^{bc}	
≥ 315 µm	110.50 ± 3.52 ^d	29.79 ± 1.52 ^d	103.53 ± 17.36°	64.0 ± 1.45 ^e	19.70 ± 2.06°	
Unsieved powder	98.61 ± 11.73 ^{bc}	28.31 ± 2.24 ^{cd}	77.57 ± 8.92 ^{ab}	61.18 ± 1.34 ^{de}	15.51 ± 1.78ª	
Hydroethanolic extract	108.50 ± 2.57 ^d	25.86 ± 1.58 ^{bc}	100.30 ± 3.92°	62.58 ± 1.77 ^e	20.07 ± 0.79°	
Ethanol extract	109.75 ± 1.48 ^d	29.92 ± 0.12 ^d	97.24 ± 2.47°	60.38 ± 0.86 ^{de}	19.45 ± 0.50°	

Table-5: Effect of powder fractions and extract of D. mespilifomis fruits on serum lipide profile

TC: total cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: triglycerides;

Values in the same column with different superscripts (a to g) are significantly different (P<0.05).

Table 6 shows the atherogenic index of rats. Oral administration of extracts and powder fractions prevented the rise of atherogenic index, the fractions powder >50 µm, 50-180 µm and solvent extracts showed strong effect. High cholesterol diet with cholic acid increases TC, LDL-C, atherogenic index and decrease HDL-C by enhancing intestinal absorption and secretion and decreasing catabolism of cholesterol [31]. Treatment with extracts and powder fractions of D. mespilifomis caused a significant decrease in mean serum TC and LDL-C and increased HDL-C, the consequence is decreasing of atherogenic index, which is considered as better indicator of coronary heart disease risk than individual lipoprotein concentration [32].

Groups	Atherogenic index
Negative control	7.32 ± 0,02 ^e
Positive control	1.13 ±0,02ª
Normal control	0.99 ± 0,09 ^a
< 50 µm	2.79 ± 0,01 ^b
50-180µm	2.66 ± 0,05 ^b
180- 315 µm	2.87 ± 0,07 ^b
≥ 315 µm	3.12 ± 0,00°
Unsieved owder	2.71 ± 0,20°
hydroalcoholic extracts	3.20 ± 0,15 ^b
Ethanol extracts	2.67 ± 0,03°

Table -6: Effect of pow	der fractions and	d extract of D. m	espilifomis fruits	on atherogenic index
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Values in the same column with different superscripts (a to c) are significantly different (P<0.05)

CONCLUSION

These results revealed that polyphenols compounds and antihyperlipidemia activity depend on powder fractions and the solvent extract. *Diospyros mespiliformis* can be safely used in the treatment of mild to moderate cases of hyperlipidemia considering its availability, cost effectiveness, and other beneficial effects.

REFERENCE

- 1. Paccaud, F., Fasmeye, V. S., Wietlisbach V. & Bovet, P. (2000). Dyslipidemia and abdominal obesity: an assessment in three general populations. *Journal of Clinical Epidemiology*, 53(4):393-400.
- 2. Tilak, K. S., Veeraiah, K., & Koteswara, R. D. K. (2001). Restoration on tissue antioxidants by fenugreek seeds (Trigonella Foenum Graecum) in alloxan-diabetic rats. *Indian Journal of Physiology and Pharmacological*, 45:408-420.
- 3. Ballantyne, C.M. (2007). Treatment of Dyslipidemia to Reduce Cardiovascular Risk in Patients with Multiple Risk Factors. *Clinical Cornerstone*, 8(6): S6-S13.
- 4. Kourounakis, A. P., Victoratos, P., Peroulis, N., Stefanou, N., Yiangou, M., & Hadjipetrou, L. (2002). Experimental hyperlipidemia and the effect of NSAIDs. *Experimental and Molecular Pathology*, 73: 135-140.
- Grundy, S. M., Cleeman, J. I., Merz, C. N., Brewer, H. B., Clark, L. T., Hunninghake, D. B., Pasternak, R. C., Smith, S. C. & Stone, N. J. (2004). National Heart, Lung, and Blood Institute; American College of Cardiology Foundation; American Heart Association. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation*, 110(2): 227-239.
- 6. Rates, M. (2001). Plants as source of drugs, Toxicon Off. Journal of International Society Toxicology. 39.
- 7. Braithwaite, M., Tyagi, C., Tomar, K., Kumar, P., Choonara, E. & Pillay V. (2014). Nutraceutical-complement modern medicine. *An overview Journal of Functional Foods.* 6 82–99.
- 8. Evans, W. C. (2008). General methods associated with the phytochemical investigation of herbal products. In *Trease and Evans Pharmacognosy* (15 ed.), New Delhi: Saunders (Elsevier),137-148.
- Wang, X.Q., Wei, F.Y., Wei, Z.F., Zhang, L., Luo, M., Zhang, Y.H., (2014). Homogenate-assisted negative-pressure cavitation extraction for determination of organic acids and flavonoids in honeysuckle (Lonicera japonica Thunb.) by LCeMS/MS. Separation and Purification Technology, 135, 80-87.
- 10. Penchev, I. (2010). Étude des procédés d'extraction et de purification de produits bioactifs à partir de plantes par couplage de techniques séparatives à basses et hautes pressions Thèse de Doctorat, Institut National Polytechnique de Toulouse, France.
- 11. Luque de Castro, M. D. & Garcia-Ayuso, L. E. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytical and Chimical Acta*, 369 (1–2): 1–10.
- Azmir, J., Zaidul, I. S. M., Rahman, A. M. M. Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N. & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117. 426–436.
- 13. Baudelaire, E. (2013). Brevet WO2013057379A1 Procédé Ptc Pour l'extraction Par Voie Sèche Des Principes Actifs Naturels. GoogleBrevets http: //www.google.com /patents/ WO2013057379A1. cl=fr.
- Becker, L., Zaiter, A., Petit, J., Karam, M. C., Sudol, M., Baudelaire, E., & Dicko, A. (2017). How do grinding and sieving impact on physicochemical properties, polyphenol content, and antioxidant activity of *Hieracium pilosella* L. powders. *Journal of Functional Foods*, 35, 666–672. https://doi.org/10.1016/j.jff.2017.06.043
- 15. Hu, J., Chen, Y., & Ni, D. (2012). Effect of superfine grinding on quality and antioxidant property of fine green tea powders. *LWT-Food Science and Technology*, 45, 8–12.
- 16. Cuji'c, N., Savikin, K., Jankovi'cT, Pljevljaku si'c, D., Zduni'c & G Ibri'c, S. (2015). Optimization of polyphenols extraction from dried chokeberry usingmaceration as traditional technique. *Food Chemistry*. 194: 135–142,http://dx.doi.org/10.1016/j.foodche.
- 17. Wafa, G., Amadou, D., Larbi, M. & Héla, O. (2014). Larvicidal activity, phytochemical composition, and antioxidant properties of different parts offive populations of *Ricinus communis* Leading *Indian Cro. Pro.* 56: 43–51.

- 18. Dewanto, V., Wu, X., Adom, K. K. & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, 50: 3010-3014.
- 19. Sun, B. S., Ricardo-Da-Silva, J. M. & Spranger, M. I. (2008). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 107(10), 4267–4274.
- 20. Hamlat, N., Neggazi, S., Benazzoug, Y., Kacimi, G., Chaïb, S., & Aouichat-Bouguerra, S. (2008). Régime hyperlipidique et processus athéroscléreux chez Rattus norvegicus. *Sciences & Technologie. C, Biotechnologies*, (27), 49-56.
- 21. Richmond, W. (1973). Medical analysis. Clinical Chemistry, 19, p. 1350.
- 22. Glick, M. R., Ryder, K. W., & Jackson, S. A. (1986). Graphical comparisons of interferences in clinical chemistry instrumentation. *Clinical Chemistry*, 32(3), 470-475.
- Makni, M., Fetoui, H., Gargouri, N. K., Garoui, E. M., Jaber, H., Makni, J., ... & Zeghal, N. (2008). Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. *Food and Chemical Toxicology*, 46(12), 3714-3720.
- Becker, L., Zaiter, A., Petit, J., Karam, M. C., Sudol, M., Baudelaire, E., ... & Dicko, A. (2017). How do grinding and sieving impact on physicochemical properties, polyphenol content, and antioxidant activity of Hieracium pilosella L. powders?. *Journal of Functional Foods*, 35, 666-672.
- 25. Onyeike, E. N., Monanu, M. O., & Okoye, C. N. (2012). Changes in the blood lipid profile of wistar albino rats fed rich cholesterol diet.
- Rains, T. M., Agarwal, S. & Maki, K. C. (2011). Antiobesity effects of green tea catechins: a mechanistic review. Journal of Nutrition and Biochemistry, 22:1–7.
- 27. Amin, K. A., & Nagy, M. A. (2009). Effect of carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetic and Metabolic Syndrome Journal*, 1: 1-17.
- Raza, M., Al-shabanah, O., El-Hadiyah, T. & Al-Majed, A. (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of swiss albino mice. *Scientia pharmaceutical*, 70:135-145.
- 29. Onody, A. M., Csonka, C., Giricz, Z. & Ferdinandy, P. (2003). Hyperlipidemia induced by a cholesterol-rich diet leads to enhanced peroxynitrite formation in rat hearts. *Cardiovascular Research*, 58: 663-670.
- Rajasekaran, A., Vellaichamy, S. & Sabarimuthu, D. (2011). Effect of *Blepharis maderaspatensis* L. Roth. Extracts on serum lipids in Triton WR-1339 and high cholesterol diet induced hyperlipidemia in rats. *African Journal of Pharmacy and Pharmacology*, 7(37), pp. 2577-2583, 8.
- 31. Heuman, D. M., Vlahcevic, Z. R., Bailey, M. L. & Hylemon, P. B. (1988). Regulation of bile acid synthesis. II. Effect of bile acid feeding on enzymes regulating hepatic cholesterol and bile acid synthesis in the rat. *Hepatology*, (4):892-897.
- 32. Vijayakumar, R. S., Surya, D. & Nalini, N (2004). Antioxidant efficacy of black pepper (Piper nigrum L.) and piperine in rats with high fat diet induced oxidative stress. *Redox Rep*, 9(2):105-110.