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Qualitative and quantitative comparison of rutin, quercetin and gallic acid concentrations in Syrian *Capparis spinosa.* L Leaves

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Abstract
Flavonoïds and their glycosides are the most common secondary metabolites found in plant kingdom. They are known to have positive effects on human health by increasing resistance to different chronic diseases. Rutin and quercetine are considered the most important flavonoïds. The current study aimed to find the optimal method for extraction of antidiabetic active ingredients from Syrian *Capparis Spinosa.*L leaves. They mainly include flavonoïds (e.g. quercetin), glycosides (e.g. rutin) and simple phenols (e.g. gallic acid). The qualitative detection of these ingredients showed that the optimal extraction method of the leaves was Soxhlet method. Ethanol (80%) was the best extraction solvent to test flavonoïds. On the other hand, ethylacetate showed more effectiveness in glycosides extraction. By using Soxhlet method and ethanol as a solvent for the extraction from (100g) *Capparis spinosa* leaves, 2.728g rutin, 8.754g quercetin and 0.543g gallic acid were sequestered. However, using the same Soxhlet method with ethylacetate as a solvent, only 0.401g rutin and 3.389g quercetin were sequestered. HPLTC method was effective for the separation of rutin, quercetin and gallic acid, and showed that leaves are rich in antidiabetic active ingredients, that it will be beneficial for the pharmaceutical industry of antidiabetic drugs.

Keywords: rutin, quercetin, gallic acid, extraction, *Capparis spinosa*, leaves, fruits.

1. Introduction
Medicinal plants are acquiring special importance concomitant with the great acceleration of the chemical component discoveries that are effective in the treatment of many diseases, making them important source of medical materials, particularly in the third world countries [1]. These chemicals are produced as a result of plant's normal metabolic activities, including primary and secondary metabolites [2, 3, 4, 5]. The secondary metabolites include alkaloids, phenols, fatty acids, resins, steroids, flavonoïds, saponins and tannins [3], which their functions are varied. For example, some secondary metabolites are toxins used to protect plants from predators, and others are pheromones used to attract insects for pollination. Beside that most of them exert a pharmacological and therapeutic effects on humans, and their complex mixtures form the botanical medicines [2].

Phenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found. They can have a simple structure with low molecular weight and single phenol ring, or can have a high molecular weight and be structured by several phenol rings. Phenols are found in plants in the free form or linked to sugar or organic acids [6]. This linkage determines the solubility of phenols in water or fat medium, that sugar and hydroxyl radicals increase the phenol solubility in aqueous solution, while methyl and isopentyl radicals make phenols lipophilic, which indicates the importance of the solvent in extraction of these components.

Gallic acid is a type of simple phenol compounds (3, 4, 5- trihydroxybenzoates) found commonly in plant tissues. It possesses an astringent activity [6, 7, 8].

Flavonoïds is a group of polyphenol compounds, which are widely distributed through the plant tissues. To date, about 300 varieties of flavonoïdes are known [7, 8]. Recent studies suggest that flavonoïdes have remarkable role in the human health, especially in the matter of resistance to various diseases [9], like antiallergenic [10], antiinflammatory, antifungal and antispasmodic effects [11]. Flavonoïds also exert other therapeutic effects such as antihepatotoxic, antitumor [12], antiulcer, and some of them provide protection against cardiovascular mortality and heart ailments [13]. Finally, the recent studies show that flavonoïds possess an antidiabetic effect [14, 15] and reduce the low density lipoproteins (LDL) [16, 7].

Flavonoïds occur in plant tissues in a free state, (Quercetin: 5, 7, 3', 4'-tetrahydroxy flavonol), or in glycosides (Rutin: 5, 7, 3, 4, tetrahydroxy flavonol-3-rhamnoglucoside) [7, 8, 11]. Quercetin and rutin are the most common flavonoïds found in plant tissues, especially plants that are used in diet, like onion and apple [6]. Quercetin and rutin possess an antioxidant activity [17, 18] making them able to protect from or treat the diseases that result from free radicals accumulation, like cancer [19, 20] and diabetes mellitus [21]. Several studies also proved that rutin and quercetin have shown a regulatory effect on hormone activities, such as regulating the thyroid hormone activity [7]. Rutin and quercetin possess an antihepatitis effect, an antifungal and antiviral actions [22, 23], also they have shown an analgesic effects [24].

Qualitative and quantitative analytical validations of chemical compounds in herbal products subjected to be used in medicinal or nutritional fields, are extremely important to avoid many side effects like, direct poisonous effects, allergic reactions and effects related to contamination [13, 11]. Analytical validations include: preliminary qualitative phytochemical screening by using specific colored reagents or radio isotopes, followed with quantitative determination of specific chemical compounds by high performance thin layer liquid chromatography (HPTLC), and using standards as markers. HPTLC is one of the most important analytical technique to determine the concentration of chemical components in plant extracts, because of its reliability, simplicity, accuracy, speedy and low cost [10, 8, 7, 25].

This research aimed to prepare leaves' extracts of Syrian *Capparis spinosa* by using different extraction methods and different solvents, then to apply on extracts a qualitative chemical detection and a quantitative separation of chemicals known with their antidiabetic effects, like rutin, quercetin and gallic acid [8, 11, 7]. We studied the effect of both, the extraction method and the solvent on the quantity of chemical extracted. We performed a statistical validation on HPTLC method to confirm the accuracy, sensitivity and repeatability of the quantitative chemical separation from the extracts.

The reason of our interest on the quantitative separation of the specific chemicals contained in Syrian *Capparis spinosa* relies to the global interest on this plant and their constituents of biological active substances, especially rutin and quercetin [23]. The latest studies indicate that *Capparis spinosa* contains more quercetin than any other medicinal plants [26, 27, 28]. *Capparis spinosa* is rich with secondary metabolites, especially flavonoïds, alkaloids, glycosides and organic acids [29, 30], making it a promising plant in the field of botanical drug industry. We hope using the botanical drugs derived from *Capparis spinosa* as a complement diet in purpose to reduce the doses of classic medicines used in the treatment of chronic diseases, such as diabetes mellitus [23].

2. Materials

Standard rutin trihydrate from Fluka**,** no.78095. Standard Quercetin from Sigma no.Q4954. Standard Gallic acid from Fluka no.91215. All other chemicals are purchased from Merk with 99% purity. TLC aluminum plates silicagel $60F_{254}$ [20×20 cm, 0.2mm thickness], TLC scanner 3 with a UV cabinet & Linomat 5, CAMAG, WINCATS Program.

3. Methods

Preparation of plant material

Leaves were collected in May and June from Damascus city**,** Syria. They were dried at ambient temperature till 5% humidity, preserved in opaque containers till extraction.

Preparation of plant extracts

- **Soxhlet method:** 25 g of dried and crashed leaves were extracted by Soxhlet apparatus with 250 ml of extraction solvent, (ethanol 80%, ethylacetate).
- **Maceration method:** 25 g of dried and crashed leaves were extracted by Maceration for 7 days at ambient temperature with 250 ml extraction solvent (ethanol 80% or ethylacetate). the extract was filtered under pressure through Bokhnar funnel.
- **Maceration & stirring method:** 25 g of dried and crashed leaves were extracted by maceration and stirring with 250 ml extraction solvent (ethanol 80% or ethylacetate) at 40˚C for 48 hours.

All extracts were evaporated by rotavapor under low pressure, washed several time with methanol until obtaining the smallest volume. Then, the extracts were transmitted to an evaporation plates for more evaporation in a water bath at 40 ˚C until the full extract dryness. After that, the evaporation plates were transmitted to a desiccator containing silica gel till the stably weight.

By the end of the extraction steps, we obtained 6 different extracts related to different solvents and methods of extraction.

Qualitative chemical detection [31, 32, 3, 33].

i) Detection of flavonoïds. The extract solution (5 mg dried extract/ ml distilled water) was prepared and filtered through Wathmann paper Nº1.

- **$FeCl_3$ test:** the test is considered positive if a greenish blue or violet color appears after adding a few drops of ferric chloride (10%) to 2 ml extract.
- **Lead acetate test:** the test is considered positive if a yellow orange precipitate appears after adding 3 ml of lead acetate (10%) to 5ml extract**.**
- **Shinoda test:** the test is considered positive if a light pink or purple color appears after adding a few drops of concentrated hydrochloric acid and a few crystals of magnesium to 2 ml extract.
- **Sodium hydroxide test:** the test is considered positive if a yellow orange color appears after adding 2 ml of sodium hydroxide (10%) to 2 ml extract, and this color disappears when hydrochloric acid is added.

ii) Detection of phenols. The extract solution (10 mg dried extract/ ml ethanol 95%) was prepared and filtered through Wathmann paper Nº1.

- **$FeCl_3$ test:** the test is considered positive if a black blue or greenish blue color appears after adding a few drops of ferric chloride (5%) to 2 ml extract.
- **Lead acetate test:** the test is considered positive if a white precipitate appears after adding 3 ml of lead acetate (10%) to 2 ml extract**.**
- **Dil. HNO_3 test**: the test is considered positive if a red color appears after adding a few drops of diluted nitric acid to 2 ml extract.

iii) Detection of glycosides. The extract solution (10 mg dried extract / ml ethanol 95%) was prepared and filtered through Wathmann Nº1.

- **Borntrager test:** to 3 ml extract, dilute sulphuric acid is added**,** boiled and filtered. To the cold filtrate equal volume of benzene is added. The organic layer is separated and ammonia is added. Ammonical layer turns pink or red which indicates the presence of anthraquinone glycosides.

- **Legal test:** to 2 ml extract, few drops of sodium hydroxide (10%) and few crystals of sodium nitroprusside are added. The appearance of pink or red color indicates the presence of cardio digitoxin glycosides.
- **Keller- killiani test:** to 2 ml extract, glacial acetic acid, one drop of ferric chloride (5%) and one drop of concentrated sulfuric acid are added. The appearance of reddish brown color at the junction of the two liquid layers, and greenish blue color at the upper layer, indicates the presence of cardiac glycosides.

Quantitative chemical detection by high performance thin layer chromatography (HPTLC) [7, 8]**.**

Standard solutions of rutin, quercetin and gallic acid (1 mg/ml methanol) are prepared. Solutions of leaves' extracts (10 mg/ml methanol) are also prepared. All solutions are filtered through Wathmann Nº.42. The plates of silica gel are activated at 105˚C for 10 minutes. By using an automatic TLC applicator Linomat 5, 5 µl of each rutin, quercetin, gallic acid standard solutions and extracts solutions are spotted on TLC aluminum plates with silica gel 60 F_{254}, as 10 mm interval between spots, and 10 mm from the plate bottom. The spot components of standards and extracts are eluted and separated on the plates by several mobile phases. After complete liquid diffusion until 15 cm height, the plates are dried at ambient temperature, and scanned at 254, 280, 366 nm by CAMAG Scanner 3. The R_F and area under curve (AUC) for each component are calculated at the wave length giving the maximal optical absorption. The estimated concentrations of quercetin, rutin and gallic acid analogues in the extracts are calculated by rating the AUC of the extract component to the AUC of the known concentration to standard.

In this research, many different mobile phases are studied to reach the separation of the three standards (rutin, quercetin, gallic acid), and their analogues in the extracts. Those mobile phases are: ethylacetate- glacial acetic acid- formic acid- distilled water (100:11:11:25), toluene- ethylacetate- formic acid (7:5:1), ethylacetate- n butanol- distilled water (10:10:4), ethylacetate- n butanol- distilled water- formic acid (10:10:4:2). We chose ethylacetate- glacial acetic acid- formic acid- distilled water (100:11:11:25) as the best mobile phase. The experiment was repeated 5 times. We validated the method of quantitative separation by using rutin standard.

Validation of HPLTC method

The analytical validation of the quantitative separation of rutin standard by HPTLC was performed by following the recommendation of ICH-Q2R(1) [34]. Validation included the calculation of linearity & correlation coefficient, precision, recovery & accuracy.

Calibration curve for rutin standard

The rutin standard solutions (1.15, 0.98, 0.82, 0.66, 0.49, 0.33, 0.16 mg/ml) were spotted on TLC plate (5 µl /spot in triplicate). Solution spots were eluted by the mobile phase chosen and then the plates were scanned as the chromatographic conditions mentioned above. The area under curve to each concentration was read at 366 nm. The average of three area under curve for each concentration was calculated. Calibration curve of rutin was constructed by plotting the average of area under curve against concentrations of rutin applied (0.16- 1.15 mg/ml). Linearity & correlation coefficient were calculated by using Graph Pad Prism v.6.01 program.

Recovery

Different spot concentrations of standard rutin (95%, 113%, 137%) were added to a pre analyzed and known concentration of rutin spot sample of *Capparis*' leave alcoholic extract (0.261 mg/ml). The spots were then analyzed by HPTLC method in triplicate.

Precision

The relative standard deviation (RSD) was used as an indicator of the method precision over the entire linear calibration range. Standard rutin (0.33 mg/ml) was spotted on a TLC plate, for 7 times, then developed and dried. The separated spots were scanned and the concentrations were calculated by using the linear calibration curve equation, and then the intra-day precision were calculated.

4. Results

Yield of extraction (w/w%): The percentage of the dry extract weight resulting from the extraction of 100 grams of the dry plant parts, expresses the yield of the extraction. The yield of extraction differed by using **different solvents**; extraction of 25 g dry *capparis*' leaves by alcohol (80%) solvent and maceration & stirring method produced 6.9 g extract, while applying the same method of extraction with ethylacetate as a solvent produced 0.61 g extract. That means respectively, 27.6% and 2.44% as percentage yields (Figure1). The yield of extraction differed also by using **different methods**; the yields of extraction were 23%, 22.96%, 27.6% after applying respectively, Soxhlet, maceration and maceration & stirring methods with the same alcoholic (80%) solvent (Figure2).

The highest extraction yield by using ethylacetate solvent was with Soxhlet method, while the highest extraction yield by using alcohol 80% solvent was with maceration & stirring method.

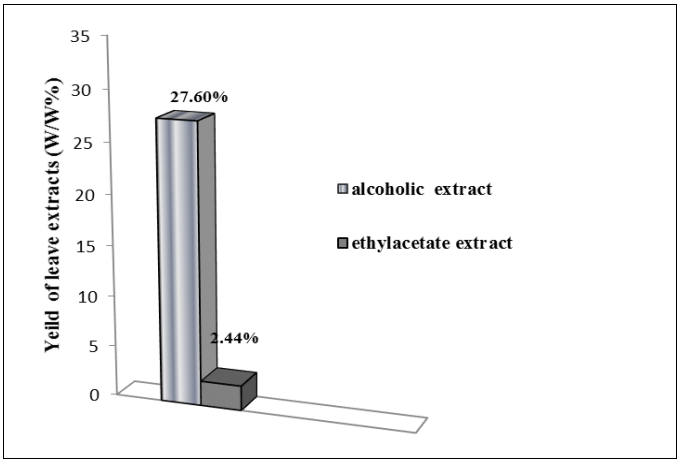

Fig 1: Difference of leave extract yield (w/w%) dependent on the extraction solvent (alcoholic or ethylacetate). Extraction was done by maceration & stirring method.

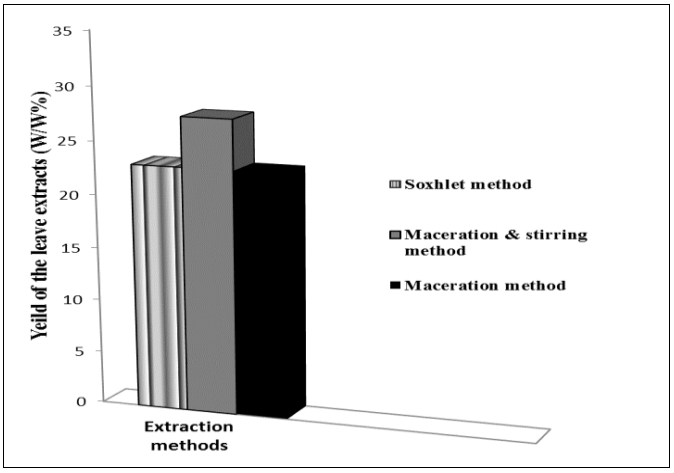

Fig 2: Difference of the leave extract yield (w/w%) dependent on the extraction method with the same solvent of extraction (alcohol 80%).

Qualitative chemical detection of *Capparis*' leave extracts

The qualitative chemical detection demonstrates the presence of flavonoïds and glycosides in all *Capparis* leave extracts (table 1). The highest estimated concentrations of flavonoïds were detected in alcoholic (80%**)** extracts, while the highest estimated concentrations of glycosides were detected in ethylacetate extracts.

Borntrager test was negative in all leave extracts, meaning that anthraquinone glycosides were absents. In contrast, Legal and Keller-Killiani tests were positives, meaning that non-saturated lactone glycosides were present in all leave extracts. Simple phenols were detected only in ethylacetate leave extract by Soxhlet method as ferric chloride and lead acetate tests were positive. In contrast, only ferric chloride test was positive in all other leave extracts, while lead acetate and diluted nitric acid tests were negatives, meaning the negativity of simple phenols presence. Table 1 shows that Soxhlet method was the best for the active substance extraction from *Capparis spinosa* leaves, either with alcohol (80%) or ethylacetate solvent; also it shows that glycosides were better extracted by ethylacetate solvent, while flavonoïds were better extracted by ethanol (80%).

Table 1: Qualitative chemical detection of flavonoïds, glycosides and simple phenols in leave extracts of *Capparis spinosa*.

Method of extraction	Simple phenol tests	Glycosides test	Flavonoïds test
Alcoholic extract by Soxhlet method	$FeCl_3$ *+++* Lead acetate **-** Dil.HNO_3 **-**	Borntrager **-** Legal *+* Keller-Killiani *++*	$FeCl_3$+++ Lead acetate +++ Shinoda +++ Sodium hydroxide +++
Alcoholic extract by maceration method	$FeCl_3$ *+++* Lead acetate **-** Dil.HNO_3**-**	Borntrager **-** Legal *+* Keller-Killiani *++*	$FeCl_3$ +++ Lead acetate +++ Shinoda +++ Sodium Hydroxide +++
Alcoholic extract by maceration & stirring method	$FeCl_3$ *+++* Lead acetate **-** Dil.HNO_3 **-**	Borntrager **-** Legal *++* Keller-Killiani *+++*	$FeCl_3$ +++ Lead acetate +++ Shinoda ++ Sodium Hydroxide +++
Ethylacetate extract by Soxhlet method	$FeCl_3$ *+++* Lead acetate *+* Dil.HNO_3 **-**	Borntrager **-** Legal *++* Keller-Killiani *+++*	$FeCl_3$ +++ Lead acetate *+++* Shinoda - Sodium Hydroxide ++
Ethylacetate extract by maceration method	$FeCl_3$ **-** Lead acetate **-** Dil.HNO_3 **-**	Borntrager **-** Legal *+++* Keller-Killiani *+++*	$FeCl_3$ - Lead acetate *++* Shinoda - Sodium Hydroxide *++*
Ethylacetate extract by maceration & stirring method	$FeCl_3$*+++* Lead acetate **-** Dil.HNO_3 **-**	Borntrager **-** Legal *++* Keller-Killiani *+++*	$FeCl_3$ *+* Lead acetate *++* Shinoda **-** Sodium Hydroxide *++*

Quantitative chemical detection by high performance thin layer chromatography (HPTLC)

Quantitative separation of rutin, quercetin and gallic acid standards: The mobile phase constituted with ethylacetate-glacial acetic acid- formic acid- distilled water (100:11:11:25) succeeded to separate the three standards (rutin, quercetin and gallic acid). The maximal optical absorptions occurred at 366 nm for rutin (R_F: 0.39; area under curve: 10621.9), figure 3, at 280 nm for quercetin (R_F: 0.79; area under curve: 508.2), figure 4, and at 254 nm for gallic acid (R_F: 0.81; area under curve: 3027.4), figure 5. All previous values were identified in comparison with methanol control, and all area under curves represent a volume of 5 µl of standard solutions (1 mg/ ml).

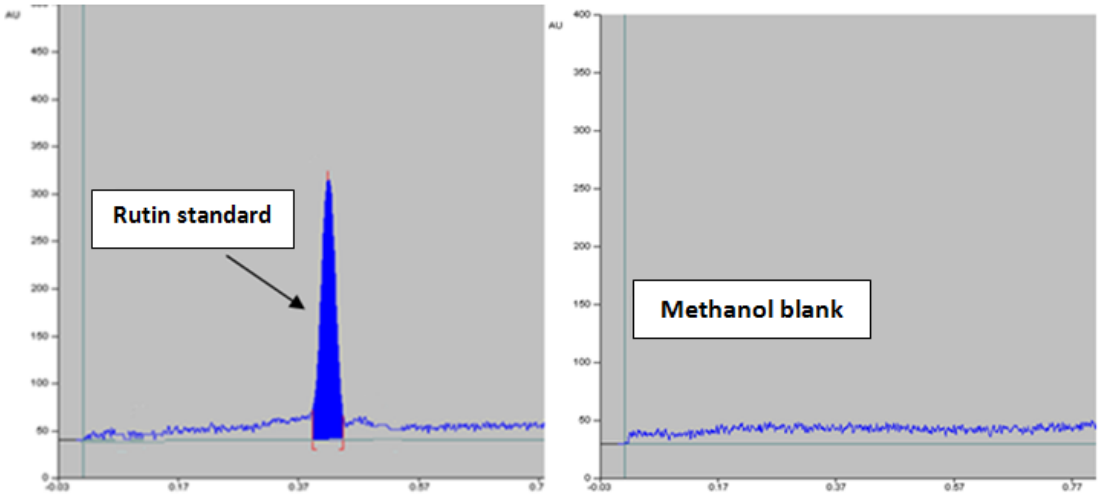

Fig 3: Maximal optical absorption of rutin standard at 360 nm in comparison with methanol blank. R_F= 0.39.

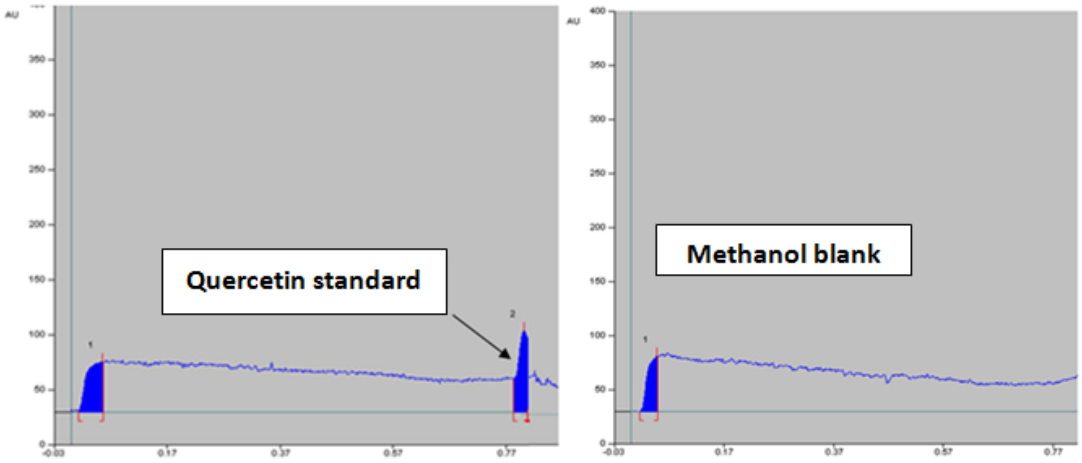

Fig 4: Maximal optical absorption of quercetin standard at 280 nm in comparison with methanol blank. R_F= 0.79.

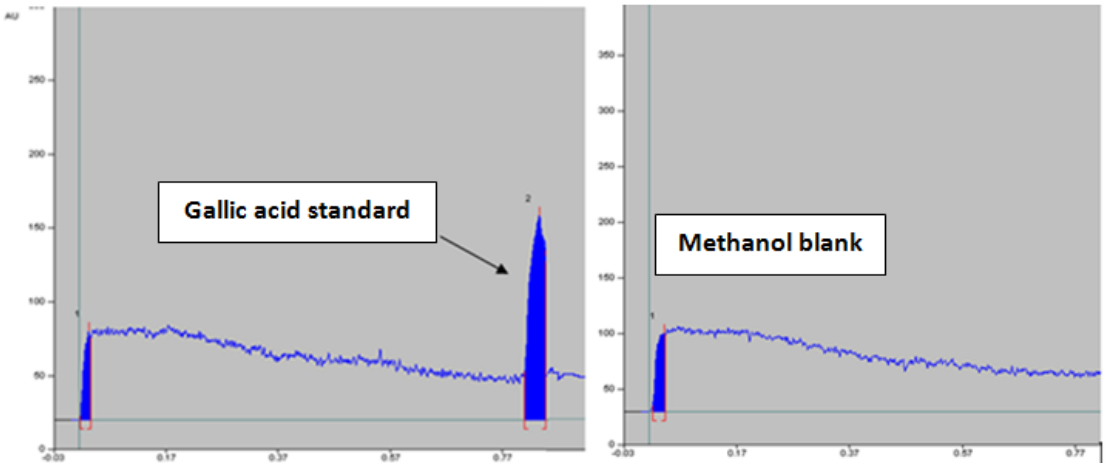

Fig 5: Maximal optical absorption of gallic acid standard at 254 nm in comparison with methanol blank. R_F= 0.81.

Quantitative separation of *Capparis spinosa* leave extract components

Alcoholic (80%) extract: The separation of the components from the extracts in conjunction with the separation of the standard have shown that, alcoholic extract by Soxhlet method contains rutin, quercetin and gallic acid analogues. This type of extract was the lonely to contain simple phenols similar to gallic acid. (Figure 6, Table 2). On the other hand, quercetin and gallic acid failed to be separated from alcoholic leave extracts by maceration or maceration & stirring methods. The rutin concentration was the highest in Soxhlet method (Figure 6, Table 2).

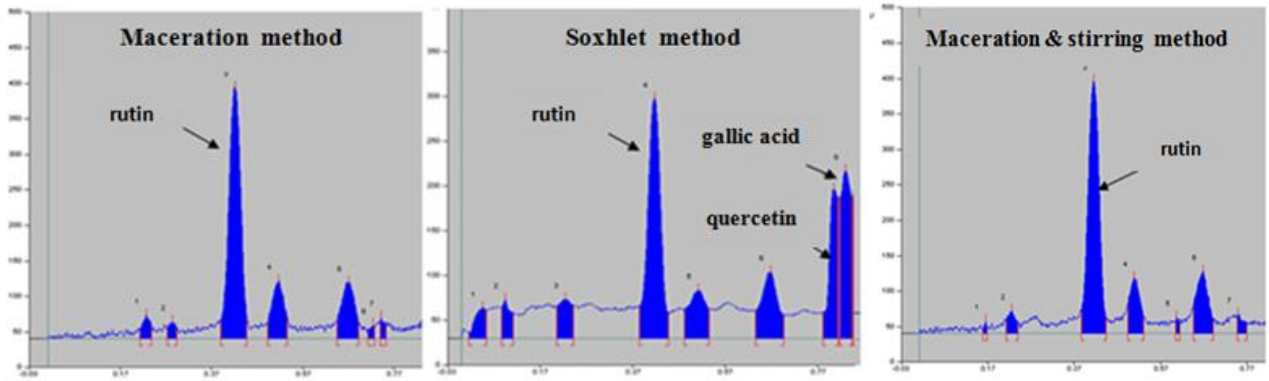

Fig 6: Quantitative separation by HPTLC of rutin, quercetin, gallic acid and unknown substances in alcoholic extracts of *Capparis spinosa* leave.

Ethylacetate extract: Quercetin and rutin were separated with ethylacetate solvent in both methods, Soxhlet and maceration, while, only quercetin was separated by using maceration & stirring method (Figure 7, Table 2).

Table 2: Quantitative separation by HPTLC of rutin, quercetin and gallic acid from the alcoholic and ethylacetate extracts of *capparis spinosa* leave extracted by three methods: Soxhlet, maceration and maceration & stirring methods.

Type of *Capparis spinosa* leave extracts	Concentration of rutin (366 nm)			Concentration of quercetin (280 nm)			Concentration of gallic acid (254 nm)		
	R_f	Area	g%	R_f	Area	g%	R_f	Area	g%
Alcoholic extract by Soxhlet method	0.39	12590.1	2.728	0.79	1934.7	8.754	0.82	712.7	0.543
Alcoholic extract by maceration method	0.39	15615.4	3.376	-	-	-	-	-	-
Alcoholic extract by maceration & stirring method	0.39	15095.4	3.923	-	-	-	-	-	-
Ethylacetate extract by Soxhlet method	0.39	6214.8	0.401	0.81	2518.1	3.389	-	-	-
Ethylacetate extract by maceration method	0.41	573.7	0.0108	0.80	4030.7	1.586	-	-	-
Ethylacetate extract by maceration & stirring method	-	-	-	*0.82*	*288*	*0.138*	-	-	-

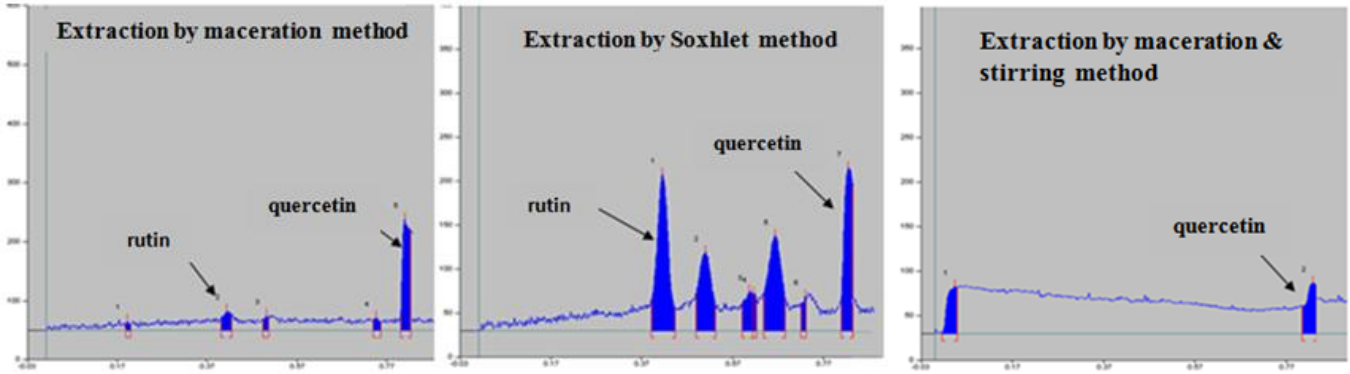

Fig 7: Quantitative separation by HPTLC method of rutin, quercetin, gallic acid and unknown substances from the ethylacetate extracts of *Capparis spinosa* leave.

Quantitative separation of unknown components from *Capparis spinosa* leave extracts. Unknown compounds were separated from some Capparis leave extracts. We determined the R_F values and area under curve to each component at 254, 280, 366 nm (Table 3). Those data will help to calculate the concentrations of those components in case of standard availability, or the availability of reference studies referring to their R_F after separation with the same mobile phase used in our study.

Table 3: Quantitative separation by HPTLC of unknown substances from the alcoholic and ethylacetate extracts of *Capparis spinosa* leaves by three methods: Soxhlet, maceration and maceration & stirring methods of extraction.

	Unknown substances at 366 nm		Unknown substances at 280 nm		Unknown substances at 254 nm	
	R_f	Area	R_f	Area	R_f	Area
Alcoholic extract by Soxhlet method	0.49	1869.6	0.64	2033.8	-	-
Alcoholic extract by maceration method	0.49	2144.4	0.21 0.26 0.64	472.8 537.6 2727.1	-	-
Alcoholic extract by maceration & stirring method	0.49	1808.6	0.21 0.55 0.63	345.6 455.1 3145.9	-	-
Ethylacetate extract by Soxhlet method	0.49	2025.7	0.59 0.63	406.6 3200.9	-	-

Validation of HPTLC method

Linearity & correlation coefficient: The calibration curve between rutin concentrations and area under curve averages gave a straight line with linear regression equation **(**Y = 14444*X - 248.0) (figure 8). The calibration curve of rutin was linear over a concentration range (0.16- 1.15 mg/ml) with a good correlation coefficient (R^2= 0.9941) (figure 8, Table 4). We can use the standard rutin linear regression equation to calculate a rutin sample concentration (X) by replacing (Y) by the area under curve.

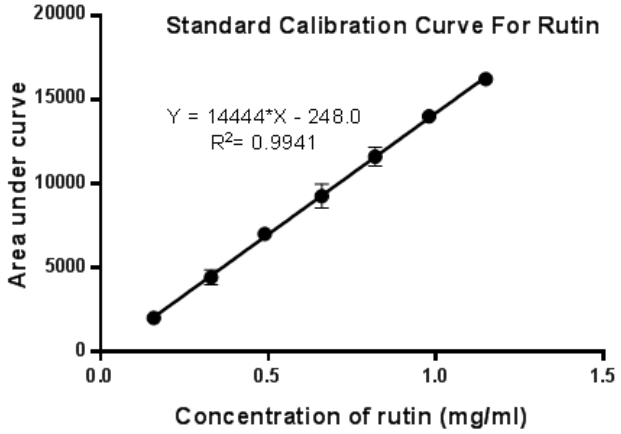

Fig 8: Standard calibration curve for rutin. It shows the relation between the triplicate average of area under curve and concentration.

Table 4: Linearity of calibration curve for rutin. C: concentration of rutin standard, A: area under curve of rutin standard concentrations calculated by HPTLC apparatus, A_m: average of area under curve in triplicate for rutin standard, A_{eq}: area under curve calculated by linear regression equation, D_{ev}: difference between A_m and A_{eq}.

C mg/ml	A	A_m	A_{eq}	D_{ev}
0.16	2083.7 1751.6 2176.9	2004.07± 223.55	2062.92	58.85 -
0.33	4946.1 4132.3 4191.9	4423.43± 453.63	4518.32	94.89 -
0.49	7166.4 7130.0 6753.6	7016.67± 228.55	6829.3	187.37
0.66	9778.1 9554.4 8458.4	9263.63 ± 706.27	9284.7	21.07 -
0.82	12103.5 10986.4 11738.3	11609.40± 569.60	11595.67	13.73
0.98	13653.2 14406.9 13981.1	14013.7 ± 377.91	13906.61	107.09
1.15	16148.8 16149.6 16387.6	16228.7 ± 137.64	16362.08	133.38 -
		sum of deviation		0

Precision & accuracy

Table 5 shows the data of recovery test which visualize the comparison between rutin concentrations prepared theoretically and that measured practically. Good recovery was obtained following enrichment of the rutin spot sample with a spot of three different standard rutin concentrations (95, 113, 137%). The percentage recoveries of the theoretical rutin concentrations were (98, 98.5, 99.5%) respectively, which demonstrate the analytical method accuracy. Tables 5, 6 show a high precision in the iterative concentration measurements, where the RSD was ranged between (0.3-0.8%).

Table 5: Recovery test. Comparison between theoretical rutin concentrations and that measured practically by HPTLC.

	Level of Recovery		
	%137	%113	%95
	Peak Area		
Repeat 1	8707.3	7601.3	6950.8
Repeat 2	8599.6	7728.3	6940.6
Repeat 3	8726.5	7600.3	6980.8
Repeat 4	8670.7	7596.2	6990.6
Mean Peak area	8676.0	7631.5	6965.7
RSD%	0.645	0.846	0.342
Prepared concentration (mg/ml)	0.618	0.555	0.508
Measured concentration (mg/ml)	0.618	0.546	0.499
Mean of recovery (%)	%100	%98.4	%98.2
Accuracy judgment	Good accuracy	Good accuracy	Good accuracy

Table 6: Precision test for HPTLC method.

	Area under curve	Concentration (mg/ml)
Repeated 1	4550.2	0.332
Repeated 2	4520.9	0.330
Repeated 3	4540.8	0.332
Repeated 4	4510.1	0.330
Repeated 5	4522.4	0.330
Repeated 6	4515.1	0.330
Repeated 7	4520.1	0.330
mean	4525.657	0.331
RSD (%)	0.302	0.300

5. Discussion

The aim of our research was to find the better extraction method of active components from Syrian *Capparis spinosa* leaves, especially those which are known to reduce blood sugar. These active components include flavonoïds like quercetin, flavonoïd glycosides like rutin, and simple phenols represented by gallic acid, which will be used in subsequent researches. We adopt the qualitative detection tests and the HPTLC method to perform the comparison between different kind of extracts, and then to identity the best one.

Our results confirmed the importance of the extraction method and the extraction solvent to reach the higher extraction yield and the higher amount of extractive active substances. The extraction of *Capparis* leave by ethanol 80% solvent assured a higher yield of extract than by ethylacetate solvent. The highest yield of leave extraction by ethanol 80% solvent was reached with stirring method at 40˚C for 48 hours (27.6 w/w%), while the highest yield of leave extraction by ethylacetate solvent was reached with Soxhlet method (6.84 w/w%). This may be due to the ability of the ethanol 80 % solvent to extract lipophilic and hydrophilic ingredients, while ethylacetate solvent extracts glycosides. The high yield of the extraction doesn't mean that the extract contains a high concentration of the effective substances. The qualitative chemical detection applied on leave ethylacetate extracts by Soxhlet or by maceration & stirring method, showed a higher proximal concentrations of flavonoïds and other glycosides in Soxhlet method than in maceration & stirring method, although the yield of the extraction were 6.84 and 27.6% respectively. The qualitative chemical detection showed also that leave extraction by ethanol 80% solvent gave a significant capacity to extract flavonoïds, while ethylacetate solvent showed better capacity to extract glycosides. That

may be due to the complex chemical formula of glycosides compared to flavonoïds, so glycosides require a stronger organic solvent to be extracted. Based on what disserted above, the selection of the extraction method and the solvent plays an important role in achieving extraction higher concentration of the effective biological substances from the plant. In this concept, and to obtain an extract containing the higher concentration of the effective biological substances, it is better to mix the two types of extract: the alcoholic extract with high concentration of flavonoïds and the ethylacetate extract with high concentration of glycosides.

Always in the field of qualitative chemical detection, the positivity of ferric chloride test when applied on leave extracts reflects the presence of phenol groups. *Capparis* leave extracts may contain simple phenol substances like gallic acid, and polyphenol chemicals represented by flavonoïds like quercetin. The higher positivity of ferric chloride test concomitant with positive flavonoïds tests (Shinoda, sodium hydroxide) and negativity of simple phenol tests (Lead acetate, Dil.HNO_3) confirmed the presence of flavonoïds, and vice versa is correct.

The negativity of Borntrager test when applied on all *Capparis* leave extracts reflects the absence of anthraquinon glycosides. On the other hand, Killer- Kellani and legal tests were positive in all leave extracts which indicates the presence of non-saturated lactone glycosides. IT is important to indicate here the non- availability of a test for detection of flavonoïd glycosides such as rutin, and because of the coexistence of most flavonoïds in mature plants as flavanoïd glycosides, their qualitative detection relays on the positivity of the specific flavonoïd tests such as Shinoda test.

HPTLC method helped to quantify the concentrations of the different chemicals contained in *Capparis spinosa* extracts, which permit to determine the best plant extraction method that can extract the highest concentrations of the effective substances. Soxhlet method showed a high capacity in the extraction of rutin, quercetin and gallic acid from *Capparis spinosa* leaves and with the alcoholic solvent, while 1.2- 1.4 folds of rutin concentrations were separated by using maceration method at an ambient temperature for 7 day and maceration & stirring method for 48 hour at 40˚C, as compared with Soxhlet method, despite that the two last methods could not extract quercetin and gallic acid. It could be that extraction by Soxhlet method and using the alcoholic solvent, facilitates the drift of rutin between other flavonoïd glycosides and its decomposition to quercetin and rutinose, which can explain the high concentration of quercetin compared with other alcoholic extraction methods. That also explain the rise of rutin concentration when using Soxhlet extraction method and ethylacetate solvent, with specifying that extraction was accomplished without decomposition of rutin. We can also argue the high concentration of quercetin compared to concentration of rutin in all leave extracts to the immaturity of *Capparis* leave used in the extraction. Flavonoïd glycosides are found in mature parts of plant.

The validation of HPTLC method used in this study showed a high precision in accordance with other reference studies. That advise the use of HPTLC to separate quantitatively the bioactive ingredients from plant extracts, because of its accuracy, low costing and facility to control the work conditions, that in comparison with other analytical methods like high performance liquid chromatography (HPLC) [7, 8, 10, 25].

As result of what discussed above, the concentrations of rutin and quercetin were determined in the different kinds of *Capparis* leave extracts, which permit us to choose Soxhlet as the best extraction method with both solvents: ethanol (80%) and ethylacetate (Figures 9, 10).

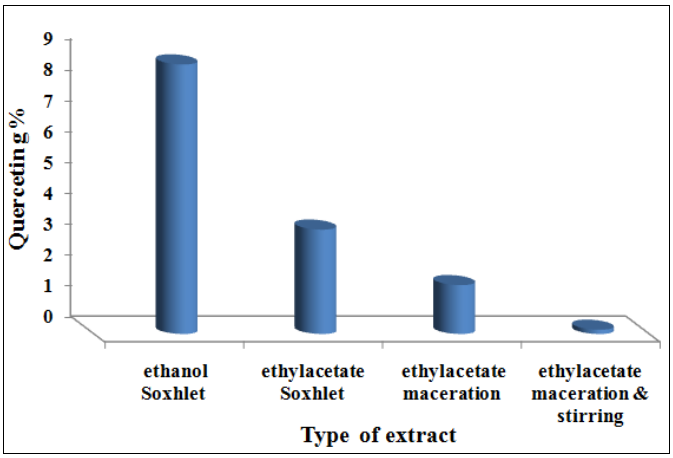

Fig 9: Concentrations of quercetin in the different kinds of *Capparis* leave extracts

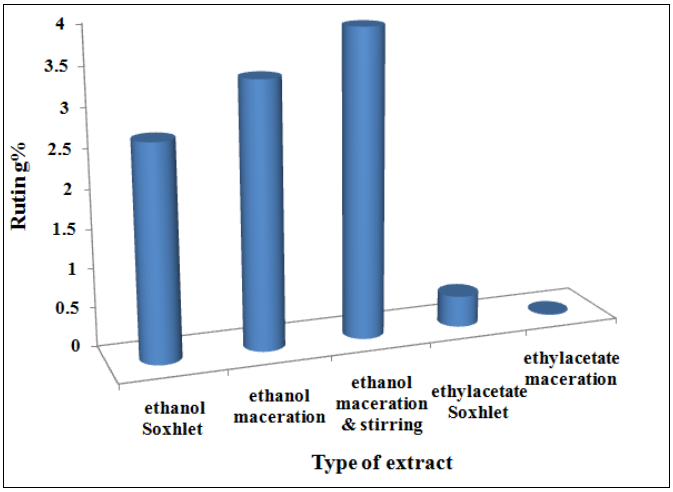

Fig 10: Concentrations of rutin in the different kinds of *Capparis* leave extracts

6. Conclusion

Capparis spinosa leave at the vegetarian stage of the plant life are rich in bioactive substances, especially rutin and quercetin. That agree with Behnaz *et al*. 2014 study results, and can be a benefit to interfere *Capparis* leave as an antioxidant in the botanical drugs which can help to protect from chronic diseases such as diabetes mellitus.

7. References

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