COMPARISON PHYTOCHEMICAL COMPOUNDS FROM TWO DIFFERENT SOLVENTS OF CRUDE *CAPPARIS SPINOSA* EXTRACTS

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ABSTRACT

An experiment was conducted to evaluate different parts of the plant *Capparis spinosa* L. extracts for their phytochemical compounds by two different solvents (ethanol &hexane) . The current results showed the superiority of the ethanolic extracts by their quantity and quality of active compounds compared to the hexane extracts. The yield percentage of ethanolic *Capparis spinosa* L extracts ranges between (3-31%), and color range between Dark brown, and olive with oily texture. Whereas, the yield of the hexane extract ranges between (1-27.9%) and it takes a longer time for extract with difficult to obtain, Ultimately, the results of the phytochemical analysis demonstrated the presence of numerous active compounds with higher degree of the sediment color in ethanol extracts such as: alkaloids, Phenols, Glycosides, Tannins, flavonoids and Saponin comparing with hexane extracts with lower color strength.

Keywords: *Capparis spinosa*, Phytochemical compounds, Hexane solvents, ethanol solvents. Plant extracts

مقارنة المركبات الكيميائية النباتية في مذيبين مختلفين لمستخلصات الخام لنبات *Capparis spinosa* L.

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المستخلص

اجريت الدراسة الحالية لتقييم المركبات الكيميائية النباتية لاجزاء مختلفة لنبات *Capparis spinosa* L. بأستخدام مذيبين مختلفين (الايثانول والهكسان). أظهرت النتائج الحالية تفوق المستخلصات الايثانولية من حيث كمية ونوعية المركبات الفعالة مقارنة بمستخلصات الهكسان. حيث تراوحت نسبة المحصلة للمستخلصات الإيثانولية لنبات *Capparis spinosa* L بين (3–31٪) ، كما تتراوح ألوانها بين البني الداكن والزيتون ذو الملمس الزيتي. في حين بلغت محصلة مستخلص الهكسان ما بين (1–27.9٪) وأستغرق وقتًا أطول للاستخلاص و صعوبة جمعها ، بالإضافة إلى قوامه شديد اللزوجة ,حيث تراوح لونه بين الأخضر الفاتح والأخضر الداكن إلى البني. .اخيرا,أظهرت نتائج التحليل الكيميائي النباتي وجود العديد من المركبات الفعاله بقوة تدرج لوني عالي في مستخلصات الإيثانول مثل: القلويات والفينولات والجليكوزيدات والعفص والفلافونويد والسابونين مقارنة بمستخلصات الهكسان ذات قوة اللون الأقل.

الكلمات المفتاحية: *Capparis spinosa* L. , المركبات الكيميائية النباتية, مذيب الهكسان, مذيب الايثانولي, مستخلصات نباتية

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INTRODUCTION

According to available data, more than three-quarters of the world's population depends mainly on plants and plant extracts for their health care needs. Numerous plants play an important role in reducing the severity of diseases and injuries all over the world (32,27). The demand for medicinal and aromatic plants has increased in the market due to their better medicinal value and pharmacological activity. It is estimated that in developed countries the medicine plants account for up to 25% of total medicines, while in fast-developing countries such as India and China, the contribution amounts to 80%. Therefore, the economic importance of medicinal plants is greater for countries like India than for the rest of the world (16,30). *C. spinosa* L., the caper bush, is a perennial winter-deciduous plant. The plant is best known for its fruit, which is usually used in the manufacture of medicines and ailments. *Capparis spinosa* L. has been shown to be anti-oxidative (21) and anti-inflammatory (41,43). anti-bacterial (5,24,25)anti-hepatotoxic (6,15). Limited articles that focused on the importance of the chemical compounds and health benefits of *C. spinosa* .Therefore, the main aim of the current search is to investigate the phytochemical compounds in crude extracts by using two different solvents of root, stem, leaves, flowers, and fruit of *C. spinose*

MATERIALS AND METHODS

1- Collection and Preparation of plant

Capparis spinosa specimens were collected handily from the Algadria region middle of Baghdad city, Iraq. That is located on longitude33°01'.94"E and latitude44°20'.41"N during autumn 2020 as seen in Figure (1). Each part of the plant was collected and stored in plastic bags for transportation to the laboratory. *C.spinosa* samples were washed with tap water to clean the dirt and then dried for three days in the shadow. Preparation of Soxhlet alcoholic extracts (ethanol & hexane) according to [1,35].In this process full 20-gram dry powder of each plant part in thimble and prepared by using a soxhlet apparatus. After completion of the process, the concentrated active constituents from macro-algae were kept in sterilized test tubes stored in a refrigerator till further use. The traces of ethanol were removed by keeping the tubes at 50˚c for 1 hr. All extracts were kept in the serial tube at 4^{o}C until used. The yield of crude extracts was expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation as follows (20):

$$\text{Yield (\%)} = \frac{\text{Amount of extracted (g)}}{\text{Amount of dry vegetal matter mass (g)}} \times 100$$

Figure 1. Position of *C. spinosa* collected site

2- Indicators of Active Compound in Plant Extracts

The preliminary phytochemical screening tests were carried out to identify the useful constituents by standard methods (6,9,15) as seen in Table(1)

Table 1. Detection methods of active compounds in algal extracts

Active compound	Test/reagent	Method	Positive result
Alkaloids	Hager reagent	Add picric acid with boiled and filtered solution of plant powder dissolved in acidic distilled water	Yellowish
Flavones	Alkaline reagent test	Dissolve algae extract in 95 % ethanol or prepare by mixing absolute ethanol and 50 % KOH. The appearance of yellow color detects Flavones	Yellowish
Glycosides	Banadact reagent	Using benedicts reagent added to extract that dissolved in distilled water appearance of red to brown color detects glycosides	Color red to brown
pH	PH-meter	Mixing 10 ml of extract and using a PH-meter to detect pH of the extract	Detect number
Resin	Hydrochloric acid	Using ethanol and leaved until boiling then filtrated appearance turbidity after adding acidic distilled water with 1 HCl	Turbidity
Saponins	Froth test	Extract was reacted with mercuric chloride appearance of a white color detect saponin	White
Tannins	Gelatin test	Add 1ferric chloride appearance of gelatin solution or yellowish	Blue

RESULTS AND DISCUSSION

Extraction methods are an approach to the separation of the active compounds of plant tissues from inactive components by using selective polar and non-polar solvents. During extraction, solutions defuse into solid plant material and solubilize compounds with similar polarity. In the present study, the phytochemical constituents of *C.spinossa* were successively extracted by two different organic solvents. Polar solvent Polarity (ethanol), Index ranging (5.2) with boiling point (78).While, the non-polar solvent (hexane) was (0.1) with a boiling point of (69) respectively, as seen in Table (3). Clearly, Hexane solvent tends to be basic more than acidic, unlike ethanol solvent, which tends to be acidic more than basic. The pH value ranged between (9.5- 4.2), the higher pH value (9.5) was in stem hexane extract. While the lower pH value (4.2) was in flower ethanol extract. The differences in the pH values are indicative of the nature of the substances in the plant extract, which greatly affect the properties of the extract and its biological efficacy [1,4]. The selection of the appropriate extraction method depends on the nature of the plant material, the solvent used, the pH of the solvent, the temperature, and the solvent for sampling. It also depends on the intended use of the finished products (36,17,8). According to the data in Table (2), the best total crude yield extract either in solvent hexane or/and ethanol extract were in Fruits % 27.9% and 31% respectively. While the less total crude yield extract was in root 1 % and 3% respectively. Finally, the higher yield percentage of stem leaves and flowers showed in ethanol solvent (13.9%, 12.8% and 11.3% respectively. The lower yield percentage was shown in hexane solvent (1.7%, 3%, and 1.3%) respectively. Numerous colors of the extracts were shown in the current study, ranging from light greenish, dark reddish, and dark brown to dark molasses with softy to sticky texture. Ultimately, most of the hexane extracts had a smooth oily texture, which may be due to the hexane naturally pulling more oil out of plant tissue products without disturbing the structure of active compounds. This shows that the extraction yield decreases with the low polarity of the solvent used in extraction (3,24). Another study, that agreed with our results, found that the extraction of essential oil from *C.spinosa* in large amounts and that note the texture of different extracts could be used in the pharmaceutical, food, and cosmetic nutrient industries [28,33]. Hexane is a hydrophobic solvent that is called concretes, and can extract lipophilic plant material such as Octanoic acid, 2-hexenal, butyl isothiocyanate, and Palmitic acid (2). While, [12] mention that the fruit of *C.spinosa* contains (79%), ash (1.6%), protein (5.8%), fat (1.6%), and crude fiber (5.4%). However, ethanol gave the lowest oil appearance due to its inefficient solvation (7,16).

Table 2 . Yield percentage of *C. spinosa extracts* in different solvents

Type of extractio	Polarity Index	Boiling point	Part of Plant	Appearance	Discretion	pH	Yield (g)%
Hexane	0.1	69.0	Roots		Brown reddish with soft texture	6.9	1
			Stems		Light green hay with soft texture	9.5	1.7
			leaves		Dark olive with glossy appear and oily texture	7.8	3
			Flowers		Dark green hay with soft texture	8.3	1.3
			Fruits		Molasses color with a glossy & sticky texture	6.2	27.9
Ethanol	5.2	78.0	Roots		Dark brown with a glossy & sticky texture	6.3	3

Stems		**Dark brownish with a greasy and sticky texture**	**5.2**	**13.9**
leaves		**Dark bluish with an oily texture**	**6.5**	**12.8**
Flowers		**Dark olive hay with soft texture**	**4.2**	**11.3**
Fruits		**Molasses color and oily texture**	**4.6**	**31**

As seen in Figure (2) the descriptive statistics of the yield were differences in the cumulative yield between the two types of solvent. The current results show a 60-minute extraction time by methanol extract gives a yield different from that obtained after 190 min by means of hexane, and that indicates that ethanol solvent needed less time and a greater cumulative yield compared to the hexane solvent. Numerous investigations support our results and have reported that the yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, the solvent and composition of the sample are known as the most important parameters (10,19).

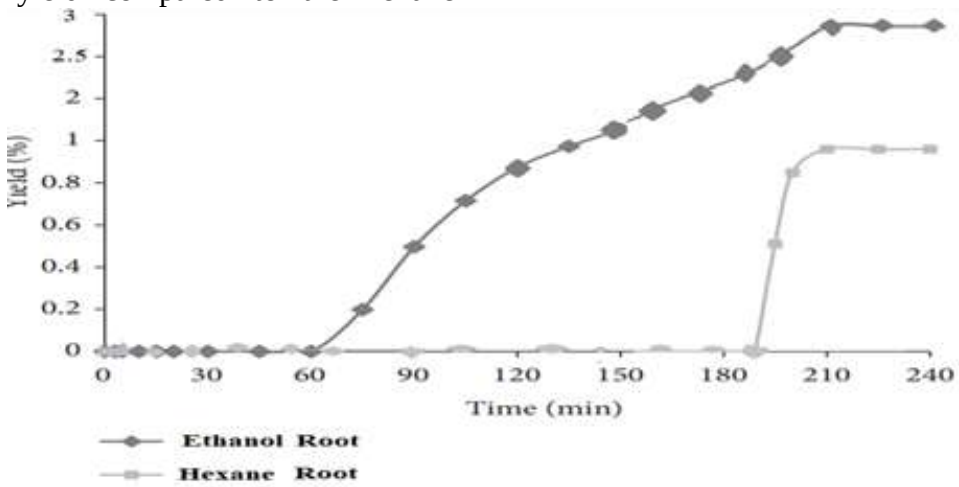

Figure 2. Yield profiles as a function of time for ethanol and hexane solvent of *C.spinosa* root extract

It was also observed from the current results of this study, present numerous bioactive phytochemicals analysis in ethanolic crude extracts with higher gradation in the degree of the sediment color compared with hexane extracts (25,42). Generally, aerial parts contain the dominant flavonoid, and that agrees with (22,43). Stem, leaves, and flowers of *C. spinosa* were rich in Alkaloids, flavonoids, Glycosides, and tannins, while roots and flowers were poor in Resins, probably due to the type of solvent that prevents capturing some polarity active compounds (3,34,40). Crude hexane extracts essential oils obtained from *C. spinosa.* Root and stem poor in phenol and tannins in addition, stem poor in Alkaloids and glycosides. Leaves poor in tannins and Resins. The flowers are mostly poor in alkaloids (31). Finally, bioactive phytochemicals analysis presents with a very low gradation of the degree of the sediment color. *C. Spinosa* has been valued as a rich source of medicinal and nutritional agents for centuries (11,13). The important medicinal, health functions and nutritional characteristics *of C. spinosa* are due to the presence of numerous compounds such as alkaloids, glucosides, reduced sugars, essential fatty acids, organic acids, vitamin C, terpenoids, flavonoids, and resins in the fruits and leaves of this species due to the effectiveness of these compounds in physiological and defensive processes against bacterial and fungal pathogens that infect the organism (2,18). Present Saponines are biochemical compounds with numerous biological roles such as anti-inflammatory allelopathic action, anti-carcinogenic, mitigating cell reinforcement, hemolytic, hypocholesterolemic resistance stimulators, and cell layer permeabilizing characteristics (14,38,43)**.** Glycosid consists of sugar that is linked to another functional group via a glycosidic bond, and plays an important role in living organisms (21,42) The results presented in this work confirmed that ethanol was the best solvent to extract phytochemicals compounds such as phenolic compounds, flavonoids, flavonols, tannins, and carotenoids. (37). It should be pointed out that there is a significant difference between the phytochemical contents of the different extracts and the plant part used. Therefore, the *C. spinosa* extracts demonstrated that the solubility of phytochemicals it depends not only on the type of solvent used but also on the part of the plant (23,26,27).

Table 3. Appearance (or/ and) disappearance of active compounds in *Capparis spinosa* extracts

Chemical Compounds	*Capparis spinosa* extracts									
	Ethanol					Hexan				
	Ro	St	Le	Fl	Fr	Ro	St	Le	Fl	Fr
Alk	+++	+++	+++	+++	++	+	-	+	+	-
Fla	++	+++	+++	+++	+++	+	+	-	+	+
Gly	++	+++	+++	+++	++	+	-	+	+	+
Phe	+++	++	++	+++	++	-	-	+	+	+
Res	_	++	+++	_	+++	+	+	-	+	+
Tan	++	+++	+++	+++	+	-	-	+	-	+
Sap	++	++	+++	++	++	+	+	+	+	+

ALk= Alkaloids, FLA= Flavones , Gly= Glycosides , Phe= Phenols , Res= Resins ,Tan= Tannins , Sap= Saponin Ro= Root, St= Stem ,Le= Leaves , Fl= Flower, Fr=Fruit, + = Weak present , ++= middle present , +++ = strong present , - = absent

CONCLUSIONS

In this study, we demonstrated that when using ethanolic solvent to obtain *C. spinosa* extracts proved to be highly yielded and present different active compounds while hexane solvent showed difficulty in obtaining the amount of yield with low gradation the degree of the sediment color. Therefore, future prospective research should screen for individual active compounds with different solvents.

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