



**ALcoholic extraction of silymarin oil from seeds of local Milk thistle plant
(*silynum marianum*) at room temperature**

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Abstract

This study conducted at central researches unit at the college of veterinary medicine in Basrah university and it aimed to extract silymarin components from local Milk thistle (*Silybum marianum*) that grow in north of Iraq(mosul) , at room temperature within different periods and isolation of silymarin oil from the plant, 50gram of grinded seeds defatted by normal hexane (500ml) using soxlet for 3 hr. and the defatted seeds powder extracted by absolute ethanol(1:3) at room temperature for 72 and 96 hr. After extraction of defatted grinded seeds of local *silybum marianum* with absolute ethanol at room temperature. Results showed that at 96 hr.of extraction significantly effect the amount of extracted mat rial(24.3720+5.307mg/g p < 0.05) , while it was 10.9840+9.2572 mg/g after72 hr. and the percentage of silymarin components were 1.109 +0.097and 2.435+ 0.0553% at 72 and 96hr. respectively and Silymarin oil that defatted from the grinded seeds yield 22.23%.

Keywords : Silymarin, ethanol.

الاستخلاص الكحولي لزيت السيليمارين من بذور نبات الحليب الشوكي (*silynum marianum*) في درجة حرارة الغرفة

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الخلاصة:-

اجريت هذه الدراسة في وحدة الأبحاث المركزية في كلية الطب البيطري في جامعة البصرة إلى استخلاص مكونات السيليمارين من الشوك الحليب المحلي (*Silybum marianum*) التي تنمو في شمال العراق (الموصل) ، في درجة حرارة الغرفة خلال فترات مختلفة. وتم عزل زيت سيليمارين من النبات إذ استخدمت، 50 غرام من البذور المطحونة منزوع الدسم بواسطة الهكسان الطبيعي (500 مل) باستخدام سوكليت لمدة 3 ساعات. ومسحوق البذور منزوع الدهن المستخلص من الإيثانول المطلق (1:3) في درجة حرارة الغرفة لمدة 72 و 96 ساعة. - بعد استخراج البذور المطحونة منزوعة الدهن من *silybum marianum* المحلي مع الإيثانول absolute في درجة حرارة الغرفة. أظهرت النتائج أنه في 96 ساعة من الاستخراج يؤثر بشكل كبير على كمية المواد المستخرجة (24.3720 + 5.307 ملغ / غم عند مستوى احتمال (p ≤ 0.05) ، في حين كان 10.9840 + 9.2572 ملغ / غم بعد 72 ساعة. وكانت نسبة مكونات سيليمارين 1.109 + 0.097 و 2.435 + 0.0553 % عند 72 و 96 ساعة. على التوالي وزيت سيليمارين منزوع الدسم من البذور المطحون 22.23 %.

Introduction

Milk thistle (*Silybum marianum*, family: Compositae) is an annual plant native to the Mediterranean area, which has now spread to other warm and dry and warm regions (1), As well as it grow in Iraq specially the north.

The most important medicinal application of milk thistle is its use as a hepatoprotectant and as supportive treatment chronic inflammatory liver disorders such as cirrhosis, hepatitis, and fatty infiltration due to alcohol(2) . and toxic chemicals like lead(3) ,(4),(5) pointed that Silymarin, derived from the milk thistle plant *Silybum marianum*, were widely used for self-treatment of liver diseases, , including hepatitis C virus (HCV), (6) examined the efficacy of silymarin on ethanol-induced oxidative stress, immunomodulatory activity. (7) showed that Silymarin, a milk' thistle flavonolignan mixture, has anti-proliferative and anti-angiogenic activities in xenografts of human prostate cancer .(4)pointed that an extract of crushed achenes of the milk thistle plant *Silybum marianum* is a multi-constituent mixture, 70-80% of which consists of a complex assortment containing the flavonolignans silybin A and B, isosilybin A and B ,silydianin,and silychristin ,and the flavonoid taxifolin .(8) used of hot water as an extraction solvent for milk thistle at temperatures above 100 degrees C was explored the maximum extraction yield of each of the silymarin compounds and taxifolin did not increase with temperature. (9)In this paper, a novel method of microwave assisted extraction (MAE) used for the extraction of apotent hepatoprotective bioactive silybinin a main flavonolignan from *Silyhum marianum*, (10) their study showed that appearance of the degradation peaks in the water extract and ethanol didnt create any cytotoxic effects

and the results of the study colfirm that (PHWE)pressurized. tot water or ethanol can be used to extract flavonolignans from milk thistle and that these extracts may possess therapeutic potential different from or beyond that of traditional organic solvent preparations. Numerous pharmacological actions of the silymarin extract have been documented in the biomedical literature, including hepatoprotective, anti-inflammatory, anti-tumor, and anti-fibrotic activities. Silymarin oil has to be removed from seeds prior to the extraction of silymarin (11).

The seed of milk thistle contains a relatively high fraction of oil .(12) found that Seeds of milk thistle (*Silybum*) contain 25% (w/w) of oil . (13)*Silybum marianum* in the Nile region (Delta) and Fayium region near water streams and has huge amounts of the oil-rich seeds, as secondary product, are produced the seeds contain about 22% oil, which is similar to many vegetable oil seeds oeven more This oil contains essential phospholipids and a relatively high content of vitamin E, it is therefore of interest as a natural source of vitamin E(14). (15)studied, fatty acids, phytosterol classes and tocopherols composition of Milk thistle seeds oil. (%). (16)An oil obtained from the seeds of Saint-Mary thistle (*Silybum marianum*) reduced the level of lipid peroxidation, increased the catalase activity.

Materials and methods

This study was conducted in Basrah — university- college of veterinary medicine. Milke thistale seeds(*Marianum silybum*) from the north of Iraq(mosul), seeds were selected according to their condition where damaged seeds were discarded before seeds in good condition were cleaned,. Seeds were grounded using grinder prior to extraction.50 grams each time were defatted in a soxhlet apparatus, using normal hexane (boiling point of 40C°) for 3 the hr (17),(18).

The oil was separated by distillation, the remain defatted seed powder was transferred into a flask fitted with a condenser and 150 ml of absolute ethanol was added and stirred for 72 hr. and 96 hr at room temperature. After filtration and concentration of the silymarin fraction under vacuum, the yellow residue was dissolved in 20 ml of toluene and evaporated for one hour., 20 ml of diisopropyle add to the crystals and deflexed for I hr. and cooled for I hr. The mixture evacuated and the remain crystals will collect and weighted. Stored in cold place(17).

Statistical analysis of the data for the effect of extraction period t-test for Equality of Means and Levene's Test for Equality of Variances by Spss program(19)

Rustles and discussion

After extraction of deffated grinded seeds of local *silybum marianum* with absolute ethanol at room temperature. Table (1) showed that at 96 hr.of extraction significantly affect the amount .

of extracted material(24.3720 ± 5.307 mg/g $p < 0.05$), while it was 10.9840 ± 9.2572 mg/g after 72 hr. and the percentage of silymarin components were 1.109 ± 0.097 and 2.435 ± 0.0553 % at 72 and 96hr. respectively.(11), (17) found that extraction with ethanol resulted in the highest silymarin yield, the maximum yields of taxifolin, silychristin, silydianin, silybinin A, and silybinin B in ethanol were 0.6, 4.0, 0.4, 4.0, and 7.0 mg/g of defatted seed, respectively (17) found extractions of defatted seed meal with boiling ethanol returned maximum yields of 0.62, 3.89, 4.04, and 6.86 mg/g defatted seed of taxifolin, silychristin, silybinin A and silybinin B, respectively. When extracting defatted seed meal with ethanol, yields of taxifolin, silybinin A and silybinin B were, respectively, 6.8-, 0.95-, 1.7- and 1.6-fold higher than when extracting whole seeds. Also (12) found that extraction of whole

seeds of milk thistle with water at 50, 70 and 85 degrees C the yield of silymarin increased with increasing water temperature and most cases, ethanol at 60 degrees C recovered the largest quantities of silymarins. However, boiling water proved to be an efficient extraction solvent for the more polar silymarin such as taxifolin and silychristin, even when using whole seeds. Extractions of defatted seed meal with boiling ethanol returned maximum yields of 0.62, 3.89, 4.04, and 6.86 mg/g defatted seed of taxifolin, silychristin, silybinin A and silybinin B, respectively.(20)The actual yield of silybin was 24.81 ± 1.93 mg/g defatted seeds, higher by 138 and 123.6% than that from ethanol extraction (2) showed that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85 degrees C, while the less polar silybinin was favored at 100 degrees C. (21) found that defatting of milk thistle seeds $m = 14.53$ g of pale yellow oil (yield $Y = 27.54$ % based ,while the isolation of silymarin from the seed the defatted seed powder ($m=37$ g) extracted by acetone for 72 hr. at room temperature and partitioned with diisopropyl ether, and evaporated the remaining solid was dried to obtain $m = 1.52$ g ($Y = 4.1$ %) of silymarin While . (22)The optimal extraction parameters to obtain the highest silymarin yield were time duration of 60 min, temperature of 112 °C, ethanol concentration of 81.5% (v/v), and a solid—liquid ratio of 1:38 (g/mL). The average experimental silymarin yield under the optimum conditions was found to be 56.67 ± 1.36 mg/g . Silymarin oil that defatted from the grinded seeds yield 22.23% table (2) as a mean for each 50 gram after 3hr. with normal hexane at 50 C degree by soxlet .. The seed of milk thistle contains a relatively high fraction of oil .(12) found that Seeds of milk thistle.

(Silybum) contain 25% (w/w) of oil. (21) pointed that defatting of milk thistle seeds m = 14.53 g of pale yellow oil (yield Y= 27.54

%).(13) found That the seeds contain about 22%oil, which is similar to many vegetable oil seeds or even more.

Table(1) silymarin extracted from *silybum marainum* in absolute ethanol at room temperature at two different period.

	time	N	Mean	Std.deviatinn
Defatted seed gm	72	5	93.6780 .92430±	2.06680
	96	5	38.7340 .35757±	.79955
Extracted silymarin\g	72	5	43560 .03763±	084240
	96	5	*94620 .206538±	.461832
Extracted silymarine mg\g	72	5	10.9840 .92572±	2.06998
	96	5	*24.3720 5.30760±	11.86815
The % of silymarin	72	5	1.10920 .097317±	.21608
	96	5	*2.43520 .530589±	1.186434

*significant effect $p \leq 0.05$

Table (2)Defatted mass and oil percentage isolated from Iraq *silybum marainm*

NO	Weight after Defatting\gm	Oil\gram	%of oil
1	39.300	8.25	16.5
2	38.374	9.5	19
3	39.123	15	30
4	43.28	10	20
5	38.2	9	18
6	38.3	15	30
7	40	10	20
8	38.2	15	30
9	38.9	10	20
10	38.1	9.9	18.8

conclusion

The results concluded that the mass(50gram) of the grinded seeds of milk thistle(silybum marianim) that grow north of Iraq after defatting by normal hexan for 3 hr. gave significant different amount of silymarin

components according to the time of extraction in absolute ethanol at 72 and 98

hr at room temperature. And a percentage .2 43520 + 530589 %

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