

EFFECT OF ESSENTIAL OIL OF *Cestrum nocturnum* FLOWERS CULTIVATED IN IRAQ AS ANTIOXIDANT AND ELONGATION COLD STORAGE PERIOD OF MINCED MEAT

Z. O. Salman

Assist. Lecturer

Dept. of Bio., Coll. of Sci. for Women, University of Baghdad, Iraq.

Dept. of Med. and med. plants, Coll. of Pharmacy, University of Baghdad, Iraq.

zainab19831210@yahoo.com

B. M. J. Alwash

Assist. Prof.

E. J. Kadhim

Assist. Prof.

ABSTRACT

The aim of this study is extraction, estimation and identification of volatile oil in flowers *Cestrum nocturnum* plant, and study the antioxidant effect of oil it's effect on the chemical properties of minced cattle meat which was stored on fridge to elongation period of cold storage. Steam distillation method was used to extraction volatile oil and gaze chromatography-Mass (GC-Mass) to estimate quantitative and qualitative active compounds in oil, Using diphenyl-2-picryl-hydrazyl(DPPH) and ascorbic acid as a positive comparison was determined the antioxidant effect. Some chemical testes was done on minced cattle meat by used 200mg/ml from oil under 4°C for (0,3,7 and 10).The results showed that volatile oil containing 39 compounds but oleic acid is more than other compounds (24,49%),then the oil has antioxidant effect 100% at 100µl/ml. The results of oil effect on minced cattle meat was showed decreased on thiobarbituric acid(TBA) and total violate nitrogen (TVN) under 4°C after 15 day, but the pH value was not changed. The volatile oil of *Cestrum nocturnum* flowers had antioxidant activity and mixing of this oil with minced cattle meat led to increase of period of save to 15 day .

Key words: extraction, save minced meat, violate nitrogen

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فعالية الزيت الطيار لازهار نبات الشبوي *Cestrum nocturnum* المزروع في العراق كمضاد للاكسدة واطالة فترة حفظ

اللحم المفروم

ايناس جواد كاظم

استاذ مساعد

قسم العقاقير والنباتات الطبية ، كلية الصيدلة ، جامعة بغداد ، العراق

بشرى محمد جابر علوش

استاذ مساعد

قسم علوم الحياة ، كلية العلوم للبنات ، جامعة بغداد ، العراق

زينب عمران سلمان

مدرس مساعد

المستخلص

هدفت الدراسة الى استخلاص وتقدير كمية الزيت الطيار في ازهار النبات للتعرف على انواع المركبات الفعالة الموجودة فيه ، ودراسة الفعالية المضادة للاكسدة الزيت الطيار، واختبار تأثير الزيت الطيار على الصفات الكيميائية للحم البقر المفروم المحفوظ بالتبريد لاطالة فترة الحفظ. استعملت طريقة التقطير البخار لاستخلاص الزيت و تقنية كروموتوغرافيا الغاز السائل لتقدير كمية ونوعية المركبات الفعالة فيه. باستعمال مادة 1-diphenyl-2-picryl-hydrazyl (DPPH) وحامض الاسكوريك للمقارنة الموجبة تم تحديد التأثير المضاد للاكسدة للزيت . اجريت بعض الاختبارات الكيميائية على اللحم المفروم باستعمال تركيز 200ملغم/مل من الزيت الطيار ومدة خزن (0و3و7و15) يوم تحت درجة حرارة 4 م°. اظهرت نتائج تحليل ال-Gc-Mass ان الزيت الطيار لازهار الشبوي تحتوي 39 مركبا وكان اعلى تركيز لمركب oleic acid اذ بلغت نسبته (24,49%)، اما بالنسبة للفعالية المضادة للاكسدة فقد اظهرت النتائج ان الزيت الطيار للازهار فعالية مضادة للاكسدة 100% عند التركيز (100 µl/ml). ان نتائج تأثير الزيت الطيار على اللحم المفروم المحفوظ بالتبريد فقد اظهرت انخفاض كل من قيم حامض الثايوباربيوتاريك وقيمة النيتروجين عند درجة حرارة 4 م° لمدة 15 يوم ، اما قيمة الاس الهيدروجيني (pH) فلم تتغير. يستنتج ان زيت ازهار الشبوي فعالية مضادة للاكسدة وان خلط الزيت الطيار بلحم البقر المفروم ادى الى اطالة مدة الخزن لمدة 15 يوم .

الكلمات المفتاحية : الاستخلاص، حفظ اللحم المثلوم، النايتروجين المتطاير

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INTRODUCTION

Cestrum nocturnum L. is a garden shrub belong to the family Solanaceae, it has many native names, such as Night Cestrum, Lady of the Night and Night Blooming Jasmine. Its flowers exude a special sweet fragrance at night, which is the main reason for folk names (21). A perennial woody bush with glossy, smooth, simple leaves is the characteristic feature of it, as well as vine-like stems and greenish, creamy white tubular flowers (18). *C. nocturnum* widely naturalized in tropical and subtropical regions throughout the world, including Australia, southern China, USA and etc the farmers cultivate it as an ornamental plant, and flowers at night that are heavily perfume (23). Most of the species of *Cestrum* have several applications in folk medicine. The presence of important bioactive compounds, alkaloids, flavones glycosides, steroidal saponins, fatty acids, essential oils, phenols, and others, which gave medicinal importance for this plant (5). *C. nocturnum* has several pharmacological activities such as antimicrobial, larvicidal, antimalarial and antioxidant (24). The leaves of the plant have been shown significant bactericidal activity, local anesthetic effect, in burns, and swellings (7,26). In west Indian and islands, the plant was used for treating epilepsy and in stupefying charm medicine. Also in several African Nations, *C. nocturnum* and *C. diurnum* are used to prevent malaria due to the presence of volatile oil hence its known to mosquito repellent (19). Oxidation is a chemical reaction that including loss of electrons or an increase in oxidation state, these reactions can produce free radicals which are crucial for life, they can also be damaging on organisms cells especially on DNA or onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis. Antioxidant compounds are molecules that inhibits the oxidation of other molecules, and scavenging free radicals (11, 16). So, industrial processing in order to prolong storage stability of foods, synthetic antioxidants are mainly used, but their usage in food products has been falling off according to the toxicologists and nutritionists who documented, the side effects of some of these synthetic antioxidants such as, butylated hydroxytoluene (BHT) and

butylated hydroxyanisole (BHA) (13), their instability, and because of a suspected action as promoters of carcinogenesis (22). However, for this reason, there is a growing interest in the studies of natural additives as potential antioxidants. The antioxidant properties of many herbs and spices are reported to be effective in retarding the process of lipid peroxidation in oils and fatty foods and have gained the interest of many research groups (8,14). There for, the essential oils and extracts of plant have been of great interest in the last years as sources of natural products, either for their uses as a preservatives of food from the toxic effects of the oxidants or as alternative remedies for the treatment of many infectious diseases (15). A several studies on the antioxidant action of various aromatic plants have been reported over the last 20 years (17). As far as our literature survey could ascertain that there are no reports on the antioxidant action of the essential oil from flower parts of *C. nocturnum* cultivated in Iraq. Thus, The research aims to estimate the quantitative and qualitative of active constituents of essential oil in flowers of *C. nocturnum* and, evaluate *in vitro* antioxidant properties of the essential oil

MATERIALS AND METHODS

The flowers of *C. nocturnum*, were collected from fields in Baghdad city /Iraq in september 2017. It was identified by the college of Agriculture, University of Baghdad and, authenticated by the Iraqi national herbarium. Then it was used directly in the extraction process.

Extraction essential oil

A Clevenger apparatus was used for the hydrodistillation extraction of the flower parts of *C. nocturnum*, one hundred gm of flowers were cutted into very small pieces, 600 ml of distilled water was adding to the flask (with total volume 2 liter). Then the Flasks was left for 12 hours, to ensure that the extraction of essential oil from the plant parts was completed, then the oil was collected with small, dark and sealed glass bottles and kept in the refrigerator at a temperature 4°C until further analysis

Gas chromatography-mass spectrometry (GC-MS) analysis

Quantitative and qualitative analysis of the *C. nocturnum* extracts was performed using a

GC-MS (Model QP 2010, Shimadzu, Japan) InertCap Pure Wax capillary column was used (30 m x 0.25 mm x 0.25 µm film thickness) with helium as carrier gas at a flow rate of 1.53 ml/min. The source was operated in positive ionization mode (electron impact energy: 70eV) and the detection was performed in full-scan mode. The inlet and the transfer line temperatures were maintained at 170°C, while the ion source was kept at 220°C. Samples were injected in split or splitless mode (2:1) and separated using a temperature gradient program as follows: 70°C for 3min, to 120°C at 15°C/min and then maintained at 120°C for 2mins; then to 200°C at 10°C/min and maintained at 200°C for further 8 mins. GC-MS spectra were evaluated by Postrun software and searched in the National Institute of Standards and Technology (NIST) MS Search V2.0 browsers(1).

Antioxidant Activity

The assessment of essential oil from *C. nocturnum* plant was done by quantifying the scavenging ability of stable free radical:1,1-Dyphenyl-2-picrylhydrazyl radical (DPPH) used as antioxidant(15). By mixing 0.5 ml of DPPH solution at 0.4 µM with 1ml of each concentration (20,40,60,80 and 100 µl ml⁻¹) of plant extract for 30 min in the dark at room temperature. Spectrophotometer was estimated the absorption of the samples at 517 nm, ascorbic acid used as standard. The scavenging activity was calculated according to the formula:

$$\text{scavenging activity \%} = \frac{A_{517 \text{ control}} - A_{517 \text{ sample}}}{A_{517 \text{ control}}} \times 100$$

Where $A_{517 \text{ control}}$ is the absorbance of DPPH only ; $A_{517 \text{ sample}}$ is the absorbance all at the extract samples. These experimental repeated in triplicate for essential oil of *C. nocturnum* plant.

Elongation period of save minced meat

Preparation of meet samples-A

The minced beef, objected in this study, was obtained freshly from the local markets, and then treated with the extracted assential oil. The concentration of (100 µl /ml) was used in the treatment and then added to 500 g of minced meat and then compared with the

control treatment (uncooked meat) The results were taken after (0,3,7,13) day at 4 ° C.

B-Chemical Estimates

1.PH measurement: The pH of the samples were measured by a pH meter. it was done by taking 10 gm from minced meat and soak it in 50 ml distilled water for 2 minutes

2.Thio-barbituric Acid (TBA) measurement : it was done by taking 10 gm from minced meat and soak it in 50 ml distilled water for 2 minutes, then added 5 mL of hydrochloric acid solution (4N) to reduce pH to 1.5. After that completed the volume to 100 ml distilled water then transferd the mixture to a distillation flask with the addition 100 mL of essential oil and 1 gm dry size to regulate boiling and prevent feces. Then the distillation device was operated and heat up until 50 mL of distillates was collected was used as a blanc solution. Distilled water the mixture was estimated at 538 nm optical then TBA was calculated on the basis of mononaldehyde amalgam / 100 g of meat (20).

3. Total violet nitrogen meseurement :

Ten ml of distilled water was added to 10 grams of minced cattle meat, then added 2 g of manganese oxide finally 250 ml of distilled water was added. The distillation flask was connected in the Kjeldahl (2%) with two drops of Methyl red. After 30 minutes of distillation, the mixture was diluted with diluted sulfuric acid (0.1 N) and the amount of total nitrogen was calculated on the basis of nitrogen mg / 100 g of meat (10).

Statistical analysis

The Statistical Analysis was done using System- SAS (2012), program was used to effect of difference factors in study parameters. Least significant difference –LSD was used to test a significant compared between means in this study

RESULTS AND DISCUSSION

The results of GC-mass analysis shows that the essential oil from *C. nocturnum* flowers are containing 39 components. (Table 1) and (Figure 1), and the Oleic acid has a higher concentration than other compounds, it reaches to 24.49 % , follow by linalool 12.94% , Hydroxycitronellol 7.40% , Acetyl carene 5.24% , Geraniol 2.97 % and Benzyl acetate 2.65 %

Table 1. Essential oil components concentrations' of *C. nocturnum* flowers

N	Name of compound	%
1	Beta-Hexenol	0.08
2	Beta-pinene	0.24
3	Limonene	0.73
4	Hydroxycitronellol	7.40
5	Linalool	12.64
6	2-Butanol, 3,3-oxybis	2.25
7	Linalyl anthranilate	0.22
8	Benzyl acetate	2.65
9	Phenylethyl chlorocetate	0.28
10	Linalool oxide	2.04
11	1,6-Heptadiene, 2,5,5-trimethyl	5.26
12	Geraniol	2.97
13	4-tert-Butylcyclohexyl acetate	0.20
14	Cinnamic alcohol	0.46
15	2 (Ethoxyethyl –sulfanyl) acetic acid	1.23
16	alpha.-Bergamotene	0.19
17	Edufan	0.57
18	3-methylpent-4-enylbenzol	0.70
19	Amyl salicylate	1.20
20	Patchoulane	1.89
21	Hexyl benzoate	1.63
22	Di-5-hexen-2-yl phthalate	9.34
23	2,7-Dimethyl-3,6-octadien-2-ol	0.94
24	Hedione	0.64
25	:Myrcenylacetat	0.64
26	4-Methyl-4-phenyl-2,4,5,6-diepoxy cyclohexanone	4.01
27	Acetyl caren	5.24
28	Bisomel	1.86
29	Galaxolide	3.63
30	Benzyle salicylate	0.76
31	Palmitic acid	1.48
32	Astratone	0.29
33	Methyl 11,14-Eicosadienoate	0.21
34	*** Oleic Acid	24.49
35	(Z)6-Pentadecen-1-ol	0.61
36	3-Heptadecanol	0.23
37	Benzyl oleate	0.18
38	Benzene, 1,2-bis-9-borabicyclo-yloxymethyl	0.33
39	5,9-dimethyl-8-decadienal	0.28

These results did not agreement with Sharif *et.al.*(24), where they stated the major characteristic components of *C. nocturnum* essential oil represented by phenylethyl alcohol, *trans*-Z-bisabolene epoxide, eicosane, methoxyeugenol, heneicosane, hexadecanoic acid benzyl alcohol, caryophyllene oxide,

eicosane and eugenol .This agreement could be due to the geographical region, difference of soil components, environment conditions ,and genome of the plant, *C. nocturnum* flowers oil has differences in concentrations and quality. Thus, these changes leads to huge effect in quantity and quality of essential oil and its components (6,3).

Antioxidant Activity

Results in (Table 2) shows that the essential oil from *C. nocturnum* flowers were tesred as antioxidant and determined by utilizing scavenging action against DPPH as free radical ,and compared with ascorbic acid as sranderd.

Table 2. Antioxidant activity of essential oil from *C. nocturnum* flowers compared with ascorbic acid

Concentratio n µl/ml	Percentage of anti-oxidation		LSD value
	essential oil	Ascorbic acid	
20	22.06 ±0.23	56.60 ± 2.37	3.638 *
40	39.74 ±0.64	80.69 ± 2.74	4.093 *
60	72.68 ±1.74	83.60 ± 3.49	3.178 *
80	86.38 ±1.65	98.20 ± 4.07	2.253 *
100	100.00 ±0.00	98.60 ± 3.05	3.708 *
LSD value	* 3.519	* 3.633	----

* (P<0.05).

The statistical analysis results shows in Table 2 that the essential oil of *C. nocturnum* flower have higher antioxidant influence by 100% in 100 µl/ml concentration than 86% in 80 µl/ml concentration as compared with the standard control ascorbic acid. The oleic acid, ,linalool, geraniol and benzyl acetate compounds which were found on essential oil of *C. nocturnum* flowers this compounds that are able to perform this reaction can be considered as antioxidant (9).The results of this study shows that the essential oil have the ability to scavenge free radicals when compared with reference standard , and this is represented by donating their hydrogen atom to quell the free radicals of DPPH, these results matched the study of (24).

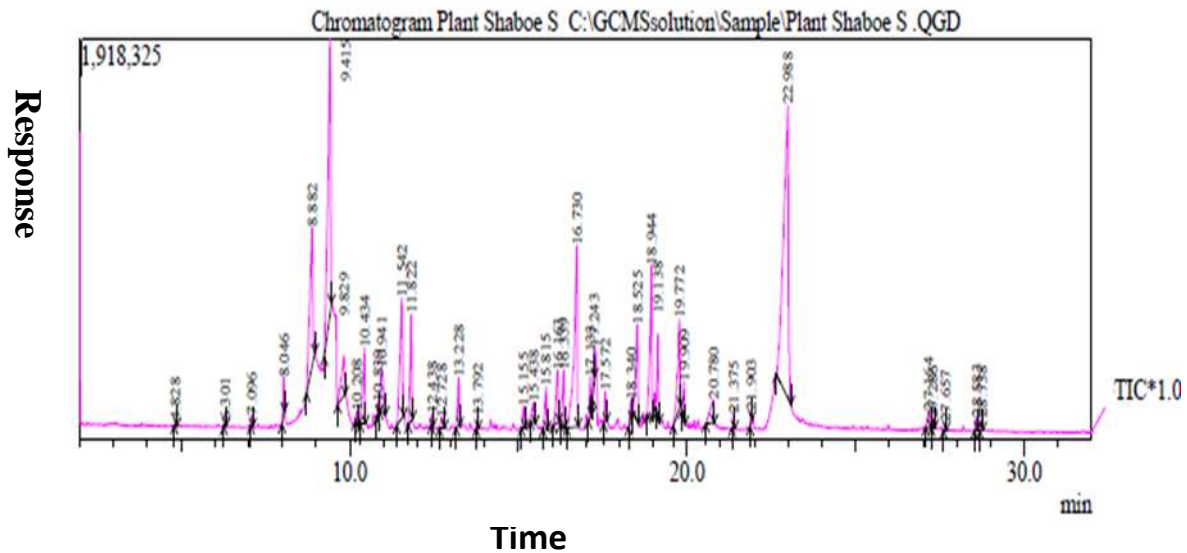


Figure 1. GC-mass analysis of essential oil of *C. nocturnum* flowers

Elongation period of save minced meat

The results in Table 3 shows the effects of essential oil from *C. nocturnum* flowers at concentration 200 mg/ml mixed with minced cattle meat in (4°c) after 3, 7, and 15 Days. These results shows the pH of the meat doesn't change after treatments ,due to the essential oil containing some acids like oleic acid and palmitic acid. These acids have ability to decrease pH to reach the equilibrium point (25).

Table 3. Effect of treatment and period with essential oil of *C. nocturnum* flowers on pH of cattle meat

Time on fridge	Mean ± SE of PH		value LSD
	After treatment	Before treatment	
time (Zero)	b5.50± 0.02	b5.53± 0.03	0.389NS
days 3	b5.60± 0.00	b5.70 ±0.00	0.307NS
days 7	ab5.66± 0.03	b5.90± 0.00	0.417NS
day 15	a6.13± 0.03	a6.56± 0.06	0.495NS
LSD	* 0.497	* 0.631	---
.(P<0.05) *			

Results in Table 4 shows the effect of essential oil of *C. nocturnum* flowers on TVN (total violate nitrogen) value of cattle meat. The best results reached to(15.90 nitrogen mg/100g meat) after 15 days when treated with essential oil compare with non-treated sample(24.23mg/100gm meat) in the same time, the followed by 13.56 and 12.63mg nitrogen/100g meat after 3 and 7 day under cold compering with control, when reached to 15.23 and 17.26 mg nitrogen/100g meat respectively. The statistical analysis indicate that the results gave significant (P<0.05) for

TVN value between the treated and not treated meat with oil under cooled condition. This results approve that the essential oil of *C. nocturnum* flowers has effected to decrease TVN, which presents the measurement of essential nitrogen release from the amino acids which degraded by bacteria(20), These resultes mighty be containing the essential oil of oleic acid, linalool, geraniol, and benzyl acetate and, these components inhibit the activities of bacteria and lead to decrease on TVN value. That means the oil of *C. nocturnum* has effect on bacteria and can saved the meat under cold degree at a long time.

Table 4. Effect of treated and period with essential oil of *C. nocturnum* flowers on TVN

Time on fridge	Mean ± SE of TVN (Nitrogen mg/100g meat)		value LSD
	After treated	Before treated	
time (Zero)	b 12.03 ±0.14	c 12.13± 0.14	1.685NS
days 3	b 12.63 ±0.06	bc15.23± 0.12	2.094*
days 7	ab13.56± 0.03	b17.26± 0.18	2.735*
day 15	a15.90± 0.15	a24.23± 0.44	4.016*
LSD	* 3.175	* 4.049	---
.(P<0.05) *			

The results of effect the essential oil from *C. nocturnum* flowers on TBN (thio barbituric acid) before and after treated with meat as shows in Table 5. The best results reached to 52.67mg/100g meat malon aldehyd after 15 days comparing with control(non treated meat) 81.00 mg/100gm meat malonaldehyd in the

same time, followed by 38.67 and 34.00 malonaldehyd mg/100g after 3 and 7 day under cold compering with control to 47.67 and 38.33 malonaldehyd mg/100g, respectively. The statistical analysis indicate that the results gave significant ($P < 0.05$) for TVN value between the treated and not treated meat with oil under cooled condition. These results indicate that the active compounds on essential oil have antioxidant activity, as shows in Table 2, like oleic acid, linalool, geraniol which might be late the oxidation of meat fat. These compounds interact with the reverse reaction, that lead to decrease in fat oxidation, and fixed the free radical by preventing the transfer of hydrogen atom. Thus, prevent the rancid component to increase, like ketones, aldehydes, and carboxylates(12).

Table 5. Effect of treated and period with essential oil of *C. nocturnum* flowers on TBN

Time on fridge	Mean \pm SE of TBA (malon aldehyd mg/100gmeat)		value LSD
	After treated	before treated	
time (Zero)	b33.00 \pm 0.57	c32.33 \pm 0.88	4.820NS
Day3	b34.00 \pm 0.00	c38.33 \pm 0.33	5.064NS
Day7	b38.67 \pm 0.33	b47.67 \pm 1.20	5.172*
day 15	a52.67 \pm 1.67	a81.00 \pm 1.73	7.947*
LSD	* 6.729	* 8.352	---
.($P < 0.05$) *			

the essential oil from *C. nocturnum* flowers cultivated in Iraq containing many types of active compounds, and has antioxidant activity that decrease the bacterial activity of meat when save under cold condition. These properties allowed to save meat mixed with essential oil in fridge for the longer time. Thus, advised using this oil as a preservative for meat and other types of food.

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