The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
E-ISSN: 2959-4820
Volume 3, Issue 3, 2025
Page No: 56-62
Website: https://najsp.com/index.php/home/index
SJIFactor 2024: 5.49 **معامل التأثير العربي (AIF) 2024: 0.71** **ISI 2024: 0.696**

Assessment of Ephedra Extracts Antibacterial Effectiveness in Vitro

Fatma Hebail[1], Najmeddin Ellali[2*], Abdussatar Saadallah[3], Ekram Rashed[4], Retaj Atiya[5], Fairouz Reda[6], Alaa Ramadan[7]
[1]Department of Chemistry, Faculty of Education Janzour, University of Tripoli, Tripoli, Libya
[2]Faculty of Pharmacy, University of Zawia, Zawia, Libya
[3,4,5,6,7]Tripoli College of Medical Science, Tripoli, Libya

تقييم فعالية مستخلصات نبات العلندة المضادة للبكتيريا في المختبر

فاطمة الهبيل [1]، نجم الدين اللالي [2*]، عبدالسار سعدالله [3]، اكرام راشد[4]، رتاج عطية[5]، فيروز رضا[6]، آلاء رمضان[7]
[1] قسم الكيمياء، كلية التربية جنزور ، جامعة طرابلس ، طرابلس، ليبيا
[2] كلية الصيدلة، جامعة الزاوية، الزاوية، ليبيا
[3-7] كلية طرابلس للعلوم الطبية، طرابلس ، ليبيا

Corresponding author: najmiellali@yahoo.com

Received: May 23, 2025	Accepted: June 25, 2025	Published: July 10, 2025

Abstract
Natural sources of medicinal plants have been receiving a lot of attention lately. The purpose of this study was to use the agar well diffusion method to assess the antibacterial activity of ephedra water and methanol extracts against four different species of bacteria: two gram negative (S. aureus, B. sub) and two grams positive (E. coil, S. typhi). The primary bioactive substances identified in ephedra were alkaloids, tannin, anthraquinone, terpene, coumarin, and saponin. The antibacterial activity indicated that the ephedra methanol extract exhibited inhibitory effect only against Bacillus subtilis and Staphylococcus aureus. However, other bacteria shown extract resistance. Ephedra extracts may be a viable option in the hunt for a natural antibacterial agent based on the current findings. The scientific knowledge gained from this investigation will help determine the antibacterial values and explore additional pharmacological features.

Keywords: Ephedra, Natural Plant, Extraction, Bioactive Substances, Antimicrobial Activity.

الملخص
تشهد المصادر الطبيعية للنباتات الطبية اهتمامًا كبيرًا في الآونة الأخيرة. كان الغرض من هذه الدراسة هو استخدام طريقة الانتشار في الآبار في الآجار لتقييم النشاط المضاد للبكتيريا لمستخلصات العلندة المائية والميثانولية ضد أربعة أنواع مختلفة من البكتيريا: اثنتان سالبتان للجرام (المكورات العنقودية الذهبية، العصوية الرقيقة) واثنتان موجبتان للجرام (الإشريكية القولونية، السالمونيلا التيفية). كانت المواد النشطة بيولوجيًا الرئيسية التي تم تحديدها في العلندة هي القلويات، والتانين، والأنثراكينون، والتربين، والكومارين، والصابونين. أشار النشاط المضاد للبكتيريا إلى أن مستخلص العلندة الميثانولي أظهر تأثيرًا مثبطًا فقط ضد العصوية الرقيقة والمكورات العنقودية الذهبية. ومع ذلك، أظهرت بكتيريا أخرى مقاومة للمستخلص. قد يكون مستخلص العلندة خيارًا قابلاً للتطبيق في البحث عن عامل طبيعي مضاد للبكتيريا بناءً على النتائج الحالية. ستساعد المعرفة العلمية المكتسبة من هذا التحقيق في تحديد القيم المضادة للبكتيريا واستكشاف ميزات إضافية.

الكلمات المفتاحية: النباتات الطبيعية والعلندة، الفصل، المواد الفعالة، النشاط المضاد للبكتيريا.

Introduction

Nowadays, more than 40% of pharmaceuticals are made from natural materials, and many of the most popular medications came from TCM's cabinets. The combination of this organic approach and traditional wisdom has led to important advancements in medicine. Herbs have been used as effective remedies for a wide range of illnesses since ancient times. They have helped create important medications like aspirin, which was made from willow bark, oral contraceptives, which were made from wild yam roots, and treatments for childhood cancer, which were made from periwinkle plants. Similarly, an analysis of ancient Chinese medicinal literature served as the foundation for Nobel Prize-winning research on artemisinin's ability to prevent malaria. Ancient vaccination traditions in societies worldwide served as inspiration for the development of the smallpox vaccine, which ultimately resulted in the disease's eradication [1]. (WHO, 2014).

According to Sodany et al. (2013), the utilization of plants for food and medicine has been practiced for thousands of years [2]. Natural products contain a variety of substances that could be used as innovative antibacterial treatments, especially in cases when drug resistance exists. Numerous secondary metabolites that have been extracted from medicinal plants might have antimicrobial qualities that aid in reducing resistance [3].

An Investigation into Plant Components Plants include a wealth of active chemical substances with a variety of medical, cosmetic, and aromatic applications, including glycosides, alkaloids, flavonoids, tannins, saponins, resins, volatile oils, fats, and lipids. Each of these many plant parts has special qualities of its own. These substances are among nature's treasures and continue to offer priceless advantages in the areas of nutrition, medicine, cosmetics, and aroma [4].

The Nafousa Mountain region in Libya is well known for its profusion of aromatic and medicinal plants, which constitute a substantial economic and health resource [5]. The area's natural vegetation cover is thought to have more than 50 species that are used for therapeutic reasons and flourish all year round. These non-timber forest products serve a variety of purposes, meet local demands, and are an important component of trade [5].

Data shows that nearly half of people in many developed nations now frequently utilize TCM in the Ephedra, commonly known as Ma Huang, is a plant that grows in the Green Mountain area. According to Hakim (2016), ephedra's ephedrine content has been utilized in Chinese medicine to treat colds, nasal congestion, bronchodilator, and other cold symptoms. Ephedrine was first extracted commercially in 1881[6]. Ephedra has several uses and advantages, including as reducing blood pressure, treating respiratory disorders like asthma and chest infections, and even controlling some cancers, especially those that are still in their early stages. Its effectiveness in fighting malignant cells and preventing their spread has been demonstrated by several investigations and tests [7].

The antibacterial efficacy of the Ephedra transitoria pathogen was investigated against a range of pathogens. Gram-positive bacteria like S. aureus and B. subtilis, gram-negative bacteria like E. coli and P. vulgaris, and fungi like A.migatus and C. albicans were all evaluated against EST Methanolic Extract utilizing the qualitative disc diffusion method [8].

A small shrub with short stems grows next to cacti and olive trees and looks like grasses and climbing plants. It rises to around one meter in height. Its numerous branches and twigs, which are the most crucial component utilized in the manufacture of medications, give it a greenish-yellow appearance. You can also utilize the entire plant or only the roots. Thick and tubular, with an almost conical form [6] [7].

Figure (1): Image 0.1 depicts the shape of the Alalanda plant.

Saudi Arabia is one of the Asian countries where alalanda and ephedra originated [9]. It is widespread in arid regions of the Arabian Peninsula, Morocco, Algeria, and Libya [10]. Alalanda /Ephedra provides several health benefits due to its active components. Its branches are rich in flavonoids, phenolic compounds with antioxidant and anticancer properties. It contains also ephedrine, chloride, minerals,

and other substances. The primary ingredient of Ephedra is Ephedrine, which dilates bronchi, constricts blood vessels, and activates the central nervous system [11]. It can also be used to lower blood pressure, relieve lung blockage and congestion, lower blood fat levels, and serve as an antibacterial [12].

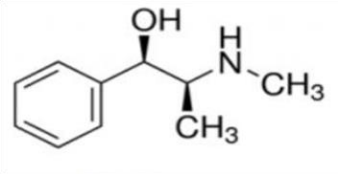

Figure (2): Ephedrine.

Table 1: Botanical Classification of *Ephedra* (Ozenda, 2009) [13].

Classe	Gnetopside
Ordre	*Ephedrales*
Famille	*Ephedraceae*
Genre	*Ephedra*
Espece	*Ephedraalata*
Sous Espece	*Ephedraalataalenda*

The purpose of this study was to evaluate the antibacterial characteristics of ethanol-prepared ephedra extract and demonstrate that it may be a valuable source of natural phenols and alkaloids with high biological activity against a variety of microorganisms (bacteria). Analyzing the active ingredients, it contains and raising awareness of its importance and applications.

Methods

Plant Material Collection

In the spring of 2024, the plant was gathered from the Nafousa Mountain region in Libya. The Department of Botany, Faculty of Science, Tripoli University, Tripoli, Libya, subsequently classified and verified it. The entire plant was taken to the lab, where it was cleaned with distilled water, allowed to dry for two weeks away from the sun and moisture, then pulverized finely with an automatic grinder and placed in sterile, airtight glass containers.

Sources Of Chemicals:

1. Merck (Germany) produces high analytical grade organic solvents, such as methanol and chloroform.
2. Oxoid (England) produced antibiotic paper discs (tetracycline, vancomycin).

Four types of bacteria were chosen to measure the biological activity of plant, there are as follows:

Table 2: types of bacteria were chosen to measure the biological activity of plant.

Gram (+ve)	Gram (-ve)
(B.sub) Bacillus subtilis	*(Sal) Salmonella typhimurium*
(S.aur) Staphylococcus aureus	*(E.coli) Escherichia coli*

Extraction test:

Preparation of plant crude extracts.

Using a sanitized electric blender, the dried material was ground into a powder and sieved through a suitable mesh screen before being stored in an airtight, dark-colored glass container. (Covered in sheets of aluminum foil). The following extraction processes were used to the powdered material:

Aqueous extraction:

a) Water: In tightly sealed jars, 20 g of ground plant material was steeped in 100 ml of distilled water at room temperature (25°C) for 6 hours in order to create an aqueous infusion, with constant trembling. Before being employed, the crude extract was stored at 4°C after being filtered through Whitman no. 1 filter paper.

b) Hot water: 20g of powered plant material was boiled with distilled water for 10 to 15 minutes while stirring in order to obtain hot water.

Organic Solvent Extraction:

In order to extract organic solvents, 20g of dry, milled plant material was extracted three times using 200 ml of the solvent (methanol) over the course of 24 hours. A rotary evaporator was used to filter the extract and evaporate it to dryness at 40°C with reduced pressure. Before being used, the crude extract was stored at 4°C after being filtered through Whitman filter paper.

Phytochemical Screening:

The principal ingredients were screened for phytochemicals using standard qualitative techniques as outlined [14]. Alkaloids, terpenes, tannins, anthraquinones, coumarone, flavonoids, and saponins. The phytochemical contents derived from the extraction of ephedra from (+ve) for faint to (+++ve) for dense turbidity are displayed

Chemical testing (Color reactions):

Standard methods were used to identify the contents in the alcoholic and aqueous extracts of powdered materials by chemical assays [15].

Coumarins Test:

Under UV light (366 nm), coumarins were discovered; after 10% KOH was sprayed, the blue fluorescence spots intensified.

Saponins Test:

Saponins are naturally occurring amphiphilic glycosides made up of a hydrophobic aglycone (sapogenin) and a hydrophilic sugar moiety (glycone). Their capacity to lower surface tension and create foam in aqueous solutions is well known. In a water bath, roughly 2g of the powdered sample was boiled with 20ml of distilled water before being filtered. To create a stable, long-lasting froth, around 5 milliliters of the filtrate were combined with 2.5 milliliters of distilled water and shaken briskly. Three drops of olive oil were added to the foam, and it was agitated vigorously once more before an emulsion was watched for. Saponins are present when emulsion forms [16].

Anthraquinone Test:

A 10% ammonia solution was added to the filtrate after 0.5g of extract and 10 ml of benzene had been agitated and filtered. Anthraquinones are identified by the development of a pink, red, or violet color on the ammoniacal phase [17].

Alkaloids Test:

Alkaloids are naturally occurring substances that contain nitrogen and are typically obtained from plants. Their pharmacological characteristics have been extensively researched, and they display a variety of biological actions. Alkaloids' basic nitrogenous structure can be found using qualitative tests, which often involve precipitation with acidic reagents. 2.5g of the powdered plant material was dissolved in 25ml of ethanol to create an alcoholic extract. The residue was then boiled with 5ml of 2N HCl and allowed to cool. Two equal quantities of the filtrate were separated after the mixture was filtered out. Wagner's reagent was applied in comparable proportions to one portion, while a few drops of Mayer's reagent were applied to the other. After that, the samples were checked for precipitation or turbidity [15]. KI solution (5g/10ml) was added after [HgCl2:1.358g] was dissolved in 60ml of water. 100 ml of water was used to dissolve KI (2g) and I2 (1.27g) [17,18].

Phenols Test:

Phenols are aromatic chemicals that have an aromatic ring directly linked to a hydroxyl group (-OH). Because of the resonance stabilization of the phenoxide ion, they have acidic characteristics. Widely present in plants, phenols are recognized for their anti-inflammatory, antibacterial, and antioxidant qualities. Both qualitative and quantitative techniques are used to test for phenols in order to identify their unique chemical reactivity [18].

Tannins Test:

Many plants contain tannins, which are polyphenol chemicals distinguished by their capacity to precipitate and bind proteins and alkaloids. Condensed tannins (flavonoid polymers) and hydrolysable tannins (derivatives of Gallic or pelagic acid) are the two primary categories into which they fall. Tannins are usually tested using qualitative analysis depending on how reactive they are to particular reagents. In a test tube, about 0.5g of the dried powdered sample was filled with 10 ml of water before being filtered. The filtrate was treated with a few drops of 0.1% ferric chloride, and its coloration was checked for brownish green or blue-black hues. The richness of tannins is indicated by the intensity of color [18].

Terpenoids Test (Salkowski test):

Terpenoids, commonly referred to as isoprenoids, are a broad and varied class of organic compounds that occur naturally and are produced from isoprene units. Based on how many isoprene units they contain, they are divided into various classes (monoterpenoids, sesquiterpenoids, diterpenoids, etc.). Terpenoids can be found in biological or chemical materials using a variety of qualitative and quantitative techniques. Five ml of plant aqueous extract were combined with \ 2 ml of chloroform, and 3 ml of concentrated H2SO4 were carefully added to create two layers. An interface that is reddish-brown in color implies that terpenoids are present [18, 19].

Flavonoids Test:

One class of polyphenolic chemicals that is commonly present in plants is flavonoids. They have important functions as plant pigments, antimicrobials, and antioxidants. Flavonoids are categorized into

multiple subclasses, such as flavones, flavanols, flavanones, isoflavones, and anthocyanins, and are distinguished by their C6-C3-C6 backbone structure. Flavonoids are tested using both qualitative and quantitative techniques depending on their distinct chemical. After adding 5 milliliters of diluted ammonia solution to plant extract aqueous filtrate, concentrated H2SO4 was added. Each extract contains flavonoids, which are indicated by a yellow tint that disappears while the extract is standing [18].

Evaluation of Extracts' Antimicrobial Properties

Hole-plate diffusion methods:

Using "hole-plate diffusion methods," the antibacterial activity of the crude extracts was assessed (Daud et al., 2005). For testing, each test organism was grown in nutrient broth (No. 2, Biolab, Difco) for 24 hours at 37°C after being maintained on nutrient agar slant. Using a pre-made calibration curve that represented the viable cell count (X ×106) versus OD 660nm (Y), cultures were regularly adjusted to a suspension of 1×106 to 2×106 CFU/ml prior to streaking. The optical density (OD) at 660 nm of each culture was measured with a UV/VIS spectrophotometer. Each culture was diluted 1:10 with new sterile nutritional agar (Meyer and Dilika 1996) for agar plates production.

Using a hollow punch, holes (8 mm in diameter) were aseptically drilled into the agar in Petri plates (12 cm in diameter) that held 30 ml of nutrient agar apiece. A sterile Eppendorf micropipette with disposable tips was used to take 150µl aliquots of the extract and put them into wells. In order to improve the diffusion of the extract into the agar, the plates were maintained at 4°C for one hour. After that, plates were incubated for eighteen hours at 37°C [20,21]. Tetracycline and vancomycin served as positive controls, while 70% methanol or sterile water served as negative controls. The results were recorded as the mean of the three experiments, and the diameters of the inhibition zones were measured in millimeters at the conclusion of the incubation period.

Result and discussion

Phytochemical screening:

Table 3 illustrates the findings of Ephedra's qualitative screening for common phytochemicals.

Table 3: The findings of Ephedra's qualitative screening for common phytochemicals.

Phytochemicals	Methanol extract
Alkaloids	+++ve
Flavonoids	+++ve
Tannins	+++ve
Terpenes	++ve
Coumarins	++ve
Saponins	+ve
Anthraquinone	++ve

ve, High, ++ve, Medium, +ve, low, -ve, None+++

Antimicrobial activity:

Aqueous extracte:

The active phytochemical elements of the aqueous extract of Ephedra, as indicated in Table 1, are examined for their antibacterial properties. The findings indicated that the plants either had a modest amount of terpene, coumarin, and anthraquinone or were rich in flavonoids and alkaloids. Other phytochemical components. According to Table 3, the water extracts' shown antibacterial activity against both G+ve and G-ve bacteria was weak to extremely weak. The plant's hot water extract exhibited almost the same activity as its cold-water extract against every tested bacterial strain, according to the results shown in Table (4) and Table (5).

Table 4: The cold aqueous plant extract's in vitro antibacterial effectiveness against sensitive, narrow-spectrum bacteria.

Aqueous Extract	Local name	Inhibition Zone (mm)*			
		Gram positive		Gram negative	
		S. aur	*B.sub*	*E. coli*	*S .typhi*
Ephedra	**Alanda**	17	8	20	14

8mm means no observed inhibition MP extracts are of 200mg/ml concentration

S. aur: Staphylococcus aureus B. sub: Bacillus subtilis E .coli: Escherichia coli

S. typhi: Salmonella typhi

Table 5: The hot aqueous plant extract's in vitro antibacterial effectiveness against sensitive, narrow-spectrum bacteria.

Hot aqueous extract of #:	Local name	Inhibition Zone (mm)*			
		Gram positive		Gram negative	
		S. aur	*B.sub*	*E. coli*	*S. typhi*
Ephedra	Alanda	19	12	12	18

Methanolic extract

The results obtained (Table 6) clearly indicate that:

a) Ephedra's methanolic extract exhibits modest antibacterial activity (DIZ= 26–32 mm on G+ve species and 18–24 mm on G-ve species).

b) The remaining methanolic extract under test demonstrated exceptional antibacterial activity against both bacterial and Gram-positive species. Given that it provides a DIZ range of 14–18 cm on G+ve bacteria, the extract appears to have relatively superior action when compared to the reference antibiotic Vancomycin (at 30 µg/disc) (Table 6).

Table 6: Ephedra's methanolic extract exhibits antibacterial action against specific sensitive bacteria in vitro.

Methanolic extract of #	Local name	Diameter of Inhibition Zone (mm)*			
		Gram positive		Gram negative	
		S. aur	*B. sub*	*E. coil*	*S. typhi*
Ephedra	Alanda	20	25	27	22
Vancomycin (30µg)		10	15	24	21
Tetracycline (30µg)		26	24	27	25

* 8mm means no observed inhibition MP extracts are of 200mg/ml concentration while, diluted Methanol, used as a negative control, produced negative outcomes for every test bacterium.

S. aur: Staphylococcus aureus *B. sub: Bacillus subtilis* *E. coli: Escherichia coli* *S. typhi: Salmonella typhi*

Table 7: Plant methanolic extracts' M I C (mg/ml) values on vulnerable bacterial species.

Plant extract of	Local name	*B. sub*	*S. aur*	*E. coli*	*S. typhi*
Ephedra	Alanda	12.50	3.125	3.125	6.25

S. aur: Staphylococcus aureus *B. sub: Bacillus subtilis,*
E. coli: Escherichia coli, *S. typhi: Salmonella typhi*

These plants' aqueous extracts exhibited mild to moderate antibacterial activity. Its alkaloid content is most likely the cause of this behavior. Given this, alkaloids' capacity to deactivate microbial adhesions, enzymes, and cell envelope transport proteins may be linked to their activity against different microbial species [22]. In comparison to other solvents like water, ethanol, and hexane, methanol was found to be the most effective solvent for the reliable extraction of antimicrobial compounds from medicinal plants in a number of studies [23]. Furthermore, Stanojevic et al. (2009) demonstrated that alcoholic extracts of Hieracium pilosella had a higher degree of phenolic content than water extracts [24]. The study's preferred extraction solvent was methanol, and the presence or absence of inhibition zones served as a qualitative indicator of the antimicrobial activity as illustrated Tables 6.

Conclusion

In conclusion, the traditional Libyan plants under investigation have demonstrated a dual function and are being examined as candidates for additional in-depth research focusing on the toxicity and molecular makeup of their active compounds. In addition, this could aid in the development of drug-design research projects to create naturally occurring lead compounds that are antibacterial and/or antioxidant.

References

1- World Health Organization (WHO). Medicinal Plants. 2014.

2- Al-Sodany YM, Bazaid SA, Mosallam HA. Medicinal plants in Saudi Arabia: I. Sarrwat Mountains at Taif, KSA. Acad J Plant Sci. 2013;6(4):134-145.

3-Rustaiyan A, Javidnia K, Farjam MH. J Med Plants Res. 2011;5(24):5713-5717.

4-Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioall Sci. 2020;12(1):1.

5- Balyan S, Sheoran A, Akash. Medicinal and Aromatic Plants and Their Utilization. Fars; 2003.

6- Hakim S. The medicinal properties and uses of Ephedra: A historical overview. J Ethnopharmacol. 2016; 180:100-115.
7- Danciu C, Muntean D, Alexa E, et al. Phytochemical characterization and evaluation of the antimicrobial, antiproliferative and pro-apoptotic potential of Ephedra alata Decne. hydroalcoholic extract against the MCF-7 breast cancer cell line. Molecules. 2019;24(1):13.
8- Parsaeimehr A, Sargsyan E, Javidnia K. A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra. Molecules. 2010; 15:1668–1678.
9- Al-Qarawi AA, Abd-Allah EF, Hashem A. Ephedra alata as biologically-based strategy inhibit aflatoxigenic seedborne mold. Afr J Microbiol Res. 2011; 5:2297–2303.
10- Ozenda P. Flora of the Sahara. 3rd ed. CNRS; 1991:662.
11- Ibragic S, Sofic E. Chemical composition of various Ephedra species. Bosn J Basic Med Sci. 2015;15(3):21-27.
12- Ephedrine: General medical uses and effects. Encyclopedia of Medicines; 2008.
13- Ozenda P. On the genesis of the plant population in the Alps: New or critical aspects. C R Biol. 2009; 332:1092–1103.
14- Abulude FO, Oladipo OT, Fagbayide JA. Phytochemical screening and mineral contents of leaves of some Nigerian woody plants. Res J Phytochem. 2007; 1:33-39.
15- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005.
16- Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. Springer; 1998.
17- Evans WC. Trease and Evans' Pharmacognosy. 16th ed. Elsevier Health Sciences; 2009.
18- Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 46th ed. Nirali Prakashan; 2008.
19- Krill C, Rochfort S, Spangenberg G. A high-throughput method for the comprehensive analysis of terpenes and terpenoids in medicinal cannabis biomass. Metabolites. 2020; 10:276.
20- Meyer JJM, Dilika F. Antibacterial activity of Helichrysum pedunculatum used in circumcision rites. J Ethnopharmacol. 1996.
21- Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48:5-16.
22- Hayder N, Bouhlel I, Skandrani I, et al. In vitro antioxidant and antigenotoxic potentials of myricetin-3-o-galactoside and myricetin-3-o-rhamnoside from Myrtus communis: Modulation of expression of genes. 2008;567-581.
23- Goyal PG, Kaushik PK. In vitro evaluation of antibacterial activity of various crude leaf extracts of Indian sacred plant, Ocimum sanctum L. Br Microbiol Res J. 2011;1(3):70-78.
24- Stanojević L, Stanković M, Nikolić V, et al. Antioxidant activity and total phenolic and flavonoid contents of Hieracium pilosella L. extracts. Sensors. 2009; 9:5702-5714.