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IN-VITRO COMPARISON OF THE ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM ENDEMIC PLANTS SPECIES

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ABSTRACT:

Onosma nigricaule and *Lathyrus karsianus* are a unique endemic plant species in Turkey. Present study reveals the difference in the antimicrobial activity pattern *of Onosma nigricaule* and *Lathyrus karsianus* different parts extracted in ethanol and methanol solvents against the pathogenic organisms *Klebsiella pneumonia* ATCC 4352 , *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, *Salmonella enteritidis* KUEN 349, *Proteus mirabilis* CCM1944, *Escherichia. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212. The results of this study suggest that the organic extracts of *Onosma nigricaule* and *Lathyrus karsianus* can be a source of natural antimicrobial agents with potential applications.

Key words: Antimicrobial activity, Minimum inhibitory concentration, *Onosma nigricaule*, *Lathyrus karsianus*

1.INTRODUCTION:

The discovery of antimicrobials is one of the most important discoveries in the field of medicine in 20th Century, which are effective against serious bacterial infections (1). But in recent years, in the fight against infectious diseases of humans and animals, therapeutic problems have been increased with the development of antimicrobial resistance due to the excessive amounts of antimicrobial usage (1,2,3). This situation is more serious in developing countries (2). According to The World Health Organization (WHO) reports; especially in tropical and developing countries around the world, more than 50% of deaths are caused by infectious diseases (4). According to WHO; more than 75% of the World population lives in the developing countries, and most of this population use traditional methods including herbal extracts and their active components as an initial treatment and they rely on them (2,5). The main reasons of committing to the traditional medicine methods are socio-economic status and difficulties to reach the modern medicines (4). The studies in recent years indicate that the traditional medicine methods for treatment of specific symptoms used by local people and antimicrobial activities in laboratories are similar to each other (4). Having antimicrobial activity of an extract of different plants has been reported by several authors (4,6). In the last 30 years, epidemiology of communicable infections has changed significantly and there is an increase in the development of antibacterial resistance in pathogenic microorganisms (4,7). This situation forces researchers to find new antimicrobial agents from various sources such as medicinal plants (7). However, antimicrobial activity of a very few plant species have been discovered systematically worldwide (7). It is known that antimicrobial activity of a plant is caused by phytochemicals which are also called bioactive *components* in the structure (8). Bioactive compounds in the form of secondary metabolites normally accumulate in plant cells, but concentrations of such compounds differs by plant parts, the season, the climate and vegetation period in particular (7). On the other hand, the obtained bioactive components from plant material is significantly dependent on the type of solvent used during the extraction procedure (1).

Onosma genus is represented by 97 species, 4 variants and 1 hybrid type in Turkey (9). 48.9% of the species belong to this genus are endemic types in Turkey (10). Some of these onosma types are used for paint

and pharmaceutical industries. Onosma nigricaule is one of the endemic species and used to treat wounds and burns (9).

Lathyrus karsianus is a kind of plant which is located in the Fabaceae family. There are more than 200 species in Lathyrus genus in the World. Plant species belonging to this family mainly consumed as food by human beings and animals, and some types are used in the pharmaceutical industry or as ornamental plants. Lathyrus karsianus is a unique endemic plant species in Turkey (9,11).

The purpose of this study is investigating the in vitro antibacterial activities of extracts obtained through two different organic solvents from *Onosma nigricaule* and Lathyrus *karsianus* endemic plant's different parts.

2.MATERIAL AND METHODS:

2.1.Plant materials and preparation of extracts

Plant samples were collected between June till September of 2012 in Kars and dried in the shade in the herbarium without being exposed to sun lights. After drying, each of the separated portions of the plant was grounded by a grinder until getting to a powder form. 20 grams of samples were collected from leaf (LkY) and body of lathyrus karsianus (LkG) and leaf (OnY), body (OnG) and flower (OnC) from Onosma nigricaule. Plant samples were extracted for 4 hours by using two different solvents (ethanol and methanol, 500 ml) in Soxhlet apparatus. The resulting extracts were concentrated in the evaporator at 40 ° C (12).

2.2.Bacterial strains:

Microorganisms were obtained from the culture collections of the Department of Microbiology at Faculty of Veterinary Medicine, Istanbul University. Nine bacterial strains, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, *Salmonella enteritidis* KUEN 349, *Proteus mirabilis* CCM1944, *Escherichia. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, were used for antibacterial testing. Bacterial cultures for antimicrobial testing were prepared by picking colony from 24-h-old Nutrient Agar (Biolab, NUA20500) plates supplemented with 7% sheep blood, and it was suspended in Tryptone Soya Broth (TSB; Oxoid, CMO129). Cultures were grown aerobically for 20 h a at 37 °C.

2.3.Dilution of plant extracts:

The agar-well diffusion method (13) was used to decide the dilution concentration of the extracts. Suspensions at the turbidity of corresponding to 0.5 McFarland standards from each bacteria were prepared. One hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar (Oxoid, CMO337) plates. The plates were allowed to dry for 3 to 5 minutes. Wells were punched in the plates using back side of a sterile pasteur pipette. The wells were filled with 50 µL and 90 µL of each extracts (200 mg/mL). The plates were incubated at 37°C for 24 hours. The plates were evaluated according to the growth inhibition zones around the wells. A common dilution range (100mg/ml to 1,56 mg/ml) of the both extracts were decided according to the results.

2.4.Detection of antibacterial activity:

Minimum inhibitory concentration (MIC) of the plant extracts was determined by the macrobroth dilution method acording to CLSI standards. Mueller-Hinton broth (Merck, 1.10293.0500; pH 7.3) with Mg^{++}(10mg/ml) and Ca^{++} (10 mg/ml) was used as the medium. Serial two fold dilutions of the extracts (100mg/ml to 1,56 mg/ml) were prepared in the media. The bacterial suspensions (10^6 CFU/ml) prepared from TSB were introduced to the tubes. Control tubes were prepared with culture medium with bacterial suspension, and with plant extracts. The tubes were incubated at 37 ºC for 16-20 hours. All measurements of MIC values were repeated in duplicate. As quality control, test was performed with

vancomycin and *E. faecalis* ATCC 29212. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC value (14,15).

To determine the Minimal bactericidal concentration (MBC), dilutions showing no visible growth for the MIC was sub-cultured onto a fresh MHA plates and incubated at 37°C for 24 hours. After incubation the concentration at which no visible growth was seen was noted as the MBC value (15).

3. RESULTS AND DISCUSSION:

The MIC values of the plant extracts against the organisms are shown in table 1.

The sample did not show any effect on metanol extracts; OnG, *S. aureus*, *S. epidermidis*, *B. cereus* and *E. coli* strains. All of the test strains showed sensitivity to the methanol extracts and S. Enteritidis, *P. mirabilis, E. faecalis, K. pneumoniae* and *P. aeruginosa* strains showed sensitivity to the OnG extracts. The highest sensitivity against LkG sample was shown by other strains excluding *S. aureus* with a 12.5 mg / ml MIC value (Fig. 1).

In the MBC evaluation of the samples' methanol extracts OnG extract was not effective on *E. coli*, *S. epidermidis, S.aureus* and *B.cereus.* Ong extract was effective on other strains whereas all other extracts were effective on all test strains (fig. 2).

All plant samples extracted with ethanol showed inhibitory activity on all test strains. When MIC concentrations were tested; the highest sensitivity is to LkY extract in *S. epidermidis* with 12,5 mg/ml concentration value, in *P.* a*eruginosa* to Lky and OnY extracts and in *B.cereus* to OnC extract (Fig. 3).

During the evaluation of the MBC for all ethanol extract samples, it has been determined that these samples are effective on all tested microorganisms. The highest activity is seen to OnC extract in *B. cereus* with a 12,5 mg/ml value (Fig.4).

etanol MLT değerleri

In our study, antibacterial activity of different solvent extracts in the same samples were also compared.

When LkY sample methanol extract and ethanol extract compared in the sense of MIC concentrations, ethanol extract in all the test strains was found to have a higher antimicrobial activity.

LkG methanol extract showed equal inhibition with ethanol extract in *S.* Enteritidis and *E. faecalis* strains whereas showed higher antimicrobial efficacy than ethanol extract in all other tested strains.

Ethanol extract samples of OnY showed higher antimicrobial effectiveness than ethanol extract against *P. aeruginosa* whereas for all other microorganisms they have same MIC concentrations.

Ong methanol extract showed equal antimicrobial effectiveness with ethanol extract in four test strains whereas showed higher effectiveness in all other tests. However, ethanol extract did not produce any MIC value in these 4 test strains.

Ethanol extract of OnC sample was found to have more antimicrobial activity than methanol extract in four test strains. For other strains antimicrobial activity was observed as equal for both extracts.

OnY methanol extract did not show any antimicrobial activity against *S.aureus, S. epidermidis, B.cereus* and *E. coli* strains, whereas all other extracts have antimicrobial activities. All plant samples belonging to the endemic plants of our country and no study of antimicrobial activities was detected. However, similar studies with different types of Onosma show that antimicrobial activities are available for such types (16,17,18).

It is very clear that onosma nigricaule and lathyrus karsianus have an effective antimicrobial potential. Therefore, both plants belong to a part of the active components of the assessment activities to be undertaken in isolation can play important roles in order to develop new antimicrobial compounds.

4. FIGURES AND TABLES:

Table: 1. MIC and (Minimal bactericidal concentration) MBC **values of the plant extracts.**

Solvent	Palnt Sample	Test	Bacterial strains								
			S. aureus	*S.epidermidis*	*B. cereus*	*S.* Enteritidis	*P.mirabilis*	*E. fecalis*	*K pneumoniae*	*P. aeruginosa*	*E.coli*
Methanol	LkY	MIC (mg/ml)	50	50	50	50	50	50	50	50	50
	LkG		25	12,5	12,5	25	12,5	25	12,5	12,5	12,5
	OnY		25	25	25	25	25	25	25	25	25
	OnG		0	0	0	50	50	50	50	50	0
	OnC		50	25	25	25	50	50	25	25	25
	LkY	MBC (mg/ml)	50	50	50	50	50	50	50	50	50
	LkG		25	25	25	25	25	50	25	12,5	25
	OnY		50	50	25	50	50	50	50	25	25
	OnG		0	0	0	50	50	50	50	50	0
	OnC		50	50	25	50	50	50	50	50	25
Ethanol	LkY	MIC (mg/ml)	25	12,5	25	25	25	25	25	12,5	25
	LkG		50	50	25	25	25	25	25	25	25
	OnY		25	25	25	25	25	25	25	12,5	25
	OnG		50	50	25	25	50	50	50	25	50
	OnC		25	25	12,5	25	25	25	25	25	25
	LkY	MBC (mg/ml)	25	25	25	25	25	25	25	25	25
	LkG		50	50	25	50	50	50	50	50	50
	OnY		25	50	25	25	25	50	25	25	25
	OnG		50	50	25	50	50	50	50	25	50
	OnC		50	50	12,5	50	50	50	50	25	50

Figure:1. Methanol extract of MIC values.

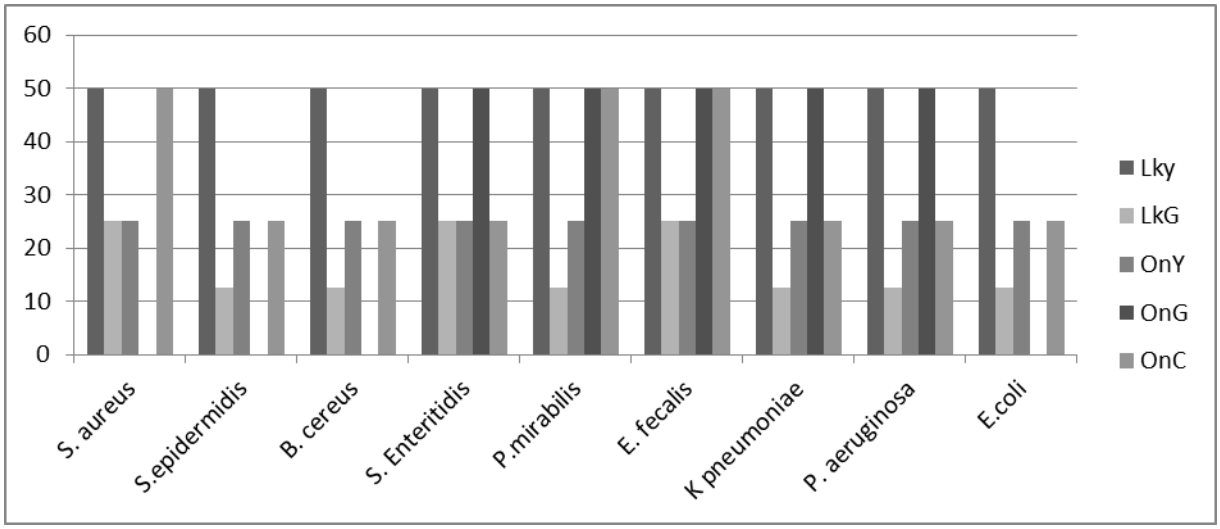


Figure:2. Methanol extract of MBCvalues.

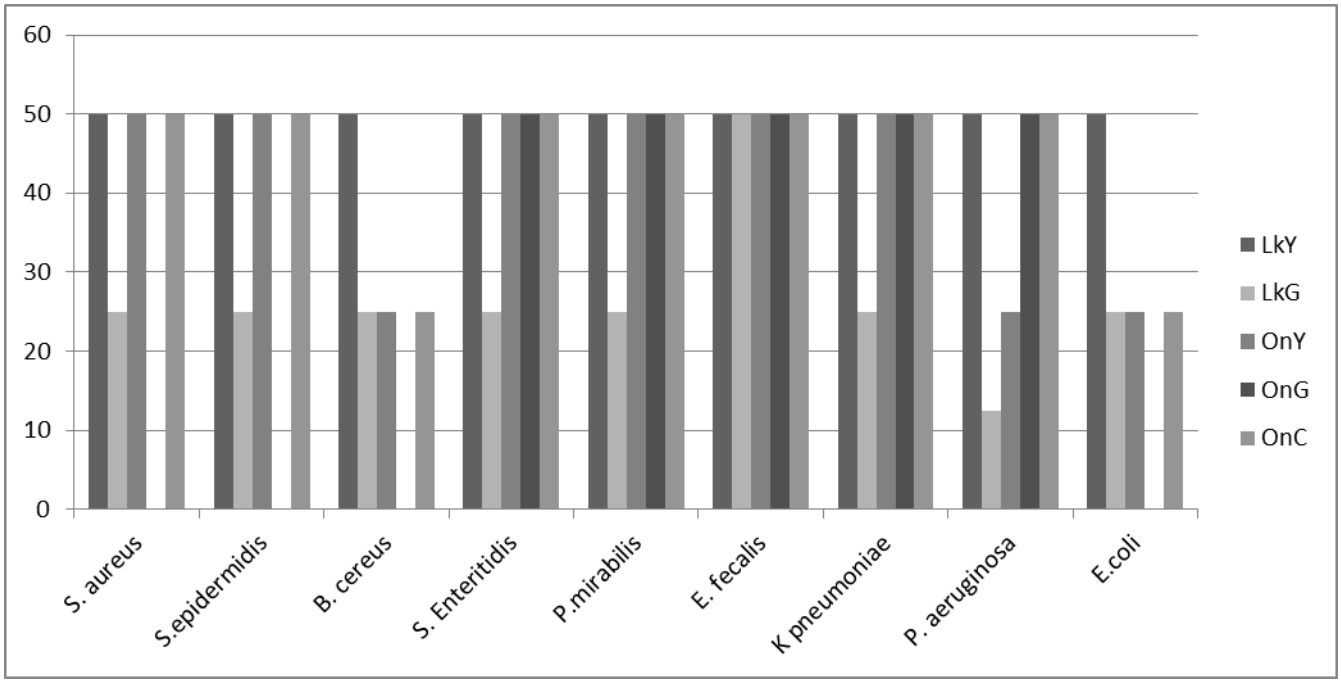


Figure:3. Ethanol extract of MIC values.

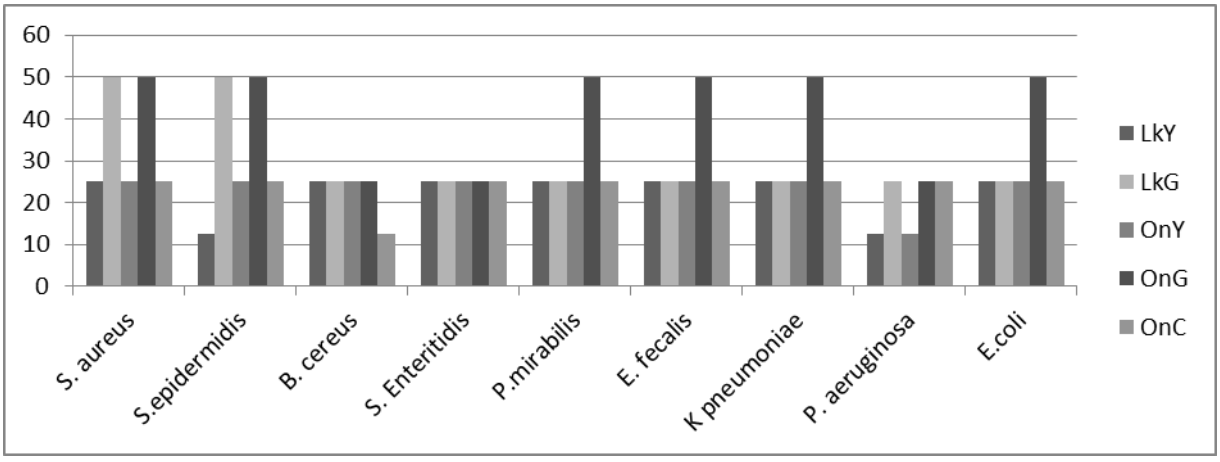
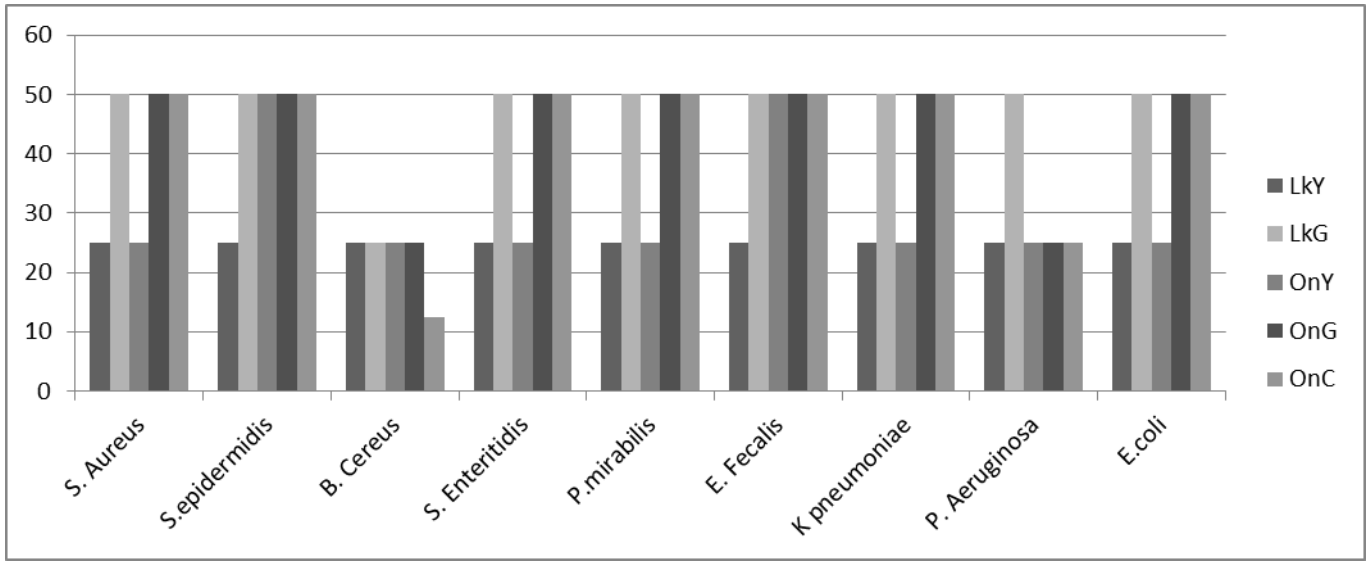


Figure:4. Ethanol extract of MBCvalues.



5. REFERENCES:

1-Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *melia azedarach* l. International Journal of Current Pharmaceutical Research 2012; 4(2): 67-73.

2- Prasannabalaji N, Muralitharan G, Sivanandan RN, Kumaran S, Pugazhvendan SR. Antibacterial activities of some Indian traditional plant extracts. Asian Pacific Journal of Tropical Disease 2012; 291-295.

3- Amanda AO, Jorge FOS, Vespasiano YKS, Elida CGM, Magda CAG, Roberto MB, Paulo OMJ, Luís IBK. Antimicrobial activity of amazonian medicinal plants. SpringerPlus 2013; 2(1): 371-376.

4- Zhang L, Ravipati AS, Koyyalamudi SR, Jeong SC, Reddy N, Bartlett J et al. Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. Asian Pacific Journal of Tropical Medicine 2013; 673-681.

5- Malini M, Abirami G, Hemalatha V, Annadurai G. Antimicrobial activity of Ethanolic and Aqueous Extracts of medicinal plants against waste water pathogens. International Journal of Research in Pure and Applied Microbiology 2013; 3(2): 40-42.

6- Ayesha M, Parwez A, Gupta VC, Mushtaq A, Amin U, Arfin S et al. A study of antimicrobial activity of few medicinally important herbal single drugs extracted in ethanol, methanol and aqueous Solvents. Pharmacognosy Journal 2010; 2(10): 351-356.

7- Selvamohan T, Ramadas SSSKV. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research 2012; 3 (5):3374-3381.

8- Wendakoon C, Calderon P, Gagnon D. Evaluation of Selected Medicinal Plants Extracted in Different Ethanol Concentrations for Antibacterial Activity against Human Pathogens. Journal of Medicinally Active Plants 2012; 1(2):60-68.

9- Ozgen U, Miloglu FD, Bulut G. Quantitative determination of shikonin derivatives with UV-Vis spectrophotometric methods in the roots of Onosma nigricaule. Reviews in Analytical Chemistry 2011; 30 (2): 59–63.

10- Akcin OE. An Investigation on the Morphology, Anatomy and Ecology of Endemic *Onosma bornmuelleri* Hausskn. Ekoloji 2004; 13(51): 13-19.

11- Erdağ D. Malathion to the oxidation parameters in mice on *Allium czelghauricum* (Liliaceae), *Lathyrus karsianus* (Fabaceae) and *Onosma nigricaule* (Boraginaceae) obtained the effects of extracts(Thesis). Graduate school of health sciences, Kars, 2012, 4-5.

12-Ozkan O, Aydin H, Bagcigil AF. In vitro evaluation of antimicrobial activities *of Salvia verticillata* and *Phlomis pungens*. Kafkas Univ Vet Fak Derg 2009; 15 (4): 587-590.

13- Igbinosa OO, Igbinosa EO, & Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatropha curcas (Linn). African Journal of Pharmacy and Pharmacology 2009; 3 (2):58-62.

14- NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Fourth Edition; Approved Standart. NCCLS Document M7-A4 (İSBN 1-56238-309-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087, 1997.
15- Doughari JH. Antimicrobial activity of Tamarindus indica Linn. Tropical Journal of Pharmaceutical Research 2007; *5* (2): 597-603.
16- Ahmad B, Ali N, Bashir S, Choudhary MI, Azam S, Khan I. Parasiticidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. African Journal of Biotechnology 2009; 8 (19):5084-5087.
17- Naz S, Ahmad S, Rasool SA, Sayeed SA, Siddiqi R. Antibacterial activity directed isolation of compounds from *Onosma hispidum*.. Microbiological Research 2006; 161,43-48.
18- Özgen U, Houghton PJ, Ogundipe Y, Coşkun M. Antioxidant and antimicrobial activities of *Onosma argentatum* and *Rubia peregrina*. Fitoterapia 2003; 74, 682–685.