The Antimicrobial Effect of The Artimisa Herba and Eordium Glycobhyllum Against A Few of Pathogenic Bacteria

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Abstract

Artimisa herba and Erodium antimicrobial activity were used against *S*erratia spp., *Bacillus cereus, E.coli,* Micrococcus *roseus, Staphylococcus aureus, Streptomyces spp.*and *Micrococcus lutaus.* The method of extraction applied in this paper was the Soxhlet- hot extraction using petroleum ether and alcoholic solvents. The bacterial inhibition by the extract was as follows:

Serratia spp was found moderately sensitive to *Artimisa herba* with failure to produce pigment. *B. cereus* was found moderately so to *Artimisa herba lal,* while it showed low response to *Artimisa herba. Escherchia coli* showed moderate inhibition to *Artimisa herba, Micrococcus roseus* responded well to and grodium moderately to grodium. *Staohylococcus aureus* well to moderately to *Artimisa herba. Streptomyces spp.* responded well only to be based on the results obtained in this study, it can be concluded that using 50% acetone extract of, *erodium glycophyllum* has high antibacterial activity against pathogenic *Staphylococcus aureus.* Hot Soxhlet extraction method is recommended as well as 96 well treys microtitre plate method. The MIC of tested *Erodium glycobhyllum* against Ttaphyllococcus *aureus* was 25 and 50 mg/ml, respectively. *Peganum* showed moderate response to Eoridium *Micrococcus luteaus* showed height moderate response to Erodium, However the exception of Erodium in high concentration, none of the tested plants showed any signs of toxicity in rats tested. The former produced liver fatty changes and haemorrhages in the kidneys. Toxicity experiments of *Erodium glycophyllum,* and *Artimisa herba* using experimental animals (rats) confirm the safety of such plants for therapy of various diseases. An antibacterial evolution for the total extract of tested medicinal plants could also reveal a more potent activity toward tested pathogenic Gram positive and Gram negative bacteria. However, the plants differed significantly in the activity towards tested pathogenic bacteria. The differences could be attributed to their structural nature.

Keywords: *Artimisa herba, Eoridum,* Pathgenic bacteria.

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1. Introduction

Medical plants are used by 80% of the world population as the only available plants medicines especially in developing countries. In most countries, the percentage of people dependent on medicinal plants for health care is estimated at over 90%. These plants and derived products an important role in the primary health care of Sudan.

in Libya, there are about 1, 825 vascular plant species, of which 134 are endemic. About 450 species are reported to be of medicinal value in the country[1]. Some important families are *Apiaceae, Asteraceae, Laminaceae, Poaceae, Fabaceae, Brasicaceae and Abiaceae*. Medicinal plants are distributed throughout the country, especially in Jabal Al-garbe, Ghedames, Gharian, Awbari and Tarhona district. Many of these plants are associated with a long history of traditional use. More than a hundred species are massively used by Bedouins or local people in folk medicine as hot or cold drinks or chewed fresh or dry. These are also used externally to cure dermal diseases, viral diseases and bacterial infections, insect or animal bites or burns and sometimes for the treatment of hair problems. These medicinal plants are very well documented in different flora. Many species of medicinal plants, such as *Asperula arvensis L., Cupressus sempervirens L., Juniperus phoenicea L., Pinus halepensis Mill., Quercus coccifera L., Tribulus longipetalus Viv., Veronica cymbalaria Bodarod* and *Vahlia dichotoma (Murray*) Kuntze have become threatened by extinction [2,3]. Owing to over-harvesting and diversion of forest land containing medicinal plants to agriculture .

The best known medicinal plant of Libya is *Silphium cyrenacicum* It is existed during Greek and Roman times. The plant was used for the treatment of many illnesses and was so important to the economy of the country that it used to be sold for its weight with silver or gold and its picture was depicted on currency coins.

There are many kinds of flower vegetables that have medicinal properties. These are flowers of traditional vegetable plants that have flowers and have also been used for cooking since ancient time. It was believed that consumption of these flowers vegetable can cure illness and infectious diseases. They also help people who suffer from diarrhea, which indicates the antimicrobial activity of these vegetables [4,5,6]. In addition, the

countries in central Africa, West Africa and Indonesia used "*khilck*" flower (*Senna siamea*) to treat patients who had stomachache [7].

The flower vegetables are composed of various chemical compounds that can be grouped as flavonoid, anthraquinone and glucoside-flavonoid is expected to be the main component that plays a major role in microbial inhibition [8]. Even essential oils were found to have inhibitory effect on pathogenic bacteria and some were found to have antimalarial and antimicrobial property [9,10].

Herbs and spices along the Mediterranean sea have been shown to possess antimicrobial actions and could serve as a source for antimicrobial agents against food spoilage and microbial pathogens [11,12,13,14,15,16].

(1) Artimisa herba

(2) Eroduim glauchophllum

1- Artimisa herba:

Artemisia genus asso (compositae) include 15 perennial aromatic herbs and shrubs that grow wild in dry or semi-dry habitats in the provine of Alberta , Canada and in Libya [17]. Asso. (syn : A. incutta Del., commonly known as the desert worm wood *(Arabic name : Sheeh) , is a dwarf shrub or semishrub)* growing widely in Jordan and in the Middle East . The plant is a perennial, strongly aromatic herb, with many basal , erect and leafy stems covered by woolly hairs . The flower heads are about 0.5 cm in diameter, not showy, and made only of tubular florets. The plant is mainly used as anthelimintic and antispasmodic and for other common uses in folk medicine such as relief of coughing , intestinal disturbances , colds and muscle relaxant by the local population [18,19].

Previous works have suggested that several suggested oils showed important antimicrobial activity against bacteria, yeas, dermatophyte and *Aspergillus strain*s [20,21,22] and have therapeutic potential , mainly in diseases involving mucosal, cutaneous and respiratory tract infections. The major constituents of many of these oils are phenolic compounds (terpenoids and phenyl propanoids) like thymol, carvacrol or eugenol, of which antimicrobial and antioxidant activities are well documented [20, 22].

Nevertheless, aromatic plants producing non – phenolic essential oils, like some *Artemisia*

species, are also used as spices and in folk remedies as antiseptics. Powdered leaves of *A. absinthium , A . biennis , A . frigida and A. ludoviciana* have been applied externally in salves and washes by North American native people for treating sores and wounds and , internally to treat chest infections [23]. In addition , *A . herba – alba* possessed antimicrobial activity against some pathogenic bacteria and yeasts using the broth micro-dilution method [17].

Recently, several species of Artemisia essential oils had inhibitory effect on the growth of bacteria *(Escherichia col , Staphylococcus aureus* and *Staphylococcus epidermidis),* Yeasts *(Candida albicans, Cryptococcus neoformans),* dermatophytes *(Trichophyton rubrum, Microsporum canis, and microsporum gypseum) Fonsecaea pedrosoi* and *Aspergillus niger* [24].

Additionally to the antimicrobial activity of *Artemisia herba-alba Asso,* [25]. In Jordan reported that the essential oil of the plant promising candidates as natural herbal constituents of antimicrobial drug combinations. In Morocco, [26]. Concluded that *Artemisia herba – alba Asso*. aqueous extract possesses antihypertensive activity in spontaneously hypertensive rats and that the underlying mechanism appears to involve, at least in part, an increase in urine and electrolyte output . However , in Tunisia, it was also reprted[27].

Artemisia oils of some species had inhibitory effects on the growth of bacteria (*Escherichia coli*, *Staphylococcus aureus* , and *Staphylococcus epidermidis*) , Yeasts (*Candida albicans* , *Cryptococcus neoformans*) , dermatophytes (*Trichophyton rubrum* , *Microsporum canis* and *microsporum gypseum*) , *Fonsecaea pedrosoi* and *Aspergillus niger*. *Artemisia biennis* oil was the most active against dermatophytes, *Cryptococcus neoformans*, *Fosecaea pedrosoi* and *Aspergillus niger* , and *Artemisia absinthium* oil the most active against *Staphylococcus strains* [24].

2- Erodium glaucophyllum (Ebrat Elrahib) :

The family Geraniaceae encompasses many promising plants from the medicinal plant of view .Some of the family members are specified in the Chinese pharmacopoeia and formulated in the Chinese herbal medicine such as Lao-guan-cao formula . The drug consists of *Erodium stephanianum , Geranium nepolense and Geranum sibiricum* and is

used to promote circulation in acute and chronic rheumatologic disorders and as detoxicant for enteritis and basillary dysentery [28]. From the chemical point of view , caffeine , tyramine , glutamic acid , choline , gallic acid , saponins , flavenoids and sugar were reported as common active constituents of *Erodium cicutarium* as well as of the family Geraniaceae [29]. Results of the antimicrobial activity against *Escherichia cili, Staphylococcus aureus* and *Candida albicans* proved comparable activity in comparison to those of ampicillin, gentamycin and mycostatin . The results of the MIC values after 48h incubation at 37Cº were determined by two-fold serial dilution assay [30]. The natural susceptibility to 71 antibiotics of 104 *S. Serratria ficaria* , *Serratia rubidaea* , *Serratia fonticola* , *Serratia odorifera* , and *Serratia plymuthica* was examined by Stock and his colleagues[31].

In this study it was found valuable to screen some of the wild medicinal plants such as *Artimisa Helichrysum stoechas* and *Erodium glycophyllum* from Libya against some Gram-positive and Gram-negative pathogenic bacteria such as: *Staphyllococcus aureus* ,Micrococcus species, *Bacillus cereus* , *Serratia species* , *Escherichia coli* , *Salmonella species* and *Pseudomonas species*.

2. Materials and Methods

1- Plant Materials:

a. *Artimisa herba*.

Artmisia is a large, diverse genus of plants with between 200 to 400 species belonging to the daisy family Asteraceae. It comprises hardy herbs and shrubs known for their volatile oils. They grow in temperate climates of the Northern Hemisphere, usually in dry or semi-dry habitats. The fern-like leaves of many species are covered with white hairs. Common names used for several species include wormwood, mugwort, sagebruch and segewort, while a few species have unique names, notably Tarrago (Artemisia dracunculus) and Southernwood (A. abortanum). Occasionally some of the species are called sages, causing confusion with the saliva sages in the family Lamiaceae. Artemisia species are used as food plants by the larvae of a number of Lepidoptera species.

Artemisia genus as so (composite) include 15 perennial aromatic herbs and shrubs that

grow wild in dry or semi-dry habitats in the provine of Alberta , Canada and in Libya[17]. About 400 species, mostly distributed in North temperate parts of the world (common on arid soils and halophytic habitats), South America and South Africa; 5 species are reported from Libya.

Artemisia herba-alba is one growing species in Libya. It is aromatic low growing , much branched from the base , up to 30 cm high shrubby perennial ; stems usually patent, at first densely cane scent later becoming glabrescent below.

The plant distributed in Wadi louti, Gharian, mountains , sand stone and limestone,common in Yefren, Wadi Ain Romia, Alghazaya towards Wazzin . Additionally to Libya , Artemisia herba-alba distributed in West Southern France , Spain, North Africa, Syria extending into West and Central Iran.

2- Preparation plant extracts:

a. Collection of plants.

a-1. Collection of plants :

The plants were collected during the spring season of 2012 from AL- Gabal AL- Gharbie mountain Libya. The plants were classified and authenticated according to Jafri and EL-ghadi (1978). In Department of pharmacognosy, Faculty of pharmacy, Tripoli University, Tripoli, Libya[32].

a-2. Extraction of tested plants

The methods for preparation extracts mainly organics solvents according to (Wangner and Bladt, 1995).

a-3 Activity of tested plant extracts :

The tests were performed by the Muller Hinton agar well diffusion method[33]. Streptococcus species, Serratia species and Gram-positive strains; Staphylococcus aureus, Micrococcus species, Bacillus cereus. The tested strains of pathogenic bacteria used in this investigation are human isolates obtained from Research Center of Biotechnology, Tripoli, Libya[34]. To dissolve and dilute the extract, 50% acetone at proper concentration was used. Control plates containing only 50% acetone. Tests were performed in duplicate.

a-4 Agar diffusion cup cut method ; Eight serial dilutions generating decreasing concentrations of 100,50,25,12.5,6.25,3.125,1.56 and 0.78 mg/ml for the acetone extract

of Artemisia herba alba and Erodium glaucophyllum were first performed..

3. Results

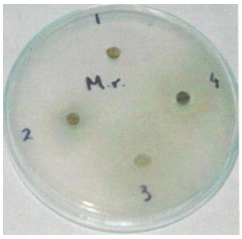
I- Screening Antibacterial Activity of some Medicinal Plants against certain Pathogenic Bacteria :

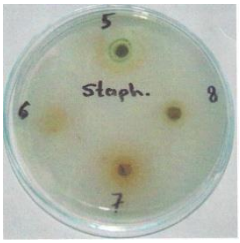
In order to test optimum condition of tested plant extraction for antibacterial activity, different solvents with increasing polarity such as water and ethanol were used. Water was used in aqueous extraction as well as ethanol. Crude extracts yield of shoot system are shown in Table (1). As shown in the same table the crude ethanolic extraction of Artimisa herba wase higher than crude *Erodium* water extracts. However, water extract appear higher solvent for Artimisa herba . This were primarly studies against certain pathogenic bacteria such as *Serratia sp., B. cereus, E. coli, Micrococcus aureus, Streptomyces sp.* And *Micrococcus loteus.* The data in table shows that failure of pigment formation of red colour in growth surrounding the clear zone of *Serratia sp*. under the effect of water extract. The preliminary results of antimicrobial activity test varied from low to high inhibition of growth against tested pathogenic bacteria by two solvents used (water and ethanol) as shown in Table (1). The aqueous extraction generally gave less antibacterial activities than ethanolic extraction. The ethanolic extraction of the shoot systems from tested medicinal plants produced high inhibition zone against certain pathogenic bacteria. Aqueous extract of *Artimisa herba* gave moderate inhibition against tested bacterial species. So it is considered that the aqueous extract of tested plants displayed no antibacterial activitity against tested pathogenic bacteria.

Table 1. Growth of different bacterial spp. In the presence of alcoholic and water extracts of some medicinal plants

Tested plants	***Erodium Gluycophyllum***		**Artimisa herba**	
Bacterial isolates	**Alcohol**	**Water**	**Alcohol**	**Water**
Serratia sp.	-	-	+*	++*
B. cereus	++	-	+	++
E. coli	++	-	++	++
Micrococcus raseus	+	-	++	+
Staphylococcus aureus	-	-	-	++
Streptomyces sp.	+++	-	-	++
Micrococcus Luteus	++	-	+++	++

: Failure in pigment formation in the growth surrounding the clear zone. : Negative inhibition, + : Low inhibition, ++ : Moderate inhibition, ++ : Moderate inhibition, +++ : High inhibition





1- Ethanolic extract of Erodium 2- Aqueous extract of Erodium

3- Ethanolic extract of *Artimisa herba herba* 4- Aqueous extract of *Artimisa herba*

M.r. : *Micrococcus roseus* E.C. : *Esherichia coli*
Staph. : *Staphylococcus aureus* B.C. : *Bacillus cereus*
M.L. : *Micrococcus loteus* Strepto. *Streptomyces sp.*

Antibacterial activity of some selected medicinal plants :

In this experiment we used two plants namely; *Artimisa herba*, and, *Erodium gluycophyllum* Using 50% acetone as solvent system, according to **Khond et al. (2009).**The highly pathogenic bacterial strains tested were *Escherichia coli; Salmonella sp.*, *Pseudomonas sp.* and *Staphylococcus aureus*.The obtained results revealed that 50% acetone was suitable solvent which has an antibacterial activity towards Gram +ve *Staphylococcus aureus* with a wide zone of inhibition at a concentration of 100 mg *Erodium glycophyllum*.Table(2).Our results showed that the 50% acetone was the only effective solvent, therefore further investigations were carried out. Other tested strains was tried which had narrow zone of inhibition at concentration 100 mg of either *Erodium glycophyllum*.

Table 2. Antibacterial activity of *Erodium glycophyllum* extracts using 50% acetone.

Bacte *Bacterialstrains*	Solvent systems			
	Con	Contralrol	55	55% Acetone
Esch erichia coli	-ve-	-ve	-ve	-ve
Salmo *Salmonella sp.*	-ve	-ve	-ve	-ve
Sta *Stahylococcus aureus*	-ve	-ve	+ve	+ve
Pseu *Pseudomonas*	-ve	-ve	-ve	-ve

-ve = no effect +ve = effect

III- Determination of minimum inhibition concentration (MIC) of 50% acetone extract:

1- Broth microdilution method :

Using the 96 well trys (Fig. I), 100 ul of 50% acetone extract of *Erodium glycophyllum* diluted two fold in decreasing concentration and 5 ul of bacterial inoculums of *Staphylococcus aureus* was distributed in the wells.

Table (1) illustrate that 25 mg/ml of acetone extract is the MIC for *Staphylococcus aureus*. However, Table (2) shows that 50 mg/ml of acetone extract is the MIC for *Staphylococcus aureus* while 100 mg/ml of *Erodium glycophyllum* extract was bactericidal for the same species.

Table 3. Determination of MIC of acetone extract of *Erodium glycophyllum* by Broth micro dilution method.

Bacterial strains	Visible turbidity				MIC
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
Staphylococcus aureus	clear	clear	turbid	turbid	50 mg/ml

Effect of Tested Plant extract on Experimental Animals:

Artimisa herba

The treated rats were closely observed for 24 hours for any behavioral changes or abnormal clinical signs. Visual observation revealed that no any abnormal behavior seen, no mortality on all doses used. Postmortem was done on the second day of the experiment, in all groups no pathological changes seen in any of the vital organs(liver and kidney).

Table 4. Mortalities and Post-mortem findings in rats treated with *Erodium gluycophyllum*

	Gr OUP / Concentration mg/kg	No. of Rats	% M Ortality After 24 hrs	Survival Rate	Mortality (%)	Changes in Tissue	
						Liver	Kidney
1	250 (mg/kg)	6	0	6	0	-ve	-ve
2	500 (mg/kg)	6	0	6	0	-ve	-ve
3	1000 (mg/kg)	6	0	6	0	++	-ve
4	2000 (mg/kg)	6	4	2	66.66	+++	++
5	Control	6	0	0	0	-ve	-ve

(-ve): No changes, (++): Fatty changes, (+++): Extensive Fatty changes.

4. Discussion

Medicinal plants, since immemorial times, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations obtained from commonly used traditional herbs and medicinal plants have been traced to the occurrence of natural products with medicinal properties.

The discovery of new drugs from natural compounds has been of significant importance in the past and obviously will continue to be just as important if not more in the future.

Plants are the oldest source of pharmacologically active compounds, and have provided human kind with many medically useful compounds for centuries. Some 7000 medicinal compounds used in the Western pharmacopoeia are derived from plants. Hence, screenings of antimicrobial plants for new agents poses an enormous challenge and are important especially with the emergence of drug resistant diseases.

Additionally to the *Artemisia* ethanolic extract showed antibacterial activity

against *B. cerius, Micrococcus roseus, E. coli, Streptomyces sp.* and *Micrococcus loteus*. This results supported the findings of Dababneh [36]. He reported that *Artemisia-herba-alba* had marked significant sensitivity towards pathogenic *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Candida albicans* using well-plate diffusion method. Recently, Hudaib and Aburjai [25] reported that the oil of Jordanian species of *Artemisia* promise candidates as natural herbal constituents of antimicrobial drug combinations. In addition, others stated that *Artemisia sp.* extract is anti-diabetic, antitumor, antifungal against *Candida albicans* and bactericidal[36].

Results of antibacterial activity of *Erodium glycophyllum* exhibit positive effect against *Staphylococcus aureus* at 100 mg. This obtained results has confirmed earlier by Gohar et al. [30]. They reported that extract of *Erodium* has antibacterial activity against *Staphylococcus aureus* , *Escherichia coli* and *Candida albicans*. Despite of the the absence of tannins in *Erodium* reported by others [30,37] proved that *Erodium glycophyllum* contains such constituent. Moreover, the use of some plants of the family *Geraniaceae* for treatment of enteritis and basillary dysentery [28] suggested the presence of antibacterial principles. Fecka et al. [38] reported that some species of the *Erodium genera* contains six natural polyphenolic compounds, brevifolin carboxylic acid, brevifolin , ellagic acid , methyl gallate, gallic acid and protocatechuic acid in methanol extract of the whole plant of *Erodium cicutarium*. Accordingly, they concluded that *Erodium* has an active component called geraniin. From the biological point of view, geraniin was reported to exhibit inhibitory activity for chitin synthase II, inhibit the level of serum cholesterol, glutamate pyruvate transaminase and inhibits the formation of 5-lipoxygenase which should be a useful principal to find a natural drug for asthma and inflammation[39].

Results from this study indicated that tested extracts possessed variable antimicrobial effects against Gram-positive and Gram-negative bacteria. However, the plants differed significantly in the activity against tested microorganisms. These differences could be attributed to the structural nature of plant constituents and microorganisms as well as concentration used. The optimal effectiveness of medicinal plants may not be due to one active constituent, but to the combined actions of different plant constituents. Moreover, the difference observed in antimicrobial activities of the investigated plant extracts suggest the

susceptibility variations of microorganisms to various chemical components. Earlier results support our findings that the compositions of essential oils depend on the plant species, the chemotypes and the climatic conditions, which lead to variation of their antimicrobial activities.

It could be concluded that the tested plant extracts exhibits a broad spectrum of activity against various microorganisms. Results of the present study should be considered for the possible application of plant extracts of natural bacteriostatic and bactericidal component in various products and as natural preservatives extending the pharmaceutical and deity products shelf life, as we believe is of great importance.

In the case of Erodium and its effect on rats, it's clear that lower concentrations did not have any deleterious effects on the rats, a part from slight fatty changes and haemorrhage in both kidneys and levers. However, when higher concentrations were used *viz*: 2000 mg/kg 66.67 % of rats died. Dead animals showed liver fatty changes and haemorrhage. Similar lesions were observed in the kidneys.

As far as *Artemisia herba,* all concentrations didn't show any visible lesions which ensure the safety of using such medicinal plants for therapy of various diseases.

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