Research Article

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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *GENISTA MICROCEPHALA* AND *ROSMARINUS OFFICINALIS* EXTRACTS FROM LIBYAN'S REGIONS

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ABSTRACT

Extracts of *Rosmarinus officinalis* and *Genista microcephala* were obtained by Soxhlet extraction. Qualitative and quantitative phytochemical evaluation of their chemical constituents were considered and showed in comprehensive encouraging results. *Rosmarinus officinalis* is a spice and medicinal herb widely used around the world. Accordingly, *Genista microcephala* phytochemicals activity of plant extracts and antimicrobial also was assessed with antibiotic susceptible and resistant microorganisms. In addition, the potential synergistic effects when associated with antibiotics were studied. Furthermore, the crude extract of plants evaluated antimicrobial activities were related with antibiotic insecure and resistant microorganisms. Whatever are more the potential synergistic effects when related with antibiotics were considered, and the crude extracts of both plants exhibited abundant antimicrobial potentials that are the aim of this study.

Keywords: *Genista microcephala*, *Rosmarinus officinalis*, phytochemical screening, antimicrobial activity, antibiotic.

INTRODUCTION

Considered medicinal and aromatic plants of the oldest plants known for human for the purpose of food and medicine through the ages, and even the present era which revealed itself in the importance of these plants and augmented their use began to appropriate in some of the food industry as preservatives and flavourings to taste and openers of appetite and other uses with high economic importance[1]. In recent times expanded applications were directly used or after extraction of effective foods industries, pharmaceuticals, perfumes, soaps and cosmetics materials manner, and that these plants received special care in many of the producing countries and that as a major source for pharmaceuticals medicinal plants and used as a raw material for the production of some chemical compounds[2, 3]. In a report of the World Health Organization has reported that about 80% of the world's population is mainly dependent on traditional medicine, though the bulk of this medicine is used crude plant extracts or active components[4]. Where medicinal plants differ from other plants to contain material that is attributable to the medicinal or physiological effect that his presence is considered medically plant contains and these active substances on the basis of chemical or natural qualities groups such as alkaloids, glycosides, tannins, volatile oils, resins and other active substances.

Genista microcephala is a small shrub with yellow flowers, fluff diffuse leaves, and mutual up to 10 × 3 mm, by bristles, isles spread. The antibiological activities of *Genista microcephala* extraction against the types of bacteria found that have the high sensitivity of these organisms. In another study found that flavonoids great importance as a result of medical properties and features instance have antioxidant activity and also of tumours and antibacterial and antiviral. Whereas *Rosmarinus officinalis* is a small evergreen tree, with market wooden cylindrical thin diameter, leaves small-scale non-pedunculated and colour green glossy. The presence of the Rosmarinic acid helps in cases of poisoning, and material Rosmarol as an antioxidant. Plant and insect repellent, where it was noted repel insects from neighbouring plants to plant *Rosmarinus officinalis* property, many compounds have been isolated from *Genista microcephala* and *Rosmarinus officinalis*, including glycoside, flavones, alkaloids, turbines, saponins. The glycosides are known to lower the blood pressure according to many reports[5]. Therefore, the presence such a variety of phytochemical compounds in the screened medical plants has a wide range of utilisation and could be certainly used for a variety of applications[6]. Flavonoids have anti-allergic, anti-inflammatory, antiviral and antioxidant activities, flavonoids are hydroxylated phenolic constituents well-known to be created by plants in response to bacterial infection and they have been found to be antimicrobial components contrary to a varied assortment of microbes in vitro. Their activity is perhaps owing to their capability to complex with extracellular and soluble proteins and to complex with bacterial cell wall[7, 8]. Alkaloids where they are an organic compound containing a base in chemical composition to an atom or more of nitrogen. And characterised as toxic alkaloids, therefore, protected by the presence of insects, animals and some alkaloids compounds act as the organisation for growth and influencing the physiological processes and organisms in plant tissue. Turbines are the basic components of the resin and Alter in an output of resin and turbines constitute the basic components of the essential oils of many plants and flowers, and these oils are considered normal fresheners are added to foods, perfumes. Saponins are chemical group containing a large number of compounds and perhaps this group

is the most famous elements nutrients and plant found in many types of vegetables, fruits and herbs, studies have shown that people who eat foods rich in saponins have less breast and prostate cancer rates, where saponins stimulate the immune system to enhance its ability to investigate and eliminate cells that may become cancerous before they grow and become cancer. Cardiac glycosides a medication used in the treatment of heart failure and some cases of arrhythmia numerous sources glycoside which is obtained from plant sources of secondary metabolites. The phytochemical screening of saponins in the ethanolic extracts of both plants not present and presents in aqueous extracts were saponins act as an antioxidant, anti-inflammatory, anti-apoptosis and immunostimulant properties, which raised estimation that these compounds could positively affect neurodegenerative disorders and delay neural ageing[9]. Phenolic compounds are found in the extracts of both plants leaves and tend to have laxative effects. As well as for terpenoids present too, which capable of preventing cancer and has anti-carcinogenic effects[10, 11]. Correspondingly the phenols possess redox properties and thus lead to antioxidant properties to the plants in which they are present. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators[12].

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh leaves of *Genista microcephala* growing wild in Alkhums Mountain located at Alkhums region Libya were collected during spring 2014. And the sample of fresh leaves of *Rosmarinus officinalis* growing wild in Tarhona region, Libya correspondingly was collected during spring 2014. Plant samples were identified by Botany Department, Science College Alkhums El-Mergeb University Al-khums, Libya. The fresh leaves were washed by tap water, shadow dried, then grinded and packed in dark plastic bags with labels of the weight and number until further use.

Preparation of extract

The crude powdered materials were extracted in appropriate solvents at a concentration (20 g leaves powder in 400 ml water and ethanol separately) proceeding Soxhlet for 4-6 hours. The extract was filtered off using a Buchner funnel and Whatman No.1 filter paper. The obtained filtrate of aqueous extract and ethanolic extracts obtained were stored in a refrigerator at 4°C until further use[13, 14].

Qualitative Analysis

Phytochemical screening was performed for the aqueous and ethanolic extracts by standard procedures to identify the presence of various phytochemicals constituents [13-15]:

Tannins test

About 10 ml of crude extracts were heated on the water bath and filtered. A few drops of 1% ferric chloride $FeCl_3$ solution were added to the filtrate. Dark green solution indicated the presence of tannins.

Saponins test

About 10 ml of crude extracts were shaken and heated to boil. Formation of a stable persistent froth and creamy mass of small bubbles indicated the presence of saponins.

Phlobatanins

About 10 ml of crude extracts were boiled with 2% HCl solution. Deposition of a red precipitate was an evidence for the presence of phlorotannins.

Flavonoids

Diluted NaOH and HCl were added to 10 ml of crude extract. A yellow solution that turns colourless indicated the presence of flavonoids.

Terpenoids

About 10 ml of crude extracts were filtered and mixed with 2 ml of chloroform $CHCl_3$. Then 3 ml concentrated sulfuric acid H_2SO_4 were carefully added to form a layer. Formation of reddish brown coloration of the interface was an evidence for the presence of terpenoids.

Cardiac glycosides

About 10 ml of crude extracts were shaken with 1ml of glacial acetic acid. A drop of ferric chloride and a drop of concentrated sulfuric acid were added. Green blue colour to the upper layer and reddish brown colour at the junction of two layers was an evidence for the presence of cardiac glycosides.

Alkaloids

About 10 ml crude extracts were heated with 2% H_2SO_4 for two minutes. The mixture was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate showed the presence of alkaloids.

Ferric chloride test for phenols

Distilled water 15 ml was added to 5 ml of crude extracts. Then, a few drops of neutral 5% $FeCl_3$ solution were added. Formation of a dark green colour indicated the presence of phenolic compounds[14, 15].

Quantitative Analysis

The quantities of the phytochemicals present were determined using the standard methods[16-19]. As shown below:
The extracts were weighed after separating the solvents by evaporated under reduced pressure and dried using a rotary evaporator at 55 °C then a percentage yield for each extract was calculated as:

$$Yield\ \% = \frac{Final\ weight\ of\ extract}{Total\ weight\ of\ ground\ plant} X\ 100$$

Alkaloid yield: 5 g of the sample was weighed into a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was added and covered, allowed to stand for 4 h. The mixture was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added by dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed[16].

Saponin yield: 20 g of the sample were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples

were heated for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 ml 20% ethanol. The combined extracts were reduced to 40 ml at 90°C. The concentrate was transferred into a 250 ml reparatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the Saponin content was calculated as a percentage[17].

Flavonoids yield: 10 g of the plant of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water-bath and weighed to a constant weight[20].

Statistical analysis

All values have been expressed as the mean ± standard deviation and the comparison of the antibacterial activity of the samples with standard antibiotics was evaluated by applying t-test. $P \leq 0.05$ values were considered to indicate statistically significant difference.

Antibacterial screening

The antibacterial activities of aqueous and ethanolic extracts were investigated against the bacterial strains by the Paper Disc Diffusion Method[21]. Three Gram-negative bacterial strains (*Escherichia coli, Salmonella* and *Pseudomonas aeruginosa*) and two Gram-positive strains (*Staphylococcus saprophyticus* and *Staphylococcus epidermis*) from standard cultures were used as test strains. Standard bacterial strains were obtained from Microbiology Medical Laboratory, Al-Khums Teaching Central Hospital Alkhums Libya. From the 50 mg/ml stock solution of aqueous and ethanolic extracts of both plants, 40 µl a divisor was transferred onto blank sterile paper discs (6 mm diameter) [22]. Dried discs were placed onto nutrient agar medium (UK) previously inoculated with a bacterial suspension and incubated at 37ºC for 24 h. Antibiotic and appropriate solvent were used as a control to determine the sensitivity of the tested strains. After incubation, plates were then examined for the presence of zones of growth inhibition, and the diameters of these zones were measured in Millimeter (mm) if any. All tests were performed under sterile conditions in duplicate and repeated two times.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analyses, it means the detection of important active compounds, were carried out in the aqueous and ethanolic extracts of *Genista microcephala* and *Rosmarinus officinalis*. The presented phytochemical compounds in the plant's extracts are shown in Table 1.

Table 1: Phytochemical analysis results

Name of plant	***Genista microcephala***		***Rosmarinus officinalis***	
Chemical Component	**Aqueous extract**	**Ethanolic extract**	**Aqueous extract**	**Ethanolic extract**
Tannins	+	+	+	+
Saponins	+	-	+	-
Phlobatanins	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	+	+	-	+
Phenols	+	+	+	+

Key: + = *present;* - = *absent*

Table 2: Percentage yield and percent composition of *Genista microcephala* and *Rosmarinus officinalis*

Plants name	**Yield (Mass %)**		**Mass percentage composition (%)**		
	Aqueous extract	**Ethanolic extract**	**Flavonoids**	**Saponins**	**Alkaloids**
Genista microcephala	64	73	18.3 ± 2	26 ± 4	22 ± 3
Rosmarinus officinalis	77	83	33.3 ± 2	41 ± 1	37 ± 3

From the obtained results in Table 1, it can see that flavonoids, terpenoids, cardiac glycosides and phenols were presented in both extracts. Only saponins were absent in ethanolic extracts of *Genista microcephala* and *Rosmarinus officinalis.* Alkaloids were absent in aqueous extract of *Rosmarinus officinalis.*

As showed in Table 2 the quantitative analysis revealed the percentage yield 83% for *Rosmarinus officinalis* ethanolic extract and 64% for *Genista microcephala* aqueous extract, where the highest yield of saponins (41/100 g) and lowest yield of flavonoid (18.3/100 g), where alkaloids were 18.3/100 g. The presence of disinfectants ingredients which is an active chemical compounds, such as tannins retains the plant from harmful insect's preserves on the plant during its growth [23]. Moreover, flavonoids known as an organic compound soluble in water, play multiple roles in plants whereupon protecting them from the harmful effects of UV rays and parasites, and used flavonoids strong indicators in taxonomy chemical plant also has many applications some of which are used pigments food as that its effectiveness against microbes. Flavonoids and tannins are major groups of compounds that act as primary antioxidants [24]. The presence of these phytochemicals confirmed the antibacterial activities of an aqueous and ethanolic extract of *Genista microcephala* and *Rosmarinus officinalis*. The preliminary evaluations of both the aqueous and ethanol extracts exhibited appreciable inhibitory activities on the tested pathogenic bacterial isolates at a concentration of 20mg m/l.

Antibacterial activity

Table 3: The antibacterial activity of aqueous and ethanolic extracts (50 mg/ml) of *Genista microcephala* and *Rosmarinus officinalis*

Plants Names / Bacteria Strains	*Genista microcephala*		*Rosmarinus officinalis*		Antibiotic *Gentamicin*
	Aqus. Extr. (mm)	Et.OH Extr. (mm)	Aqus. Extr. (mm)	Et.OH Extr. (mm)	
E. coli	Nil	Nil	Nil	Nil	11
P.aeruginosa	8	9	10	7	15
Salmonella	7	8	9	9	17
S. saprophyticus	9	10	8	7	18
S. epidermidis	7	11	8	10	12

Note: Discs injected with 40µl of ethanol solvent served as controls against each bacterial strain and showed nil zone of inhibition for *E. coli*, and zone of inhibition mellemeter (mm) for *Salmonella*, *S. saprophyticus, S. epidermidis* and *P.aeruginosa.*

Through an assessment of research findings note that the presence of chemical compounds in extracts may have energetic to measure biological activity against five species of harmful bacteria nature of the human being (*E.coli, Salmonella, S. saprophyticus, S. epidermis* and *P.aeruginosa*). The inhibitory attractiveness of the aqueous and alcoholic extract of *Rosmarinus officinalis* for some types of bacteria was more influential on the germs. Somewhat affected by Gram-negative bacteria and Gram-positive bacteria. The effectiveness of the alcoholic extract of *Rosmarinus officinalis* in the inhibition of microbiology it contains tannins and saponins compounds were considered and effective inhibition of these compounds for a wide range of microorganisms including bacteria. Both plants spread widely in the Alkhums region and on the fertile land in Tarhuna and other areas in Libya. Proceeding from this study that the natural herbs as possible to be a source of products able to control the microbial resistance toward antibiotics and are of great importance for the development of effective and safe medicines and reasonable. Respectively they also contained the important activity proportion against antibiotic resistant bacteria, in opposition; both extracts from *Genista microcephala* and *Rosmarinus officinalis* did not show any antimicrobial activity against *E. coli*, which is already known and have multi-resistant to drugs. Among the phytochemicals, the ethanolic extract of *Genista microcephala* which is extracted showed the highest antimicrobial activity. The lowest variation was observed for aqueous extract of *Genista microcephala* against *Salmonella* and *S. epidermidis* and also an ethanolic extract of *Rosmarinus officinalis* against *P.aeruginosa* and *S. saprophyticus* [25]. In concisely the results of agar disc diffusion method are presented as in Table 1 several phytochemicals have been known to possess antibacterial properties. Such as tannins, alkaloids, saponins, flavonoids and sterols have found to own growth inhibitory impact on *Salmonella* [26]. Tannins form irreversible complexes with proline-rich protein resulting in inhibition of cell synthesis of bacteria [27].

CONCLUSION

The Phytochemical screening and antimicrobial activities of the *Genista microcephala* and *Rosmarinus officinalis* extracts were confirmed. With regard to antibacterial activity are active against Gram-positive and Gram-negative of bacterial strains. The obtained extracts were showed effectiveness by their incorporation into various foods, cosmetics and pharmaceutical products for which a natural aroma, colour, and the antioxidant/antimicrobial additive is desired. These properties are also needed by the food industry in order to find possible alternatives to synthetic preservatives. Further studies are necessary to investigate the incorporation of extracts into appropriate food formulations and evaluate flavour, chemical changes and antioxidant and antimicrobial activities in the whole food system [28].

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