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Reviewing Phlomis rigida Labill From Turkey as a Antimicrobial Efficacy

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Abstract

This work researched the inhibitory properties of the extract of *Phlomis rigida* Labill plant including chloroform on gram-negative and positive bacteria, yeasts and dermatophyta; the related plant is used for therapeutic purpose in public. For the disk diffusion method, this extract is effective on *Pseudomonas aeruginosa* that is one of the negative bacterias by 11.33 ± 0.57 mm inhibition areas. Regarding other bacterias, the effect of the related extract is so low in avoiding the improvement of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus megaterium* by 8.33 ± 0.57 mm inhibition area. It has highly effective on *Trichopyton sp.* and *Candida albicans* that are from dermatophytes fungis and yeast by 16.33 ± 0.57 mm inhibition area. About yeast and dermatophytes fungi it has a remarkable impact on *Candida glabrata* (14.33 ± 0.57 mm inhibition area), *Epidermophyton sp.* (12.33 ± 0.57 mm inhibition area). Antimicrobial activity of this natural extract against all the microorganisms that were tested by MIC method by concentrations vary by 100 uL and 6.25 uL. Acording to this; the results showed good inhibitory effect with 6.25 µL for *C. albicans*, *Trichophyton* sp. with 12.25 µL for *C. glabrata*. In the other hand it is effective with 25 µL for *P. aeruginosa*, *Epidermophyton sp*. with 50 µL for *E. coli*, *S. aureus*, *B. megaterium*. Being used plants for medical purposes as from since the early ages show that these plants can be used as alternatives of available antibiotics; including important medical components is the other reason for being an alternative at the same time. Concerning results, *P. rigida* can be utilized as a therapeutic drug for the healing of infections caused by microorganisms. In this study, we believe that the P. rigida is important in the search for new antibiotics and will contribute to the literature and the world of drug as it affects different types of microorganisms at the lowest level for the first time.

Keywords: *Phlomis rigida* Labill, therapeutic medicine, dermatophyta, bacteria, yeast

1. INTRODUCTION

Inspite of developments in the medical world related to people are progressing; Pandemics caused by bacteria, fungi, viruses cause serious health problems and even loss of life in our world [1].

Plants have been a natural source of drug compositions and pure products from past to present [2].

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Every year, two or three antibiotics from microorganisms become ineffective. With the realization that the duration of antibiotics has been limited in the last decade, new sources of antibiotics have been directed especially to herbal antibiotics [3].

The genus Phlomis has an important effect on the emergence of natural therapeutic products [4]. Ulukanlı and Akkaya (2011) informed that Phlomis species in Turkey are recognized with the names specific to that region as ‘‘Ballıkotu, Calba, Çalba or Şalba” [5].

Phlomis genus belonging to Lamiaceae family has more than 100 species in the world. In folk medicine, many Phlomis species are mostly consumed as herbal teas (Dağçayı) in the treatment of many diseases as tonic, carminative, appetizing and stimulating and pain reliever [6].

In addition, it is present several studies showing that this species has various biological and pharmacological activities for some plants such as malaria, antimicrobial, anti-allergic, antifibriel effects, immunosuppressive and free radical scavenging properties. Different secondary medicinal products have been previously identified on the Phlomis genera [7-9].

It is seen that there are studies about antimicrobial and biological characteristics of diverse Phlomis species in the literatüre [10-13].

According to our literature search, this is the first report on the antibacterial, antidermatophyte and antifungal features of *P. rigida*. It may have natural therapeutic drug on diseases caused by microorganisms in humans. This study researched the medical use areas of *P. rigida* plant by reviewing its antimicrobial characteristics by aiming new active ingredients for disease treatments.

2. MATERIAL-METHOD

2.1. Screening of antimicrobial Characteristics

2.1.1.The collection and preparation of the sample

Phlomis rigida Labill’s collection (Lamiaceae) was made during appropriate vegetation (in June 2018) in Bingöl kuruca bight on northern slopes in the Turkey. This plant sample has been maintained in the Herbarium of Munzur University (UC - 145) in Tunceli, Turkey (UC - 145). The Flora of Turkey was utilized for the taxonomic diagnosis [14]. The diagnosed plant was made suitable for grinding. The grinded plant (2 g) was treated in 10 mL chloroform (98.1 %) solvent by keeping on a rotary shaker (100 rpm) for 24 h. The remaining extract was dissolved again in its own solvent. Thus, the plant extract was obtained. These plant materials were filtered under suitable aseptic conditions and left at 4 ° C for further study. Then, 100 µL (20 mg L^{-1}) of plant extracts were injected into 6 mm diameter (Schleicher & Shüll No: 2668, Germany) blank antibiotic paper discs to try the test isolates separately.

2.1.2. Microbial strain

The bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50071, *Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32), yeasts (*Candida albicans* FMC 17, *Candida glabrata* ATCC 66032) and dermatophyte (*Trichophyton sp.*, *Epidermophyton sp.*) were tested as species for the current study. The tested pathogens were taken by the Department of Biology, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.1.3. Inhibitory characteristic test

The agar disc diffusion method was performed in order to detect inhibitory effect. Mueller Hinton Agar, Yeast Malt Extract Agar and Sabouraud Dextrose Agar were prepared separately in erlen-meyer bottles under laboratory conditions and brought to 45-50 ° C pouring temperature, with the culture of microorganisms to be prepared as explained, will be added at the incidence of % 1 (10^6 cells mL^{-1} of bacteria, 10^4 cells mL-1 yeast and cells mL^{-1} dermatophyta fungi as per Mc Farland standard). 15 ml medium by shaking well is poured in to sterile petri plates and homogenously distributed. The discs (6 mm

diameter) with treated 100 microliters of plant extract were added to the appropriate agar media inoculated with microorganism. Then, petri dishes was stored at 4 ^{0} C for 2 h. The cultivated petri dishes were left in the incubator at 37 ± 0.1 ^{0} C at 24 h for bacterial isolates and also at 25 ± 0.1 ^{0} C at 72 h for yeasts and dermatophyte. The antibacterial, antifungal, antidermatophyta characteristic of plant extract was evaluated by observing the inhibition area on the disks [15]. Micostatin and ampicillin sulbactam were used as positive control. Chloroform injected discs were tested as negative control.

2.1.4. Minimal inhibition concentration

Minimal inhibitory concentrations (MIC) were detected using the Broth dilution assay. The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA). The passages of microorganisms were prepared with 12- hour broth cultures and the passages were set at a blur of 0.5 Mc Farland Standard. The plant sample was first rarefied to the maximum value 100 µL to be evaluated, and then serial 2-fold subtilizations were acquired in a value serial from 6.25 to 100 µL (1562 - 25000 µg) in 10 mL aseptic test tubes including nutrient broth for bacteria and sabouraud dextrose broth for yeast and dermatophyta fungi. MIC values of this plant against analyzed microorganisms were revealed with a micro-well dilution method [16]. The propagation of microorganisms was determined by an EL x 800 universal microtiter plate reader at 600 nm with optical density quantity. After incubation for 18 - 24 h at 37±1^0 C for bacteria, 25 ± 0.10 C at 72 h for yeast and dermatophyte pathogens. It was defined as the smallest value of that sample for the nominal value of the plant material used to prevent proliferation of microorganisms. This is the last tube symbolization (mg mL^{-1}) whose demectric is not microbial growth.

2.1.5. Statistical analysis

Statistical comparisons were made between the extract and control groups (chloroform, ampicillin sulbactam, micostatin) in relation to measurable preventive activity against bacteria, yeast and dermatophytes. SPSS 15.0 soft ware was used for statistical evaluation (SPSS Inc., Chicago IL). The values were achieved by analysis of variance (ANOVA) and the lowest significant difference (LSD) tests were specified as mean ± SE. $P<0.0001$, $P<0.001$, $P<0.01$, $P>0.05$ were evaluated for the variations between extract and control groups. P values given as footnotes below Table 1 and 2 were considered extremely effect, highly effect and moderately effect. This study was conducted in three repetition.

3. RESULTS

The datas of the antimicrobial measurement showed that this extract has highly effective, effective, very low effective against the tested microorganisms (Table 1-2). *P. rigida* extract has highly effective with 16.33 ± 0.57 mm inhibition area on *C. albicans*, *Trichophyton sp.*, from yeast and dermatophyta fungi ($P<0.001$). In the microorganisms: It is effective in destroying the proliferation of *P. aeruginosa* (11.33 ± 0.57 mm inhibition area), *C. glabrata* (14.33 ± 0.57 mm inhibition area), *Epidermophyton sp.* (12.33 ±0.57 mm inhibition area) (P<0.001).

In the gram negative and positive bacteria; In the gram negative and positive bacteria; It is very low effective with 8.33±0.57 mm inhibition area against *E. coli*, *S. aureus* and *B. megaterium* (P> 0.05). This means that it contributes as a natural drug raw material for treatment works on microorganisms, notably dermatophytes that derange the human health.

In conclusion, antimicrobial characteristic of *P. rigida* is pretty high yeast and dermatophyte fungi compared to standard antibiotic and chloroform.

The reason why we use chloroform as a solvent in the antimicrobial efficacy test is that it is among the effective solvents used in researches on this subject and the chloroform extract generally shows a high antimicrobial effect. This is because chloroform produces inhibitory compounds in the composition of plants [17, 18].

Table 1
The antimicrobial characteristic of *Phlomis rigida* Labill by the agar disc diffusion method

Microorganisms	Inhibition area (mm)		
	P. rigida	Control	
		Chloroform	Standart antibiotics
E. coli	8.33±0.57[a]	9.00±1.57[c]	14.33±0.57*
S. aureus	8.33±0.57[a]	9.33±0.57 [c]	14.33±0.57*
B. megaterium	8.33±0.5[a]	10.33±0.57[c]	13.00±1.00*
P. aeruginosa	11.33±0.5[d]	10.33±0.57[c]	12.33±0.57*
C. albicans	16.33±0.5[d]	8.33±0.57c	12.33±0.57*
C. glabrata	14.33±0.5[d]	8.33±0.57[c]	9.66±0.57**
Epidermophyton sp.	12.33±0.5[d]	9.33±0.57[c]	9.66±0.57**
Trichophyton sp.	16.33±0.5[d]	8.33±0.57[a]	9.66±0.57**

Extract positive control; ampicillin sulbactam (*) and mikostatin (**) (120 µL and 20µg/disc), the negative control; chloroform. Inhibition zone > 20 mm (extremely effect; *P< 0.0001*; cd), 15 – 19 mm (highly effect*; P< 0.001*;d), 9-14 mm (effective; *P<0.001*;d), very low effect (a: *P>0.05*)

The antimicrobial features of this plant extract in concentrations ranging from 100 µL to 6.25 µL of was evaluated against all of the tested microorganisms with MIC. Table 2. shows the MIC value of all pathogen microrganisms for this extract. The MIC values ranged from an average of 6.25 µL to 50 µL. Acording to this; the results showed good inhibitory effect with 6.25 µL for *C*. albicans, *Trichophyton sp.* with 12.25 µL for *C*. glabrata. In the other hand it is effective with 25 µL for *P. aeruginosa*, *Epidermophyton sp.* with 50 µL for *E. coli*, *S. aureus*, *B. megaterium*.

So that means; this natural extract showed its antimicrobial affect at the lowest inhibition value tested against the development of yeast and dermatophyte fungi (6.25-12.25 µL). This once again proved that this plant extract, which we use with the MIC method, is very effective against the development of microorganisms causing diseases in humans.

Table 2
The antimicrobial characteristic of *Phlomis rigida* Labill by the minimum inhibition concentration (MIC in 100 µL)

Microorganisms	Inhibition area (µL)
	MIC values
	P. rigida
E. coli	50
S. aureus	50
B. megaterium	50
P. aeruginosa	25
C. albicans	6.25
C. glabrata	12.25
Epidermophyton sp.	25
Trichophyton sp.	6.25

The antimicrobial feature of *P. rigida* has been found to create a rather high inhibition zone against bacteria, yeast and dermatophyte fungi compared to standard antibiotics and chloroform used for control purposes. Therefore, it can be a natural antimicrobial agent.

Microorganisms developing resistance against antibiotics, it requires the continuity of the search for new natural therapeutic compounds. Just to clarify, our study is highly significant effect on both dermatophyte fungi and microorganisms by the smallest concentration even.
Plants for the isolation of natural antimicrobial compounds have been the focus of many researchers' previous scientific studies on this topic. It is the first report in the literature about antimicrobial analysis of *P. rigida* in studies performed to date.

In many studies conducted in- vitro, it has been determined that extracts of various Phlomis species have antimicrobial effects on the development of microorganisms. It has been stated in the studies conducted on diverse Phlomis species from different regions, that the vast majority of pathogenic microorganism species including bacteria and various fungi have an preventing characteristic on development [2].

Nikan et al. (2017) stated that essential oil from *P. rigida* is the most active oil that prevents the development of *S. aureus* and *C. albicans* [19]. In a study by Kulani et al. (2013), *P. rigida* oils were determined an preventing and biological characteristic, chemical content [20].

The different species of Phlomis essential oils showed an preventing effect on the disease-causing microorganisms in the development of pathogenic bacteria. In addition, methanol extracts from several Phlomis kinds also have the same effect. Extracts obtained with different solvents of *Phlomis* species have a preventive effect on the development of *Candida* species [4].

Another study found which *P. kurdica* essential oil prevented the development of food-borne B. cereus, while *H. pylori* was the most resistant [9].

Ristic et al. (2000) stated in their study that the ethanol extract and essential oils of *P. fruticosa* demonstrated preventing effect on the tested some bacteria and fungi. The largest inhibition zones were found for *B. subtilis*, *E. coli* and *M. luteus*. Substantial activity against *P. aeruginosa* and *S. faecalis* was not found [21].

In the study by Aligiannis et al. (2004), essential oils obtained from Phlomis species other than our study were found to have moderate activity on bacteria and have a stronger activity on pathogenic fungi [22].

Morteza-Semnani et al. (2006) expressed that the methanol extracts of different species of Phlomis prevented the development of *Streptococcus sanguis* and *S. aureus* at high levels. It also does not have any antifungal effects [23].

In another study, the methanol extracts from *P. rigida* were identified to be more effective than *H. bracteosa* as percent of inhibition [24].

It was determined in the study by Toroğlu et al. (2013), that extracts of *P. oppositiflora* using different solvents showed inhibitory activity on bacteria and fungi. In addition, essential oil and methanol extract have inhibitory activity in *E. coli* [6].

This study verifies the places used in folk medicine practices in a way. This plant has a potential to be a natural antimicrobial agent to be used as a medicine for human health and life quality.

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Authors' Contribution

All authors have contributed in experimental study and writing of the manuscript equally.

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The authors declare that this document does not require an ethics committee approval or any special permission.

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The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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