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Antibacterial Potential of Clonally Propagated *Prunella vulgaris* L. under *in vitro* Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aims to determine the antibacterial efficacy of *Prunella vulgaris* plant produced by micropropagation method.
Place and Duration of Study: The study was carried out during May and June 2018 at Kütahya Dumlupınar University Biotechnology Laboratory and Manisa Celal Bayar University Microbiology Laboratory.
Methodology: Shoot explants isolated from *in-vitro* germinated sterile plantlets were cultured in MS medium containing 3 mg/l BAP and 1 mg/l IBA. The plantlets have been subculture for 10 times at an interval of two weeks and harvested. The plantlets were dried in the shade at room temperature for antibacterial activity studies. Ethanol and chloroform extracts from micropropagated plants were assayed against nine bacteria species (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* CCM 5445, *Proteus vulgaris* ATCC 6896, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13047, and *Kocuria rhizophila* ATCC 9341). Penicillin G, novobiocin, ampicillin, chloramphenicol and erythromycin as test antibiotics were used for comparison.

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Results: Extracts of *P. vulgaris* showed 20 to 28 mm inhibition zones against *Proteus vulgaris* and *Salmonella typhimurium. Prunella vulgaris* found more effective on gram-positive bacteria in compared to gram-negatives.
Conclusion: *Prunella vulgaris* found more effective on gram-positive bacteria than gram-negatives. The present investigation clearly indicates that the antibacterial activity varies with the *P. vulgaris*. Further, the active phytocompounds of this plant against some bacteria should be characterised, and their toxicity should be evaluated in *in-vivo*.

Keywords: Antimicrobial; micropropagation; plant tissue culture; Prunella vulgaris.

1. INTRODUCTION

Prunella vulgaris L. (Labiatae) has some medical importance. This plant is known as "self-heal", was popular in traditional European medicine during the 17th century as a remedy for alleviating sore throat, reducing fever and accelerating wound healing [1]. In China, it was employed in folk medicine as a traditional antipyretic remedy [2]. Phytochemical studies indicate that *P. vulgaris* contains phenols, saponins, alkaloids, tannins, terpenes and anionic polysaccharide prunellin [3]. Prunellin exhibits anti-HIV activity [4], and it also displayed specific activity against the *Herpes simplex* virus type 1 and 2 [5]. *P. vulgaris* aqueous-ethanol extracts have also been shown to exhibit the scavenger effects on DPPH [6].

In recent years, studies on the production of plants by biotechnological methods have gained importance that meets the food and medical needs of the rapidly increasing world population, unlike traditional methods. After application, such as sand culture, a large number of plants can be produced in a short time by plant tissue culture methods which enable plants to obtain a complete plant from a single cell, tissue or organs [7]. Micropropagation is one of the plant tissue culture methods, that can produce plants in desired amounts from organise meristems or somatic cells which have matured or immatured. Micropropagation based on the totipotency feature, which is described as the ability of genetic information in plant cells to produce a complete plant. Plant cells which have totipotency feature can be oriented to shoot, root or callus by plant growth regulators [8]. On this count, a whole plant can be obtained from a single cell or tissue. Auxin and cytokinins are the most commonly used plant growth regulators in micropropagation applications [9]. The micropropagation protocol of *P. vulgaris* has been determined in the previous studies [10].

However, scarce information is available regarding the antibacterial activity of *P. vulgaris* which is obtained by micropropagation method. The study aims to determine the antibacterial efficacy of *P. vulgaris* plant produced by micropropagation method.

2. MATERIALS AND METHODS

Prunella vulgaris seeds used in the study were obtained from Hekim Sinan Medicinal Plant Research Center of municipality of Kütahya, Turkey. Firstly, the sterile plantlets of *P. vulgaris* seeds were subjected to surface sterilisation by using 70% ethyl alcohol for 3 minutes, followed by 0.5% NaOCl for 5 minutes, and kept in 3 separate sterilised water jars for 3 minutes after rinsing. After that, the plantlets were transferred to 30 ml MS jars without any growth regulators [11]. Shoot tips were excised from aseptic seedling and were cultured on MS medium supplemented with 3 mg l^{-1} BAP and 1 mg l^{-1} IBA for micropropagation studies. The plantlets that have been subculture 10 times at an interval of two weeks were harvested and then dried in the shade at room temperature for antibacterial activity studies [12].

The plants were reduced to coarse powder. Two gram of powder was extracted with 20 ml of ethanol and chloroform at 25°C. Sample solutions were prepared by dissolving the extracts in the same solvents (1 ml). Antibacterial studies were carried out by the agar well diffusion method. Bacterial strains grown on nutrient agar at 37°C for 24 h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards. 50 µl inoculum was added to 25 mL melted Mueller Hinton Agar and cooled at 45°C. The solution was poured into petridishes and maintained for 1h at room temperature. 6 mm diameter wells were cut in the agar plate; 60 µl of extract concentration with a negative control (ethanol and chloroform) were loaded in the wells. The plates were incubated at 37°C for 24h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed. Commercial antibiotics (penicillin G, novobiocin,

Table 1. Antibacterial activity of *Prunella vulgaris* extracts

Bacteria	Inhibition zones (mm)						
	Extracts*		Antibiotics**				
	Eth.	Chl.	Pe. G.	Nov.	Amp.	Chlo.	Eryt.
S. aureus	14	16	24	32	20	18	12
E. coli	12	10	6	6	26	9	28
B. cereus	18	12	10	25	28	12	15
B. subtilis	14	10	8	13	32	11	30
S. typhimurium	**24**	**20**	6	40	6	13	28
P. vulgaris	**28**	**22**	10	26	12	9	22
E. faecalis	10	12	24	28	30	18	16
E. cloacae	10	10	12	22	12	11	30
K. rhizophila	12	14	20	28	10	12	15

**Eth: Ethanol, Chl: Chloroform*

***Pe.G: Penicillin G, Nov: Novobiocin, Amp: Ampicillin, Chlo: Chloramphenicol, Eryt: Erythromycin*

ampicillin, chloramphenicol and erythromycin) were used as positive control [13].

3. RESULTS AND DISCUSSION

Plant tissue culture techniques enable the production of plant tissue or cells in sterile environments under controlled conditions, allowing the growth and development of the cells or tissues to be manipulated for a variety of applications. Among these methods, micropropagation could be produced pharmacologically active molecules at the desired amount and constant quality at any time in laboratory conditions [14,15]. Several studies on the antimicrobial potential of medicinal plants produced by plant tissue culture attract the attention [16-18].

In this study, the antimicrobial potential of *Prunella vulgaris* shoots obtained through propagation has been examined. The extracts of *P. vulgaris* showed various antibacterial activities against the tested bacteria. All extracts showed antibacterial activity against at least one of the tested microorganisms with inhibition zones ranging from 14 to 28 mm (Table 1). Ethanol extract of *P. vulgaris* showed inhibition zones of 28 mm and 24 mm against *Proteus vulgaris* and *Salmonella typhimurium,* respectively. Chloroform extract showed 22 mm and 20 mm inhibition zones against *Proteus vulgaris* and *Salmonella typhimurium,* respectively. These results are differed from the tested antibiotics. *Prunella vulgaris* found more effective on gram-positive bacteria in compared to gram-negatives. Rasool et al. [19] reported that methanol, ethanol and chloroform extracts of wild and *in-vitro* grown plants of *P. vulgaris* showed different levels inhibition zones against five bacterial strains. The Inhibition zone values were found to be similar with the present study. The earlier study [19] also reported that wild *P. vulgaris* plants were determined more effective on bacteria than *in-vitro* plants.

4. CONCLUSION

The antibacterial effects of plantlets micropropagated by organogenesis method of *Prunella vulgaris* were investigated. The extracts of *P. vulgaris* showed various antibacterial activities against the tested bacteria. All extracts studied in this study showed antibacterial activity against at least one of the tested microorganisms with inhibition zones ranging from 10 to 28 mm. The present investigation clearly indicates that the antibacterial activity varies with the *P. vulgaris*. Further, the active phytocompounds of this plant against some bacteria should be characterised and their toxicity should be evaluated in *in-vivo*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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