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LC-MS/MS Analysis and Biological Activities of Methanol Extract from *Sagina apetala* Ard.

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Abstract

This is the first study on the investigating phenolic compounds of methanol extract (ME) of *Sagina apetala* and examining its cell-based antioxidant and antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The determination of phenolic compounds of ME was performed by LC-MS/MS and 25 main compounds were identified. For the cell-based antioxidant activity of ME, Vero cell line (Cercopithecus aethiops kidney epithelial, Monolayer) was used as the model cell line and ME was showed 61.22% cell viability. ME, also showed insignificant antibacterial activity against both gram-positive and gram-negative bacteria. In conclusion, this study in the species provides the basic data for future studies for the species.

Keywords: Antibacterial activity, Antioxidant activity, Phenolic compounds, *Sagina apetala* Ard.

1. Introduction

Caryophyllaceae is a family containing about 90 genera traditionally used for treatment of many diseases in different ethnic communities. Especially, it is widely used in Chinese traditional medicine to treat a wide variety of ailments [1]. Caryophyllaceae family members are rich source of natural products- triterpenes, phenolics and alkaloids are the main metabolites of this family- and these compounds are known to be pharmacologically active [2].

Among the most highly studied metabolites of the Caryophyllaceae family are flavonoids, which are classified as polyphenolic compounds and show a wide variety of biological and pharmacological activities such as antioxidant, anti-oedemic, anti-inflammatory, antimicrobial, and immunomodulatory effects [3].

Sagina L., affiliated with the family Caryophyllaceae, consists of about 35 species and 4 of them are represented in flora of Turkey. The diversity center of this genus is Europe, and it is found on almost all continents except Antarctica [4-6].

In the literature, there are a few number of studies on *Sagina* species and most of them are on the *S. japonica* [7, 8]. In one of this study, antitumor activity of the petroleum ether extract obtained from *S. japonica* was analyzed on three human cancer cell lines (K562, Hela and MCF-7) by MTT and SRB methods. Moreover, in this study, phytochemical investigation was performed, and twenty-five components were identified by GC-MS [9]. In another study, the antitumor activity of flavones, saponins and essential oils from *S. japonica* was tested on mice with tumor cell lines U14 and S-180 [10]. The study on *S. merinori* species was focused on antioxidant activity, investigation of total phenolic contents of *S. merinori* and uses of *S. merinori* for the skin care [11]. However, there is no study on the identification of phytochemical content and examination of the biological activities of *S. apetala*.

The aims of this study were to determine the phenolic contents by LC-MS/MS of ME from *Sagina apetala* Ard. and determined the antimicrobial and cell-based antioxidant activity of that extract.

2. Materials and Methods

2.1 Chemicals

Chemicals, solvents, and all materials required for LC-MS/MS analyzes and *Pseudomonas aeruginosa* ATCC 27853 (gram-negative) and *Staphylococcus aureus* ATCC 25923 (gram-positive) for antibacterial activity analyzes were obtained from Sigma Aldrich, USA. DMEM- high glucose (E0500-180) and 10% Fetal Bovine Serum (FBS, A0500-3010) for Cell-based Antioxidant Activity analyzes were obtained from Cegrogen Biotech, Germany, 0,5% DMSO (APA3672.0250), 1% Sodium Pyruvate (L0473), 1.0% Penisilin-Streptomisin (A2213) were obtained from Applichem, USA, Merck, Germany, Biochrom, Germany, respectively.

2.2. Plant Material

Sagina apetala Ard. (whole plants) were collected from Bozdağ, İzmir, Turkey, identified and a voucher specimen has been deposited in Ege University Herbarium (EGE- HERB 28494) (Figure 1).

Figure 1. *Sagina apetala* Ard.

2.3. Preparation of the Extract

The air-dried and powdered plant material (50.00 g) was extracted with methanol (3 × 100 mL) at room temperature. After carrying out filtration and evaporation procedures, 0.71 grams of methanol extract (ME) were obtained.

2.4. LC-MS/MS Analysis

LC–MS/MS analyzes of ME of *S. apetala* were carried out in Manisa Celal Bayar University Scientific Technical Application and Research Center (DEFAM) by LC–MS/MS Agilent Technologies 1260 Infinity liquid chromatography system hyphenated to a 6420 Triple Quad mass spectrometer. The column used for chromatographic analysis was Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 µm) and formic acid, water, methanol, ammonium acetate, acetonitrile and acetic acid was applied gradiently. Gradient program with 0.1% formic acid/methanol, 5 mM ammonium acetate/ acetonitrile with 0.1% acetic acid and, 10 mM ammonium formate with 0.1% formic acid/acetonitrile with 0.1% formic acid, respectively. The 2,0 µL of the sample was applied into the column that fixed at 25 °C with the 0.4 mL/min flow rate of mobile phase.

2.5. Cell-Based Antioxidant Activity

Cell based antioxidant activities of ME was carried out by Ege University Central Research Test and Analysis Laboratory Application and Research Center (MATAL). Vero cell line (Cercopithecus aethiops kidney epithelial, Monolayer) were maintained in control medium consisting of DMEM- high glucose with 10% FBS and 1.0% P-S. The cell (1×10^5 cell/mL) was cultivated in medium and incubated in a 96-well plate for 24 h (37 °C and 5% CO_2, 95.0 % humidified environment) , Afterwards, the cell was exposed to 2 μM H_2O_2 (diluted with DMEM) for 3 h in order to stimulate oxidative stress. At the end of the incubation time, the cell viability was determined using MTT test (570 nm). Cell based antioxidant capacity of the extract was determined comparing to the cell viability of the control [13, 14]. Each assay in this experiment was repeated three times.

2.6. Determination of Antibacterial Activity

The antibacterial activity of ME was analyzed with the agar well diffusion test method [15]. This analyzes were carried out by Ege University Central Research Test and Analysis Laboratory Application and Research Center (MATAL). The antimicrobial activities of the ME were determined against two microorganisms: *Pseudomonas aeruginosa* ATCC 27853 (gram-negative) and *Staphylococcus aureus* ATCC 25923 (gram-positive). Bacteria were stored at -20 °C in media containing 16% glycerol. They were incubated in Muller-Hinton Broth (MHB) at 37 °C for 16-24 hours until a turbidity of 0.5 McFarland was reached. The ME was diluted to 50% with MHB medium and a 20 µL of a microorganism culture solution was added to microplate [16]. After the extract was placed in the petri dish, it was waited for another 15 minutes and incubated for 24 hours at 37 °C. Standard antibiotic disc of ofloxacin (OFX, 5.0 µg/disc) was used positive control. Each assay in this experiment was repeated three times [17].

3. Results and Discussion

3.1. LC-MS/MS Analysis

The phenolic compounds of ME from *S. apetala* were identified by LC-MS/MS and the obtained chromatogram was given in Figure 2. The results showed that *S. apetala* is very rich in phenolic compounds. 25 phenolic compounds were detected in the extract screened over 30 standards (Table 1).

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The most abundant phenolics in the ME are as follows: Rosmarinic acid (392.23 mg/kg extract), protocatechuic acid (141.98 mg/kg extract), apigenin 7-glucoside (121.50 mg/kg extract), 4-Hydroxybenzoic acid (115.48 mg/kg extract), *p*-coumaric acid (73.92mg/g extract). Besides these major compounds, pyrocatechol, (+)-catechin, taxifolin, 2-hydroxycinnamic acid and eriodictyol were not observed in the ME.

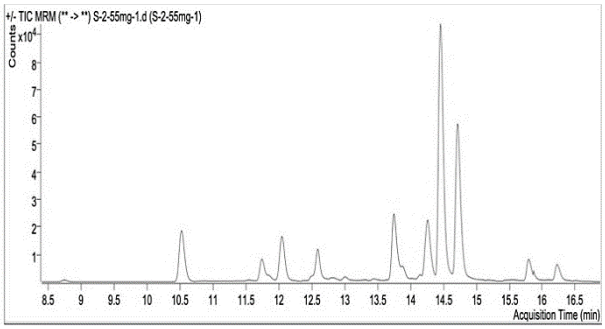

Figure 2. LC-MS/MS Chromatogram of *S. apetala* ME.

Table 1. Phenolic Compounds of *S. apetala* ME

Analytes	Transition	Retention Time	Quantification (mg analyte/kg extract)
Gallic acid	168.9 -> 125.0	8.7	4.47
Protocatechuic acid	152.9 -> 108.9	10.5	141.98
3,4-Dihydroxyphenylacetic acid	167.0 -> 123.0	10.8	1
Pyrocatechol	109.0 -> 52.9	10.9	ND
(+)-Catechin	289.0 -> 245.0	11.0	ND
Chlorogenic acid	355.0 -> 163.0	11.8	33.42
2,5-Dihydroxybenzoic acid	152.9 -> 109.0	11.9	0.99
4-Hydroxybenzoic acid	136.9 -> 93.1	12.1	115.48
(-)-Epicatechin	291.0 -> 139.1	12.3	0.12
Caffeic acid	179.0 -> 135.0	12.6	30.37
Syringic acid	196.9 -> 181.9	12.7	31.92
3-Hydroxybenzoic acid	137.0 -> 93.0	12.8	5.58
Vanillin	151.0 -> 136.0	13.0	28.73
Verbascoside	623.0 -> 160.8	13.4	2.85
Taxifolin	303.0 -> 285.1	13.7	ND
p-Coumaric acid	162.9 -> 119.0	13.8	73.92
Sinapic acid	222.9 -> 207.9	13.8	0.96
Ferulic acid	193.0 -> 134.0	13.9	35.79
Luteolin 7-glucoside	447.1 -> 285.0	14.2	13.26
Hesperidin	611.1 -> 303.0	14.3	5.13
Rosmarinic acid	359.0 -> 160.9	14.5	392.23
Hyperoside	465.1 -> 303.1	14.5	12.64
Apigenin 7-glucoside	433.1 -> 271.0	14.7	121.5
2-Hydroxycinnamic acid	162.9 -> 119.1	14.8	ND
Pinoresinol	357.0 -> 151.0	14.9	3.73
Eriodictyol	287.0 -> 151.0	15.1	ND
Quercetin	301.0 -> 151.0	15.6	0.53
Kaempferol	285.0 -> 229.1	15.8	2.52
Luteolin	287.0 -> 153.1	15.8	24.5
Apigenin	271.0 -> 153.0	16.2	16.65

Phenolic compounds play an important role in living organisms and establish the high majority of compounds contained in medicinal plants. For instance, in this study, rosmarinic acid, which the most abundant phenolic compound in the ME, showed biological activities such as antimicrobial, antiviral, antioxidant, anti-inflammatory, anti-angiogenic, anti-depressant, antihyperglycemic, anti-allergic, antithrombotic, anticarcinogenic, and anti-aging [18]. Protocatechuic acid is known to exhibit anticancer, antiproliferative, antioxidant, and antiadipogenesis activities [19, 20]. Apigenin, which is important flavonoid, has been reported to exhibit potential anticancer activity [21], as well as antifungal [22], antibacterial [23] and antiparasitic [24] properties. 4-hydroxy benzoic acid has biological activities such as antimicrobial and antioxidant [25]. Finally *p*-Coumaric acid has been researched for antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, anti-diabetic and anti-hyperlipaemia [26].

3.2. Cell-based Antioxidant Activity

The antioxidant activity of the Me was examined cell based antioxidant activity test. The ME, which is rich in phenolic compounds and flavonoids, was expected to show high antioxidant activity. Antioxidant activity is often associated with phenolic compounds and flavonoids [27]. The protective effect of extract against the oxidative stress was determined by observing the cell viability of H_2O_2-damaged Vero cell, which was pre-treated with the extract. After the treatments with H_2O_2 (2 μM) for 24 hours, the cell viability of Vero cell pre-treated with the extract was determined as 61.22%, as given in Figure 3.

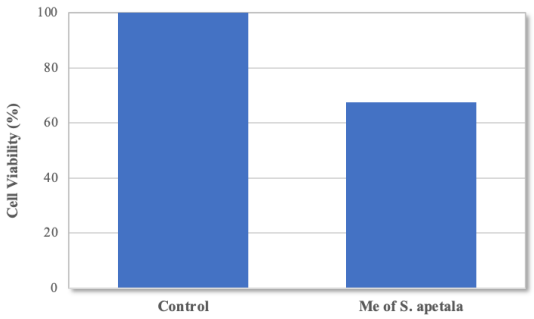

Figure 3. Antioxidant activity of *S. apetala* ME.

3.3. Antibacterial activity of *S. apetala* ME

In this study, two different bacterial strains were used to determine the antimicrobial activity. The results of the antibacterial activities of the standard antibiotics and the ME against the tested microorganisms as given in Figure 4. At a concentration of 50 µg, the ME showed any activity against Gram-negative *Pseudomonas aeruginosa* ATCC 27853 and gram-positive Staphylococcus aureus ATCC 25923 bacteria in this study.

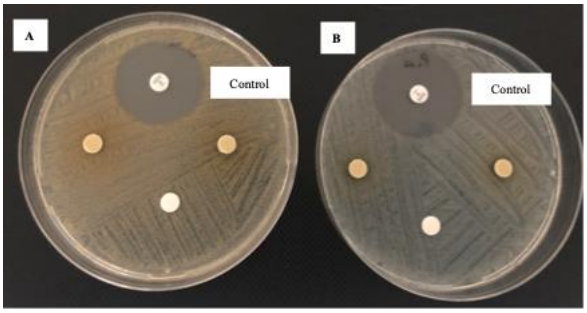

Figure 4. Antibacterial activity of *S. apetala* ME against *S. aureus* (**A**) and *P. aeruginosa* (**B**).

4. Conclusion

In this study, the chemical composition, antibacterial and antioxidant activity of *S. apetala* were reported for the first time. While medium cell based antioxidant activity was observed in ME, insignificant antibacterial activity was observed. This result may be related to the high phenolic compounds of ME. As a result, investigation of the phenolic compounds, antibacterial and antioxidant activities of the ME obtained from *S. apetala* may contribute to the biological activity tests. This study also sheds light on future studies on other species belonging to the Caryophylaceae family.

Author's Contributions

Rabia Nur ÜN: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ethics

There are no ethical issues after the publication of this manuscript

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