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Contributed Paper

# Fatty Acid, Essential Oil and Phenolic Compositions of *Alcea pallida* and *Alcea apterocarpa* with Antioxidant, Anticholinesterase and Antimicrobial Activities

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## ABSTRACT

This study was the first phytochemical and biological activity report on *Alcea pallida* and *Alcea apterocarpa* extracts. The main constituents of the essential oils were identified as arachidic acid (34.2%) for *A. pallida*, and hexatriacontane (25.3%) for *A. apterocarpa*. The main constituents of the fatty acids obtained from petroleum ether extracts of *A. pallida* and *A. apterocarpa* were identified as palmitic acid (31.2%) and oleic acid (25.6%), respectively. The phenolic compositions of the samples were determined using HPLC (LC-20 liquid chromatographic system). *A. pallida* and *A. apterocarpa* showed the same peaks which were ascorbic, caffeic, salicylic and p-hydroxybenzoic acids and quercetin, respectively. Salicylic acid showed the highest abundance. Among the eight extracts, the acetone extract of *A. pallida* possessed the best ABTS cation radical scavenging activity and moderate butyryl-cholinesterase activity at 200 µg/mL. The *A. pallida* acetone extract exhibited 53.12% inhibition in DPPH free radical scavenging activity method at 100 µg/mL concentration. The acetone extract of *A. pallida* showed weak antimicrobial activity against *Escherichia coli, Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and moderate activity against *Candida albicans* (inhibition zone diameter 16 mm). The acetone extract of *A. apterocarpa* showed moderate activity against *C. albicans* (inhibition zone diameter 14 mm) and *S. aureus* (inhibition zone diameter 13 mm);

weak activity against *E. coli.*, *S0 pyogenes*, and *P. aeruginosa.*

**Keywords:** Malvaceae, *Alcea pallida*, *Alcea apterocarpa*, essential oil, fatty acid, HPLC, antioxidant, anticholinesterase, antimicrobial

## 1. INTRODUCTION

The genus *Alcea* L. which is a member of the Malvaceae family, consists of 20 species in Turkey and about 70 species worldwide, distributed in Mediterranean and Iran-Turanian phytogeographical region [1, 2]. *Alcea* species are named Hatmi [3], in Anatolia, and *Alcea pallida* Waldst. et. Kit. named as Gül Hatmi, Fatmagül, Deli ebe gümeci, Fatmaana gülü, Fatmacik [4], Deli Fatmaana, Sığirkuyruğu [5] and *Alcea apterocarpa* (Fenzl) Boiss. named as Huri, Hürin, Hüri [6]. These species are widely used to treat wound healing, intestinal and stomach ailments, conjunctivitis, cough, fungal infection and wart [4- 6].

Malvaceae family plants are exceptional among leafy plants due to their high content of polyprenols, which are chemotaxonomic markers [7, 8]. They also exhibited high content of cyclopropane acids, which were not observed in plants of other families [9]. So far, there are only a few studies about *Alcea* species which afforded polyprenols, terpenoids [10] and polysaccharides [11].

Since ancient times, people have benefited from plants not only as food supply, but also as smell, flavor, fuel, weapon and medicine. Especially extracts derived from medicinal plants have been used to treat many diseases and accordingly, healing has emerged as a profession. Nevertheless, in the 1800s, first active substances derived from plants, produced synthetically, as a result pharmaceutical industry was born and the old traditional methods were left aside. However, especially in the last 30-35 years, an increased interest emerged towards traditional methods known as "alternative medicine" namely the therapeutic usage of plant extracts, since the treatment of synthetic drugs used in modern medicine failed to reach the desired success and despite having many negative side effects synthetic drugs usually only have a positive impact [12-15].

A literature survey showed that there have been no previous phytochemical and biological reports on *A. pallida* (AP) and endemic *A. apterocarpa* (AA). The aim of this study was to evaluate the antioxidant, anticholinesterase and antimicrobial activities of the petroleum ether, acetone, methanol and water extracts of them with their total phenolic and flavonoid contents. The petroleum ether extracts of *A. pallida* and *A. apterocarpa* were analyzed to determine their fatty acid compositions by GC/MS. The essential oils were analyzed to determine its composition by GC/MS. Their phenolic compositions were determined using HPLC. ABTS cation radical decolorisation and DPPH free radical scavenging activity methods were carried out to indicate the antioxidant activity. The anticholinesterase and antimicrobial potentials of the extracts were determined by Ellman and disc diffusion methods, respectively.

## 2. MATERIALS AND METHODS

### 2.1 General Experimental Procedures

A Thermo pH-meter, a BioTek Power Wave XS, an Elma S15 ultrasonic bath and a vortex (LMS Co. LTD) were used for the activity assays. Ethanol, hexane, diethyl ether, chloroform, toluene, dichloromethane,

methanol, potassium acetate, sulphuric acid, aluminium nitrate nonahydrate, aluminium chloride, ABTS, sodium acetate, nutrient broth, boric acid, nutrient agar, butylated hydroxytoluene were purchased from Merck (Germany), DPPH, $H_2O_2$, quercetin, pyrocathecol, acetic acid, sodium methoxide, Tween 40, DTNB, linoleic acid, neocuproine, EDTA, acetylcholinesterase, butyrylcholinesterase, caffeic acid, ascorbic acid from Sigma (Germany), a-tocopherol, acetylthiocholine iodide, p-hydroxybenzoic acid from Aldrich (Germany), galanthamine hydrobromide, salicylic acid from Sigma-Aldrich (Germany), BHT from Fluka (Germany), sterile blank disc and antbiotic disc from Oxoid (United Kingdom), petroleum ether, sodium dihydrogen phosphate, sodium carbonate, sodium hydrogen phosphate, ammonium acetate from Reidel de Haen (Germany).

**2.2 Plant Material**

Whole plants of *Alcea pallida* Waldst. et. Kit. and *Alcea apterocarpa* (Fenzl) Boiss. were collected from Turkey "(Istanbul and Kahramanmaras, repectively) in July 2012 by Dr. Abdulselam Ertas, and identified by Dr. Yeter Yesil. These specimens have been stored at the Herbarium of Istanbul University (ISTE 98062 and ISTE 98074, respectively).

**2.3 Isolation of Essential Oil**

Essential oils were obtained using a Clevenger apparatus from the whole parts of *A. pallida* and *A. apterocarpa*, which were crumbled into small pieces and soaked in distilled water for 3 h. The obtained essential oils were dried over anhydrous $Na_2SO_4$ and stored at +4 °C for a sufficient period of time.

**2.4 GC/MS Conditions (Essential oil)**

The essential oils were diluted using $CH_2Cl_2$ (1:3 volume/volume) prior to GC/FID and GC/MS analysis. GC/FID performed using Thermo Electron Trace GC FID detector and GC/MS performed using same GC and Thermo Electron DSQ quadrupole for MS. A nonpolar Phenomenex DB5 fused silica column (30 m × 0.32 mm, 0.25 µm film thickness) was used with helium at 1 mL/min (20 psi) as a carrier gas. The GC oven temperature was kept at 60 °C for 10 min and programmed to 280 °C at a rate of 4 °C/min and then kept constant at 280 °C for 10 min. The split ratio was adjusted to 1:50, the injection volume was 0.1 µL, and EI/MS was recorded at 70 eV ionization energy. The mass range was $m/z$ 35-500 amu. Alkanes (C8-C24) were used as reference points in the calculation of Kovats Indices (KI) by the same conditions [16, 17]. Identification of the compounds was based on comparing their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as data from the published literature. GC/FID and GC/MS were replicated three times. (Mean RSD % <0.1)

**2.5 Esterification of Total Fatty Acids with GC/MS Conditions**

Esterification of the petroleum ether extracts was carried out according to Kilic et al. [18]. Helium was used as carrier gas at a constant flow rate of 1 mL/min. 1 µL of sample was injected. The GC temperature program was set as follows; 150 °C hold for 5 min, ramp to 250 °C at 3 °C/min and hold for 10 min. The temperature of the MS transfer line was set at 230 °C. Using scan mode a mass range from 50 to 650 $m/z$.

Column: Rtx-5Sil MS, 30 m, 0.25mm ID, 0.25 µm. Thermo Scientific Polaris Q GC-MS was used in this study.

**2.6 Preparation of Extracts**

Whole plant materials were dried and powdered, 100 g of each plant material was sequentially macerated with petroleum ether, acetone, methanol and water for 24 h at 25 °C. After filtration, the solvents were evaporated to obtain the crude extracts. The yields of the petroleum ether extracts were calculated as APP 1.5%, AAP 2.5%, the acetone extracts as APA 2.1%, AAA 1.2%, the methanol extracts as APM 7.8%, AAM 6.7%, and the water extracts as APW 4.2%, AAW 3.5% (w/w).

**2.7 Preparation of Extracts to Determine Phenolic Compounds using HPLC**

The dried and powdered plants were extracted with MeOH about 24 h at room temperature. The extract was filtrated and evaporated under vacuo. Dry filtrate was diluted until 1000 ng/L and passed through the microfiber filter 0.2 µm.

**2.8 Chromatographic Conditions for Detection of Phenolic Compounds**

The instrument was used a Shimadzu LC-20 liquid chromatographic system equipped with a UV detector and controlled by Lab Solutions software was used. Detection was achieved on Cronusil-S ODS2 C18 column (25 cm × 0.46 cm; 5 µm). For the identification of phenolic compounds via HPLC a gradient mobile phase of formic acid in methanol and water. Mobil phase A included methanol:water:formic acid (10:88:2; v,v) and mobil phase B included methanol:water:formic acid (90:8:2; v,v). The elution was 75% mobil phase A and 25 % mobil phase B initially, and amount of mobil phase B increased by % 10 by five for 18 min. A linear gradient continued (20% mobil phase A and 80% mobil phase B) until 30 min and finally 75:25 (A: B; %) for 10 min. The flow rate was 1 $mLmin^{-1}$. The detection wavelength was 280 nm, and the sample injection volume was 10 µL.

**2.9 Determination of Total Phenolic and Flavonoid Contents of Extracts**

The amounts of phenolic and flavonoid contents in the crude extracts were expressed as pyrocatechol and quercetin equivalents, respectively, and they were calculated according to the following equations [19, 20]:

Absorbance = 0.0146 pyrocatechol (µg) + 0.0249 ($R^2$ = 0.9982)

Absorbance = 0.1494 quercetin (µg) - 0.0961 ($R^2$ = 0.9992)

**2.10 Antioxidant Activity**

We used DPPH free radical scavenging activity and ABTS cation radical decolorization methods to determine the antioxidant activity of the extracts [21-24].

**2.11 Anticholinesterase Activity**

A spectrophotometric method developed by Ellman, Courtney, Andres, and Featherstone [25] was used to determine the acetyl- and butyryl-cholinesterase inhibitory activities of the extracts.

**2.12 Antimicrobial Activity**

Five different microorganisms including Gram-positive bacteria (*Streptococcus pyogenes* ATCC19615 and *Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922), and yeast (*Candida albicans* ATCC10231) were purchased from Refik Saydam Sanitation Center (Turkey) and were used for detecting the antimicrobial

activity of the samples. The disc diffusion method was employed for this purpose [26]. Imipenem and nystatin were used as positive controls for bacteria and yeast, respectively.

**2.13 Statistical Analysis**

The results of the antimicrobial, antioxidant and anticholinesterase activity assays were mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's *t*-test, $p$ values < 0.05 were regarded as significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Identification by GC MS Analysis

The essential oil compositions of *A. pallida* and *A. apterocarpa* were determined by GC/FID and GC/MS analysis. Twenty-four components were determined, constituting 99.6% of the essential oil composition of *A. pallida*. The major components of the essential oil were identified as arachidic acid (34.2%) and

**Table 1.** Chemical compositions of the essential oils from *A. pallida* (AP) and *A. apterocarpa* (AA).

| RI[a] | Rt (min)[b] | Constituents[c] | Composition% AP | Composition% AA |
|---|---|---|---|---|
| 865 | 10.87 | Isononane | - | 1.1 |
| 1249 | 24.06 | 1,3-Di-tert butyl benzene | 1.1 | 2.6 |
| 1447 | 29.47 | β-Farnesene | 1.0 | 2.1 |
| 1480 | 30.34 | τ-Muurolene | 1.4 | 0.6 |
| 1484 | 30.48 | Valencene | 2.2 | 4.1 |
| 1498 | 30.87 | α-Selinene | 8.0 | 6.5 |
| 1505 | 31.01 | β-Himachalene | 0.8 | 2.1 |
| 1528 | 31.83 | α-Muurolene (Amorphene) | 2.0 | 1.5 |
| 1677 | 35.02 | Cadalene | 1.4 | 1.3 |
| 1746 | 35.52 | 2-Methyl heptadecane | 1.4 | 1.7 |
| 1778 | 36.08 | pentadecanol | - | 1.3 |
| 1800 | 36.45 | Octadecane | 1.8 | 1.5 |
| 1890 | 36.74 | 2-Methyl-1-hexadecanol | 1.9 | 1.9 |
| 2172 | 37.46 | 2-Eicosanol | - | 1.1 |
| 2185 | 38.35 | Z-8-Octadecen-1-ol acetate | 1.6 | 4.4 |
| 2171 | 38.98 | Butyl phthalate | 0.7 | 1.1 |
| 2109 | 40.00 | Heneicosane | 1.8 | 3.6 |
| 2259 | 40.13 | 2,5-Di-tert octyl-p-benzoquinone | 5.2 | 6.6 |
| 2366 | 40.59 | Arachidic acid | 34.2 | 1.2 |
| 1986 | 40.66 | Hexadecanoic acid | 6.6 | 1.0 |
| 2407 | 40.84 | Tetracosane | - | 2.5 |
| 2413 | 41.13 | 3-Ethyl-5-(2-ethylbutyl)octadecane | 0.7 | 1.4 |
| 2700 | 43.30 | Heptacosane | 7.0 | 1.1 |
| 2852 | 43.64 | 1-Hexacosanol | 1.9 | 2.6 |
| 2896 | 43.84 | Choleic acid | - | 1.0 |
| 2900 | 44.10 | Nonacosane | 6.3 | 1.1 |
| 3094 | 44.41 | Ethyl iso-allocholate | 3.0 | - |
| 3508 | 45.11 | 17-Pentatriacontene | 0.6 | 1.4 |
| 3600 | 46.50 | Hexatriacontane | - | 25.3 |
| 4400 | 47.12 | Tetratetracontane | 7.0 | 15.4 |
| | | Total | 99.6 | 99.1 |

[a] RI Retention indices (DB-5 column)

[b] Retention time (as minutes).

[c] A nonpolar Phenomenex DB-5 fused silica column

α-selinene (8.0%) (Table 1). Twenty-nine components were determined, constituting 99.1% of the essential oil composition of *A. apterocarpa*. The major components were hexatriacontane (25.3%) and tetratetracontane (15.4%) (Table 1).

The fatty acid compositions of the petroleum ether extracts of *A. pallida* and *A. apterocarpa* were determined by GC/MS analysis. As shown in Table 2, seventeen components were identified, constituting 99.4% of the *A. pallida* petroleum ether extract. The main constituents of the fatty acid obtained from the petroleum ether extract were identified as palmitic acid (31.2%) and linolenic acid (15.9%). As shown in Table 2, fifteen components were identified, constituting 99.9% of the *A. apterocarpa* petroleum ether extract. The main constituents of the fatty acid obtained from the petroleum ether extract were identified as oleic acid (25.6%) and linoleic acid (24.8%).

**Table 2.** GC-MS analysis of the fatty acids of *A. pallida* (AP) and *A. apterocarpa* (AA).

| Rt (min)[a] | Constituents[b] | Composition% AP | Composition% AA |
|---|---|---|---|
| 9.69 | Octanedioic acid | 2.1 | - |
| 12.00 | Lauric acid | 5.3 | - |
| 14.39 | 10-Undecenoic acid | - | 0.8 |
| 18.60 | Myristic acid | 3.2 | 1.6 |
| 24.94 | Palmitoleic acid | 0.1 | 0.7 |
| 25.27 | Palmitic acid | 31.2 | 21.7 |
| 29.27 | Tetradecanedioic acid | 1.6 | - |
| 29.75 | Phytol | 2.8 | 2.0 |
| 30.64 | Linoleic acid | 11.2 | 24.8 |
| 30.77 | Oleic acid | 10.2 | 25.6 |
| 30.86 | Linolenic acid | 15.9 | 6.9 |
| 31.00 | Vaccenic acid | - | 1.8 |
| 31.54 | Stearic acid | 7.3 | 5.1 |
| 36.55 | Eicosane | 0.8 | - |
| 37.38 | Arachidic acid | 2.5 | 2.7 |
| 38.19 | 6-Hexadecenoic acid | - | 2.4 |
| 39.36 | Docosane | 1.1 | 1.1 |
| 40.29 | Heneicosanoic acid | - | 0.9 |
| 42.65 | Pentacosane | 1.3 | - |
| 43.82 | Behenic acid | 1.7 | 2.8 |
| | Total | 99.4 | 99.9 |

[a] Retention time (as minutes).
[b] A nonpolar Phenomenex DB-5 fused silica colum.

### 3.2 Determination of Phenolic Contents using HPLC

After identifying the peaks of standard compounds using by HPLC at 280 nm, the retention times were established. Then, after injection of *A. pallida* and *A. apterocarpa*, extracts, the peaks of five phenolic compounds were indicated in the chromatogram (Figure 1). Both of them showed the same peaks which were ascorbic acid, caffeic acid, salicylic acid, p-hydroxybenzoic acid and quercetin, respectively. Salicylic acid showed the highest abundance.

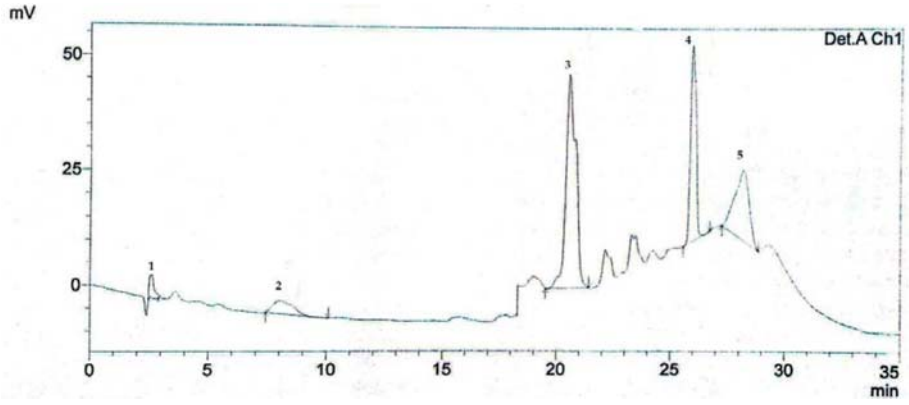


**Figure 1.** A representative chromatogram of phenolic compounds in *A. pallida* and *A. apterocarpa***.** 1: Ascorbic acid (tR: 2.225), 2: Caffeic acid (tR: 7.537), 3: Salicylic acid (tR: 20.199), 4: p-hydroxybenzoic acid (tR: 26.015), 5: Quercetin (tR: 28.950).

### 3.3 Antioxidant and Anticholinesterase Activities

Antioxidant activity tests were carried out at four different concentrations (10, 25, 50 and 100 µg/mL). The antioxidant activity of the petroleum ether (APP and AAP, respectively), acetone (APA and AAA), methanol (APM and AAM), and water (APW and AAW) extracts, prepared from the whole plants of *A. pallida* and *A. apterocarpa,* were investigated through two antioxidant activity test methods by using DPPH free radical scavenging and ABTS cation radical decolorisation assays with their total phenolic and flavonoid contents. Among the eight extracts, the acetone extract of *A. pallida* was found to be the richest in phenolics and flavonoids content. As a result, all of the extracts were found to be at least moderately rich in phenolics. The results were shown in Table 3.

**Table 3.**Total phenolic and flavonoid contents of *A. pallida* (AP) and *A. apterocarpa* (AA) extracts[a].

| Extracts | Phenolic content (µg PEs/mg extract)[b] | Flavonoid content (µg QEs/mg extract)[c] |
|---|---|---|
| APP | 132.19±2.42 | 14.7±0.39 |
| APA | 175.00±1.22 | 19.66±1.58 |
| APM | 106.51±0.18 | 13.30±0.45 |
| APW | 152.74±0.76 | 12.19±0.09 |
| AAP | 125.34±2.42 | 10.52±0.07 |
| AAA | 144.18±0.06 | 18.71±1.10 |
| AAM | 96.23±0.08 | 10.94±0.10 |
| AAW | 109.93±0.04 | 11.47±0.17 |

[a] Values expressed are means ± S.D. of three parallel measurements ($p < 0.05$)
[b] PEs, pyrocatechol equivalents (y=0.0146x + 0.0249 $R^2$=0.9982)
[c]QEs, quercetin equivalents (y=0.1494x – 0.0961 $R^2$=0.9992).

As shown in Figure 2, the *A. pallida* acetone extract exhibited 53.12% inhibition in DPPH free radical scavenging activity method at 100 µg/mL concentration. As shown in Figure 3, the acetone and water extracts of *A. pallida* and *A. apterocarpa* exhibited 87.89, 82.87, 63.08 and 62.48% inhibition in ABTS cation radical scavenging assay at 100 µg/mL concentration, respectively. It is significant that the acetone extract of *A. pallida* exhibited stronger ABTS cation radical scavenging activity than standard compounds a-TOC and BHT.

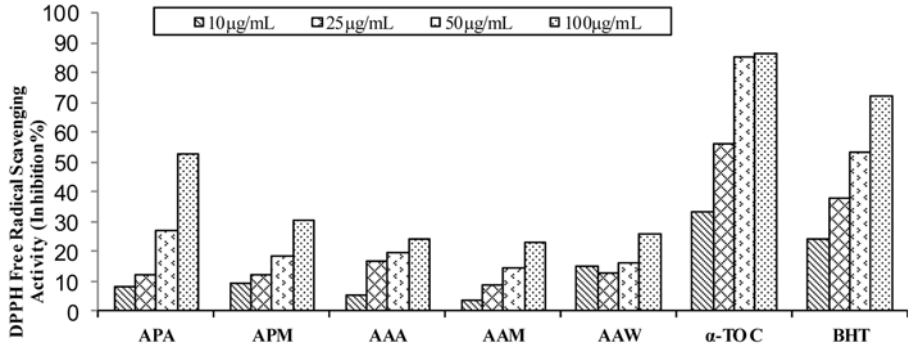


**Figure 2.** Inhibition (%) of DPPH free radical scavenging of the *A. pallida* (AP) and *A. apterocarpa* (AA), α-tocopherol and BHT. Values are means ± S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

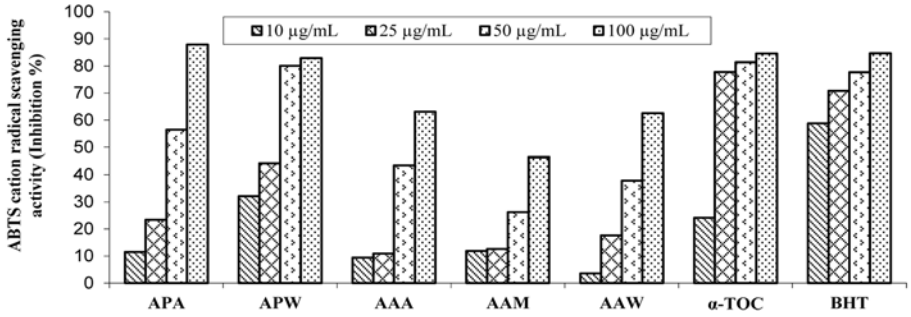


**Figure 3.** Inhibition (%) of ABTS cation radical scavenging of the *A. pallida* (AP) and *A. apterocarpa* (AA), α-tocopherol and BHT. Values are means ± S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

As shown in Table 4, at 200 µg/mL concentration, the four extracts of *A. pallida* exhibited moderate butyryl-cholinesterase inhibition except for the water extract while they were not showed any meaningful activity against acetyl-cholinesterase enzyme. Among the tested eight extracts, the *A. pallida* acetone extract showed the highest inhibition against butyryl-cholinesterase enzyme.

**Table 4.** Anticholinesterase activity of *A. pallida* (AP) and *A. apterocarpa* (AA ) extracts and galanthamine at 200 µg/mL[a].

| Samples | Inhibition %against AChE | Inhibition %against BChE |
|---|---|---|
| APP | NA | 54.49±1.57 |
| APA | NA | 65.99±0.84 |
| APM | NA | 53.26±1.24 |
| APW | NA | NA |
| AAP | NA | 15.45±0.55 |
| AAA | NA | 49.63±1.12 |
| AAM | NA | 57.07±0.37 |
| AAW | NA | NA |
| Galanthamine[b] | 79.11±0.59 | 81.55±0.48 |

[a] Values expressed are means ± S.D. of three parallel measurements ($p$<0.05)
[b] Standard drug
NA: Not active

### 3.4 Antimicrobial Activity

The antimicrobial activities of *A. pallida* and *A. apterocarpa* extracts against different microorganisms were assessed according to inhibition zone diameter. Results are presented in Table 5. The petroleum ether and water extracts of both plants showed no activity at all the five tested microorganisms (Data not shown). The acetone and methanol extracts were active on all microorganisms tested with different zone diameters indicating weak (inhibition zone < 12) and moderate antimicrobial activity (inhibition zone < 20-12). The acetone extract of *A. pallida* showed weak antimicrobial activity against *E. coli*, *S. pyogenes*, *S. aureus*, *P. aeruginosa* and moderate activity against *C. albicans* (inhibition zone diameter 16 mm). The methanol extract of *A. pallida* showed weak antimicrobial activity against all tested microorganisms at all concentrations. The acetone extract of *A. apterocarpa* showed moderate activity against *C. albicans* (inhibition zone diameter 14 mm) and *S. aureus* (inhibition zone diameter 13 mm); weak activity against *E. coli*, *S. pyogenes*, and *P. aeruginosa*. The methanol extract of *A. apterocarpa* showed weak antimicrobial activity against all tested microorganisms at all concentrations except for against *E. coli* at 300 µg/disc concentration. The extract showed moderate activity with 13 mm inhibition zone diameter.

**Table 5.** Zones of growth inhibition (mm) showing the antimicrobial activity of *A. pallida* and *A. apterocarpa* extracts.

| | | | | Microorganisms | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | E. coli | S. pyogenes | S. aureus | P. aeruginosa | C. albicans |
| Inhibition zone diameter (in mm) | *A. pallida* | Acetone extract | 100 µg/disc | 10±0.2 | 10±0.2 | 9±0.3 | 10±0.6 | 14±0.4 |
| | | | 200 µg/disc | 10±0.4 | 11±0.1 | 10±0.6 | 10±0.3 | 14±0.2 |
| | | | 300 µg/disc | 12±0.1 | 13±0.4 | 11±0.2 | 11±0.5 | 16±0.3 |
| | | Methanol extract | 100 µg/disc | 9±0.3 | 11±0.2 | 9±0.1 | 9±0.2 | 10±0.3 |
| | | | 200 µg/disc | 11±0.2 | 11±0.2 | 10±0.2 | 11±0.1 | 10±0.2 |
| | | | 300 µg/disc | 12±0.2 | 11±0.3 | 10±0.2 | 11±0.3 | 11±0.4 |
| | *A. apterocarpa* | Acetone extract | 100 µg/disc | 11±0.2 | 11±0.2 | 8±0.3 | 8±0.4 | 12±0.2 |
| | | | 200 µg/disc | 11±0.3 | 13±0.3 | 11±0.3 | 9±0.4 | 14±0.2 |
| | | | 300 µg/disc | 12±0.3 | 13±0.2 | 11±0.3 | 9±0.2 | 14±0.1 |
| | | Methanol extract | 100 µg/disc | 11±0.1 | 10±0.2 | 9±0.3 | 9±0.0 | 9±0.3 |
| | | | 200 µg/disc | 11±0.1 | 10±0.1 | 11±0.3 | 10±0.1 | 9±0.2 |
| | | | 300 µg/disc | 13±0.2 | 10±0.3 | 11±0.2 | 11±0.2 | 11±0.1 |
| | | | IPM (10 µg) | 19±1.4 | 39.5±0.7 | 27.5±0.7 | 12±0 | - |
| | | | Nystatin (30 µg) | - | - | - | - | 25±0.5 |

Benli et al. (2007) investigated the antimicrobial activity of six endemic plants containing *A. apterocarpa* against 14 microorganisms. They reported that the seed and sepal extract was found to be effective against *P. aeruginosa* (36 mm zone diameter) but no antimicrobial activity was observed in leaf extract [27].

## 4. Conclusion

The present study is the first fatty acids, essential oils, phenolic contents and biological activities reports on *A. pallida* and *A. apterocarpa*. It is noteworthy that the acetone extract of *A. pallida* exhibited stronger ABTS cation radical scavenging activity than the standard compounds, a-TOC and BHT. Furthermore, the acetone extract of *A. pallida* exhibited moderate butyryl-cholinesterase inhibition (65.99%) and moderate activity against *C. albicans* (inhibition zone diameter 16 mm). Thus, the acetone extract of *A. pallida* as the most active extract should be investigated in terms of both phytochemical and biological aspects to find natural active compounds responsible for antioxidant, antimicrobial and antibutyrylcholinesterase activities

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