International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (2), pp. 001-009, February, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antioxidant and antimicrobial activities of Turkish endemic *Achillea* species

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Accepted 23 July, 2017

This study was undertaken to examine *in vitro* antioxidant, radical scavenging and antimicrobial activities of extracts of *Achillea schischkinii* Sosn. and *Achillea teretifolia* Waldst. and Kitt (*Asteraceae*). The plant materials were extracted in methanol, water and chloroform using rotary evaporator apparatus. The extracts were screened for antioxidant activity using the ABTS radical scavenging capacity, DPPH radical scavenging capacity, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities and compared to standard antioxidants. The results obtained in this study indicate that *A. schischkinii* and *A. teretifolia* are potential sources of natural antioxidants, antimicrobial activity screening was performed by the disc diffusion method against 6 bacteria strains and 2 yeast species. *A. teretifolia* displayed strong inhibitory effects against *Pseudomonas aeruginosa.* The *A. teretifolia* extract also showed antimicrobial activity against *Saccharomyces cerevisae*. I contrast, the *A. schischkinii* extract showed no antimicrobial activity against gram-positive and gram-negative bacteria nor against yeast.

Key words: *Achillea schischkinii*, *Achillea teretifolia*, antioxidant, antimicrobial.

INTRODUCT ON

Many plants contain a variety of useful compounds that have not yet been identified. Natural plant resources that contain antimicrobial and antioxidant effects have been extensively studied and utilized as additive agents in food (Rey et al., 2005; Riznar et al., 2006). Therefore, the development and commercialization of novel functional compounds derived from these plants must be pursued to improve the functionality and safety of foods. The application of natural ingredients containing antioxidants and antibiotics may prove useful (Lanzotti, 2006). Antioxidants and antibiotics derived from natural resources are perceived by consumers as being better and safer than synthetics (Park et al., 2008). In addition to providing additional taste and flavor to foods, certain spices have been used as remedies in traditional medicine for centuries (Srinivasan, 2005). The auto-oxidation of lipids, as well as the enzymatic oxidation of fats, oils and fat containing foods during storage and processing, are responsible for rancidity and deterioration of food quality. There is a profound interest in biologically active molecules from food which prevent the deleterious effects of free radicals and also prevent the deterioration of foods due to lipid oxidations and microbial spoilage. Spices and condiments are important food taste and aroma enhancers. They are used as an integral part of human diet to impart flavor, taste and color to the food. Foodborne illness caused by consumption of contaminated foods with pathogenic bacteria and/or their toxins has been of great concern to public health. Controlling pathogenic microorganisms would reduce foodborne outbreaks and assure consumers a continuing safe, wholesome and nutritious food supply (Karanika et al., 2001; Allahghadri et al., 2010).

Oxidative stress, the consequence of the imbalance between prooxidants and antioxidants in an organism, is considered to play a very important role in the pathogenesis of several degenerative diseases, such as

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diabetes, cancer and cardiovascular diseases, including atherosclerosis. Reactive oxygen species (ROS), including hydroxyl radicals, superoxide radicals and singlet oxygen, as well as reactive nitrogen species, are continuously generated in the cell, as a result of normal human metabolism and can be harmful, as they can attack biological macromolecules, cause membrane and DNA damage and enzyme inactivation. The mechanisms by which free radicals interfere with cellular functions are not yet fully understood, but one of the most important processes seems to be the formation of lipid hydroperoxides (Dröge, 2002). Data support the idea that diet could have beneficial effects against diseases and several patterns of diet or individual foods have previously been highlighted (Nijveldt et al., 2001).

Antioxidants can protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation. Hence, a need has appeared to identify alternative natural and safe sources of food antioxidants and the search for natural antioxidants, especially of plant origin, has notably increased in recent years. Antioxidants are often added to foods to prevent the radical chain reactions of oxidation. They act by inhibiting the initiation and propagation steps, leading to the termination of the reaction and delaying the oxidation process (Gülçin, 2006a; Shahidi, et al., 1992). At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and *tert*-butyl hydroquinone. However, BHA and BHT have been restricted by legislative rules due to doubts over their toxic and carcinogenic effects.

There is a growing interest in natural and safer antioxidants for food applications and a growing trend in consumer preferences towards natural antioxidants, all of which have given impetus to the attempts to explore natural sources of antioxidants (Gülçin, 2006b, 2007). Recently, there is increased interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant compounds are widely distributed in plants and are capable of terminating a free radical-mediated oxidative reaction plant antioxidants would therefore potentially have beneficial activities in protecting the human body from such diseases (Havsteen et al., 2002; Mohamed et al., 2010).

The genus *Achillea* (Asteraceae) is represented by 44 species (50 taxa) in the flora of Turkey, and 21 (28 taxa) of them are endemics (Arabacı, 2006). Some *Achillea* species have ethnopharmacologic importance and are known to be used in folk remedies for various purposes (Baytop, 1997). Although there are a lot of studies reporting the composition of the essential oils of *Achillea* species, there is only one report referring to *A. teretifolia* (Unlu et al., 2002). Dried aerial parts of *A. teretifolia* were found to contain 0.4% (calculated per weight of dried plant material) of essential oil. 37 compounds, representing 83.53% of the essential oil of *A. teretifolia*, was identified by GC-MS. The major components of the oil were found to be piperitone (21.37%), linalool (18.99%), 1,8-cineole (6.79%), -terpineol (5.88%) and borneol (4.29%). The essential oil was found to be significantly rich with oxygenated monoterpenes (71.39%). Compared with this work, the 2 essential oils show different compositions. Furthermore, antimicrobial studies indicate that *E. coli*, *B. cereus* and *S. aureus* were inhibited by the oil of *A. aleppica* subsp. *aleppica* moderately with a MIC value of 62.5 g/ml. The oil showed a strong inhibitory effect against *C. albicans* with a MIC value (62.5 g/ml) that was equal to ketoconazole. *A. schischkinii* oil, on the other hand, was less active against the test micro-organisms except for *S. epidermidis*. The objectives of this study were to determine the essential oil composition of *Achillea aleppica* DC. subsp. *aleppica* and *A. schischkinii* Sosn. (an endemic species in Turkey) and their antimicrobial, antiinflammatory and antinociceptive activities. The results showed that *A. schischkinii* oil did not show any noticeable antiinflammatory activity (Iscan, 2006).

Furthermore, the antioxidant activity and radical scavenging capacity of *A. schischkinii* and *A. teretifolia* has not previously been published. In this study, *in vitro* antioxidant, radical scavenging and antimicrobial properties of the methanol, water and chloroform extracts of 2 *Achillea* species growing in the eastern part of Turkey were investigated.

MATER ALS AND METHODS

Chemicals

Ferrous chloride, -tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS), 3-(2-Pyridyl)-5,6-bis (4-phenyl- sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trichloracetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Plant material and extraction procedure

A. schischkinii and *A. teretifolia* were collected from Elazı region in the eastern Anatolia of Turkey when flowering (June 2009). The taxonomic identification of plant materials was determined using by Flora of Turkey (Davis, 1975). Fresh plant material was washed with tap water, air dried and then chopped into small fragments, which was shade-dried and reduced to a coarse powder in a mortar and pestle. The aerial parts of the plant samples (50 g) were separately extracted with 150 ml chloroform ($CHCl_3$) and methanol (MetOH) at room temperature for 3 times. The organic solvents were evaporated to dryness under vacuum at low temperature using a rotary evaporator. To obtain the water extracts, 50 g plant sample was kept in 250 ml boiling water for 10 min and subsequently filtered. Then resultant water extracts were lyophilized using freeze-dryer. The dried extracts were dissolved in methanol, chloroform and water to a final concentration of 100 mg/ml and used as such for the antimicrobial and antioxidant testing.

Microorganisms used

In vitro antimicrobial studies were carried out against 6 bacteria strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* COWAN 1, *Bacillus cereus* FMC 3, *Pseudomonas aeruginosa* DSM 50071, *Klebsillea pneumonia* FMC 5, *Enterobacter aerogenes* CCM 2531), two yeast (*Candida* sp. and *Saccharomyces cerevisiae*), which were obtained from microbiology laboratory in Fırat University-Turkey.

ABTS radical scavenging capacity

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging capacity was determined according to the method of Re et al. (1999). $ABTS^{\bullet+}$ was produced by reacting 2 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1 M sodium phosphate buffer (pH 7.4). Then, 1 ml of $ABTS^{\bullet+}$ solution was added to 3 ml of *A. schischkinii* and *A. teretifolia* extracts in ethanol at 100 g/ml concentrations. The absorbance was recorded 30 min after mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance.
The scavenging capability of test compounds was calculated using the following equation:

% Inhibition= $[(A_o - A_1)/A_o] \times 100$

where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of *A. schischkinii* and *A. teretifolia* extracts or standards.

DPPH radical scavenging capacity

The free radical scavenging capacity of *A. schischkinii* and *A. teretifolia* extracts was measured by 2,2- diphenyl-1- picryl-hydrazil ($DPPH^{\bullet}$) using the method of Shimada et al. (1992). Briefly, a 0.1 mM solut ion of $DPPH^{\bullet}$ in ethanol was prepared and 1 ml of this solution was added 3 ml of *A. schischkinii* and *A. teretifolia* extracts solution in water at different concentrations (50, 100 and 250 µg/ml) . 30 min later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.
The capability to scavenge the $DPPH^{\bullet}$ radical was calculated using the following equation:

$DPPH^{\bullet}$ Scavenging Effect %= $[(A_o - A_1)/A_o] \times 100$

where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of *A. schischkinii* and *A. teretifolia* extracts.

Superoxide anion scavenging capacity

Measurement of superoxide anion scavenging capacity of *A. schischkinii* and *A. teretifolia* extracts was based on the method described by Liu et al. (1997) with slight modification. 1 ml of nitroblue tetrazolium (NBT) solution (156 mmol/l NBT in 100 mmol/l phosphate buffer, pH 7.4), 1 ml NADH solution (468 mmol/l in 100 mmol/l phosphate buffer (pH 7.4)) and 100 µl of sample solution of *A. schischkinii* and *A. teretifolia* extracts in water were mixed. The reaction was started by the addition 100 µl of phenazine methosulphate (PMS) solution (60 mmol/l PMS in 100 mmol/l phosphate buffer, pH 7.4) . The reaction mixture was incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging capacity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

%Inhibition= $[(A_o - A_1)/A_o] \times 100$

where A_o is the absorbance of the control and A_1 is the absorbance of *A. schischkinii* and *A. teretifolia* extracts or standards (Ye et al., 2000).

Hydrogen peroxide scavenging capacity

The ability of the *A. schischkinii* and *A. teretifolia* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically using the extinction coefficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Extract dilutions (50, 100 and 250 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *A. schischkinii* and *A. teretifolia* extracts and standard compounds was calculated:

% Scavanged $[H_2O_2]$= $[(A_o - A_1)/A_o] \times 100$

where A_o is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of *A. schischkinii* and *A. teretifolia* extracts or standards.

Metal chelating activity

The chelating of ferrous ions by the *A. schischkinii* and *A. teretifolia* extracts and standards was estimated by the method of Dinis et al. (1994) . Briefly, extract dilutions (50, 100 and 250 µg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were run in triplicate and the average is reported here. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula given bellow:

%Inhibition= $[(A_o - A_1)/A_o] \times 100$

where A_o is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of *A. schischkinii* and *A. teretifolia* extracts or standards. The control does not contain $FeCl_2$ and ferrozine, complex formation molecules.

Antimicrobial activity

Antimicrobial tests were carried out by disc diffusion method using 100 l of suspension containing 10^6 per/ml of bacteria and 10^4 per/ml yeast inoculated into Mueller Hinton agar (Difco) and Malt extract agar (Difco) respectively. The discs (6 mm diameter) were impregnated with 50 l of extract and placed into the inoculated agar. Petri dishes were placed at 4°C for 2 h. The inoculated plates were then incubated at 37 ± 0.1°C at 24 h for the bacterial strains

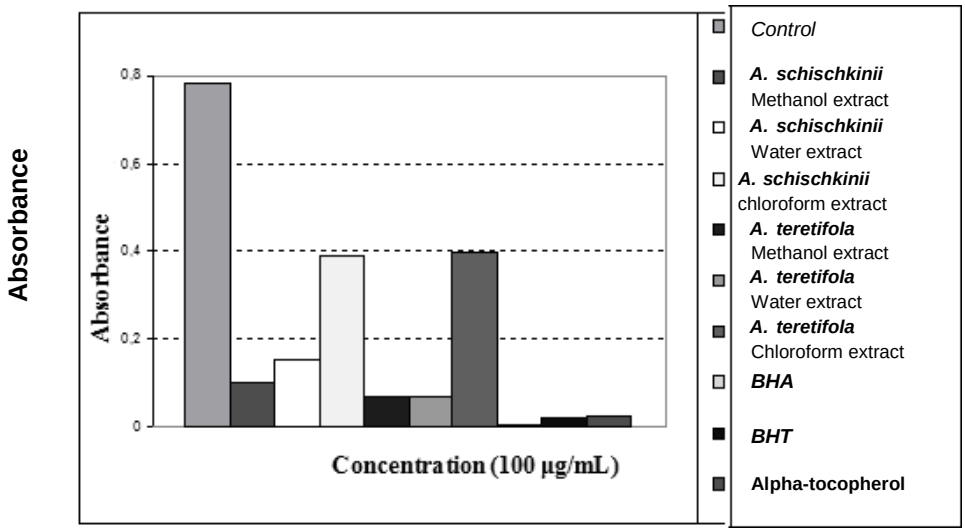

Figure 1. ABTS$^{\bullet+}$ radical-scavenging capacity of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia*, BHA, BHT and -tocopherol.

and at 25 ± 0.1°C at 72 h for yeast. Antimicrobial activity was determined by measuring the zone of inhibition against the test organisms.

RESULTS AND D SCUSS ON

ABTS radical-scavenging capacity

All the tested compounds exhibited effective radical cation scavenging activity. The scavenging effect of *A. schischkinii* and *A. teretifolia* (100 µg/ml concentrations) and standards on ABTS$^{\bullet+}$ decreased in the order (Figure 1): BHA> BHT> -tocopherol> *A. teretifolia* methanol extract > *A. teretifolia* water extract > *A. schischkinii* methanol extract > *A. schischkinii* water extract > *A. schischkinii* chloroform extract > *A. teretifolia* chloroform extract (99.8, 97.3, 96.9, 91.5, 91.2, 87.3, 80.9, 50 and 49%, colour reduction respectively) at the concentration of 100 g/ml (Table 1).

DPPH radical scavenging capacity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidative activity of antioxidants (Duh et al., 1999). Figure 2 illustrates decrease in the concentration of DPPH radical due to the scavenging ability of the extracts of *A. schischkinii* and *A. teretifolia*. -tocopherol was used as a standard. The scavenging effect of methanol, water and chloroform extracts of *A. Schischkinii* and

Table 1. (%) ABTS$^{\bullet}$ radical-scavenging capacity of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* and of BHA, BHT and -tocopherol.

Extracts (100 µg/ml)	ABTS assay (%)
A. schischkinii methanol extract	87.3
A. schischkinii water extract	80.9
A. schischkinii chloroform extract	50
A. teretifolia methanol extract	91.5
A. teretifolia water extract	91.2
A. teretifolia chloroform extract	49
BHA	99.8
BHT	97.3
-tocopherol	96.9

A. teretifolia and standards on the DPPH radical decreased in that order: -tocopherol> *A. teretifolia* methanol extract > *A. teretifolia* water extract> *A. schischkinii* water extract> *A. schischkinii* methanol extract> *A. teretifolia* chloroform extract> *A. schischkinii* chloroform extract. 100 µg of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* (Figure 3) exhibited 68, 69.2, 59.2, 75.9, 69.6 and 60.1% DPPH scavenging capacity, respectively. In contrast, at the same dose, -tocopherol exhibited 95% DPPH scavenging capacity. These results indicate that *A. schischkinii* and *A. teretifolia* extracts have a noticeable effect on scavenging free radical.

Superoxide anion scavenging capacity

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling

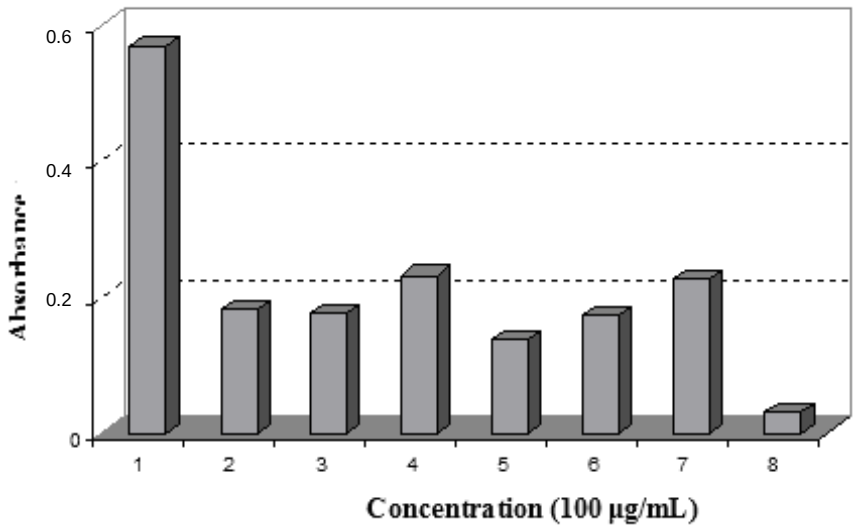

Figure 2. DPPH$^{\bullet}$ radical scavenging capacity of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* (100 µg), -tocopherol. (1. Control 2. Methanol extract of *A. schischkinii* 3. Water extract of *A. schischkinii* 4. Chloroform extract of *A. schischkinii* 5. Methanol extract of *A. teretifolia* 6. Water extract of *A. teretifolia* 7. Chloroform extract of *A. teretifolia* 8. -tocopherol.)

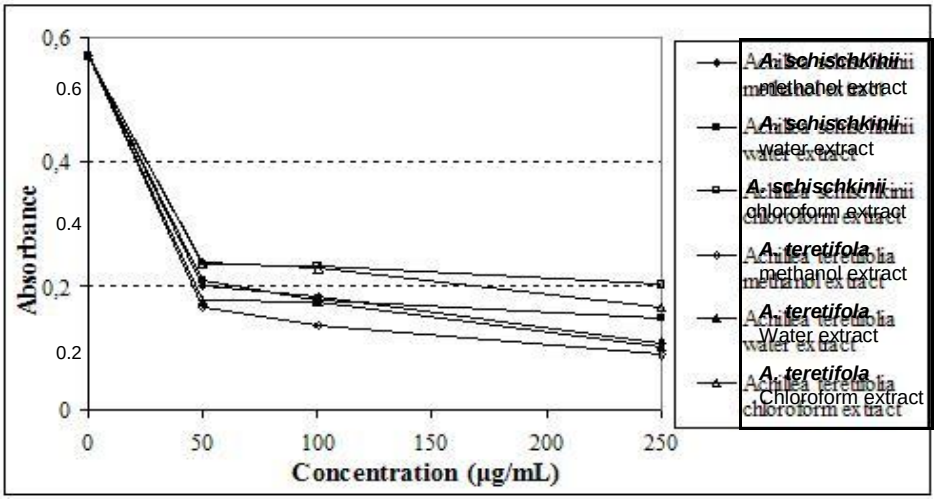

Figure 3. DPPH$^{\bullet}$ radical scavenging capacity of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia*.

reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Figure 4). Table 2 shows the percentage inhibition of superoxide radical generation of 100 µg/ml of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* and comparison with same doses of BHA, BHT and -tocopherol. The extracts of *A. schischkinii* and *A. teretifolia* have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than -tocopherol. The percentage inhibition of superoxide generation by 100 g doses of BHA, BHT, - tocopherol, methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* was found as 94.1, 80.4, 54.5, 77.1, 93.2, 45, 81.8, 93.6 and 57.7% and greater than that same doses of, respectively. Superoxide radical scavenging activity of those samples followed the order: BHA> *A. teretifolia* water extract> *A. schischkinii* water extract> BHT> *A. teretifolia* methanol extract> *A. schischkinii* methanol extract> -tocopherol> *A. teretifolia* chloroform extract > *A. schischkinii* chloroform extract.

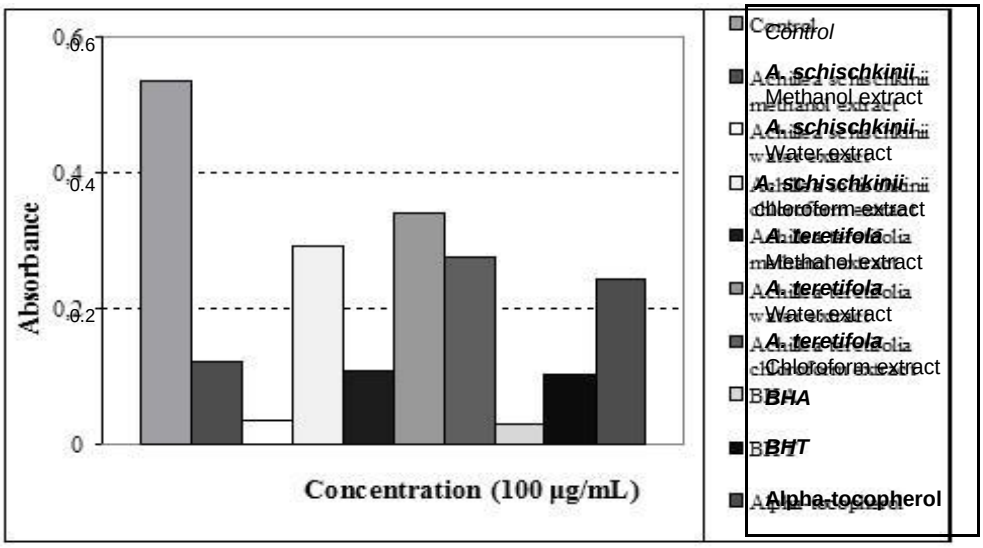

Figure 4. Superoxide anion radical scavenging capacity of extracts of *A. schischkinii* and *A. teretifolia*, BHA, BHT and -tocopherol by the PMS–NADH–NBT method.

Table 2. (%) Superoxide anion scavenging activity of extracts of *A. schischkinii* and *A. teretifolia*, BHA, BHT and -tocopherol.

Extracts	%Superoxide anion scavenging activity (100 µg)
A. schischkinii methanol extract	77.1
A. schischkinii water extract	93.2
A. schischkinii chloroform extract	45.0
A. teretifolia methanol extract	79.9
A. teretifolia water extract	93.6
A. teretifolia chloroform extract	48.0
BHA	94.1
BHT	80.4
-tocopherol	54.5

Hydrogen peroxide scavenging activity

The ability of extracts of *A. schischkinii* and *A. teretifolia* to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). The scavenging ability of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* on hydrogen peroxide is shown Figure 5 and compared with BHA, BHT and -tocopherol as standards. The extracts of *A. schischkinii* and *A. teretifolia* were capable of scavenging hydrogen peroxide in an concentration dependent manner. The extracts of *A. schischkinii* and *A. teretifolia* had stronger hydrogen peroxide scavenging activity (Figure 5) . Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because of it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Thus, removing H_2O_2 is very important for antioxidant defence in cell or food systems.

Metal chelating activity

The chelating of ferrous ions by the extracts of *A. schischkinii* and *A. teretifolia* was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator (Yamaguchi et al., 2000). The *A. schischkinii* and *A. teretifolia* extracst and standard compounds interfer with the formation of ferrous and ferrozine complex (Figure 6), suggesting that they have chelating activity and are able to capture ferrous ions before ferrozine does.

As shown in Figure 6, the formation of the Fe^{2+} ferrozine complex is incomplete in the presence of methanol, water and chloroform extracts of *A. schischkinii*

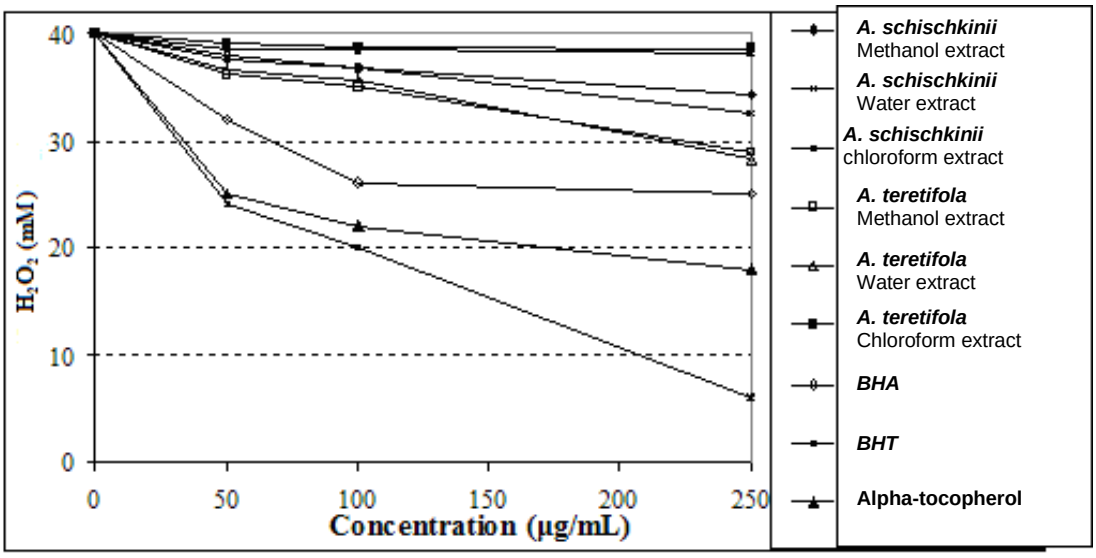

Figure 5. Hydrogen peroxide scavenging capacity of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia*, BHA, BHT and -tocopherol.

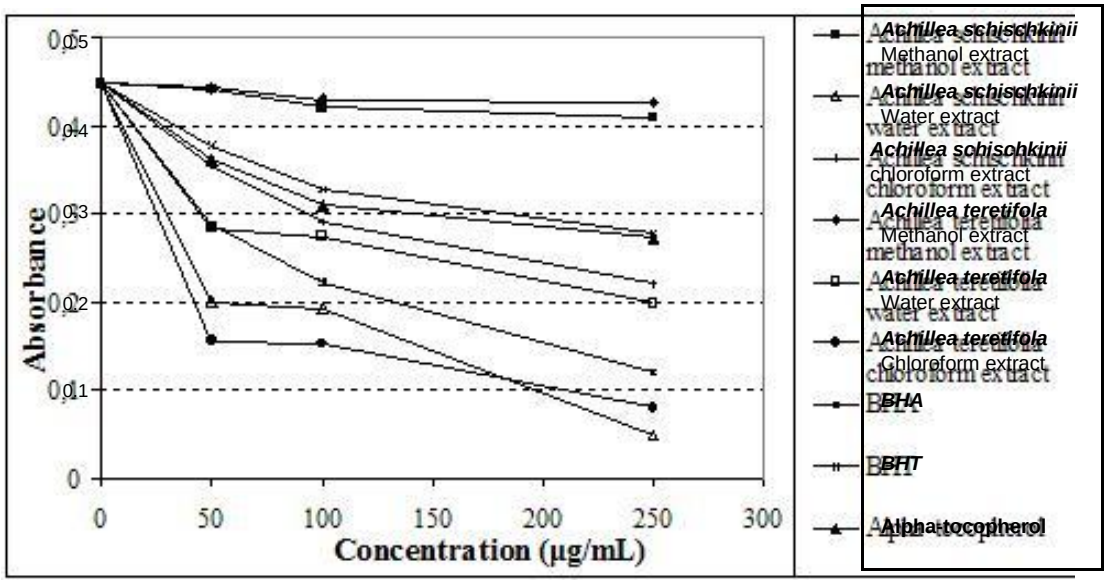

Figure 6. Metal chelating capacity of different amount of methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia*, BHA, BHT and -tocopherol.

and *A. teretifolia*, indicating that extracts of *A. schischkinii* and *A. teretifolia* chelate with the iron. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased dose dependently (from 50 to 250 µg/ml) . The percentages of metal scavenging capacity of 250 µg concentration of methanol, water and chloroform extracts of *A. schischkinii* and methanol, water and chloroform extracts of *A. teretifolia*, BHA, BHT and -tocopherol were found as, 9, 89, 50.8, 4, 55.5, 82.3, 72.9, 37.9 and 39.2%, respectively.

Metal chelating capacity was significant, since it reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh et al., 1999). It was reported that chelating agents, who form s-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). The data displayed in Figure 6 reveals that *A. schischkinii* and *A. teretifolia* extracts have a marked capacity for iron binding. This suggests that their action as peroxidation

Table 3. Result of the screening of three plant extracts by means of the agar disc diffusion method. (Bacteria tested inhibiton zone diameter (mm).

Microorganisms	*A.teretifolia*	*A.schischkinii*	Ampicillin* Nystatin**	Control
E.coli	12	-	12*	-
S.aureus	13	-	17*	-
B.cereus	15	-	17*	-
P.aeruginosa	16	-	11*	-
K.pneumonia	13	-	15*	-
E.aerogenes	11	-	11*	-
S.cerevisiae	-	-	20**	-
Candida sp.	14	-	18**	-

Control: (methanol); Ampicillin 20 µg; Nystatin 100 µg

protector may be related to its iron binding capacity.

Antimicrobial activity

The antimicrobial effect of *A. schischkinii* and *A. teretifolia* extracts obtained by extraction techniques were tested against 2 gram-positive bacterial species (*B. cereus*, *S. aureus*), four gram-negative bacterial species (*E. coli*, *E. aerogenes, P. aeruginosa, K. pneumonia*) and 2 yeast species (*S. cerevisiae*, *Candida* sp.). Methanol had no inhibitory effect on any of the test microorganisms in the control treatment. The results of these tests, as well as the effects of 2 control antibiotics, are presented in Table 3.

The extracts of *A. teretifolia* extracts showed all of the tested microorganisms from high to low respectivelly, having an inhibition zone of 12 - 16 mm; *B. cereus* 15 mm, *S. aureus* 13 mm, *E. coli* 12 mm, *E. aerogenes* 11 mm, *K. pneumonia* 13 mm, *P. aeruginosa* 16 mm. When the results were compared to those of standard antibiotic (ampicilin), it was determined that they have strong effect against *P. aeruginosa.* The extract obtained from *A. teretifolia* showed antimicrobial activity against *S. cerevisae* yeast.

The extracts of *A. schischkinii* showed no antimicrobial activity against gram positive and gram negative bacteria. In addition, the extract of *A. schischkinii* no inhibitory activity against *S. cerevisiae* and *Candida* sp. yeast.

In conclusion, the methanol, water and chloroform extracts of *A. schischkinii* and *A. teretifolia* showed strong antioxidant activity as seen by their ABTS radical, DPPH radical, superoxide anion scavenging, hydrogen peroxide scavenging and metal chelating activities when compared to standards such as BHA, BHT and -tocopherol. The results of this study show that the methanol and water extracts of *A. teretifolia* can be used as a easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry due to their strong antimicrobial and antioxidant activities.

In addition, the data of the present study indicate that the extracts of *A. schischkinii* contains antioxidants but not antimicrobial compounds. These could possibly be used in stabilising food against oxidative deterioration. The individual components responsible for their antimicrobial and antioxidant activities have not been identified in this study and will be the aim of further studies within this laboratory. Further investigations on the isolation and identification of antioxidant components in the plants may lead to chemical entities with potential for clinical use.

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