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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF RHIZOME EXTRACTS FROM MALAYSIAN SPECIES OF *ALPINIA GALANGA* AND *ALPINIA OFFICINARUM*

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Abstract

Ethanolic extract from dried rhizomes of Malaysian species of *Alpinia galanga* (AGEE) and water extracts from fresh and dried rhizomes of Malaysian species of *A. galanga* (AGWF and AGWD) were evaluated for their inhibitory effect against eight bacterial isolates *viz. Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Micrococcus luteus*, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Pseudomonas merobilis* and two fungal isolates *viz*. *Aspergillus niger* and *Candida albicans*. They showed disparate activities ranging from nil, week (< 12 mm), moderate (12 to ≤ 20 mm) and strong (≥ 20 mm) inhibitory effects. Ethanolic and water extracts from dried rhizomes of Malaysian species of *Alpinia officinarum* (AOEE and AOWE) were evaluated for their antioxidant activity by DPPH radical scavenging assay. Both AOEE and AOWE showed promising radical scavenging activity with IC_{50} values of ~ 0.18 and ~ 0.13 mg/mL, respectively. The positive control, butylhydroxytoluene (BHT) showed an IC_{50} value of ~ 0.13 mg/mL.

Keywords: *Alpinia galanga*, *Alpinia officinarum*, ethanolic *extract, water extract, BHT, radical scavenging assay, antioxidant, antibacterial, antifungal*.

Introduction

The by-products of normal metabolism are reactive oxygen species (ROS) such as hydroxyl, alkoxyl, peroxyl etc. free radicals [1] and they have been caused oxidative cleavage in lipids, DNA, proteins etc. [2]. This oxidation process is responsible for human degenerative diseases such as cancer, cataracts, cardiovascular diseases, brain dysfunction etc. Radical scavengers such as glutathione, glutathione peroxidase, tocopherols, transferrin etc. are employed to prevent this oxidation process [2, 3]. Fortunately, our dietaries such as fruits, vegetables and beverages derived from plants are rich sources of phenolics and flavonoids which are powerful antioxidants too [4-7]. On the other hand, the dermatophytic fungal infection has been created more public health concerns in the past decades [8]. The currently available antifungal agents have high level of resistance and the effective treatment is usually costly and time consuming [9, 10]. Furthermore, the antifungal agents such as chlorhexidine and imidazole derivatives have only limited applications [11]. Similarly, some drug-resistant bacteria such as multidrug-resistant *Shigella sonnei* (Shigellosis), multiantibiotic-resistant *Salmonella typhimurium* DT 104, methicilin-resistant *Staphylococcus aureus* (MRSA) etc. have been caused an outbreak of community-acquired foodborne illness [12, 13].

Alpinia galanga belongs to the Zingiberaceae family. Known by other names such as greater galangal, Lengkuas and Arattai, *A. galanga* is one of the most widely used herbs from ginger family [14-16] and has been used in culinary in Asian continent [17], particularly in south and southeast Asia [14, 18, 19]. *A. galanga* has therapeutic applications in traditional medicine [14] and has been employed to get rid of bad breath, throat infections and skin infections and as a digestive stimulant, bronchial catarrh, rheumatism etc. [17, 20]. Several bioactive pure compounds have been reported from *A. galanga* [21-25].

Alpinia officinarum also belongs to the Zingiberaceae family. *A. officinarum* is known by other names such as lesser galangal, Lengkuas and Chittarattai. *A. officinarum* has therapeutic applications in traditional medicine in India, Vietnam, Thailand and other south and southeast Asian countries. *A. officinarum* has been employed to get rid of arthritis, ring worm, dyspepsia, loss of appetite, infectious acne, venereal diseases, gastrointestinal disorders etc. [26-28]. *A. officinarum* has been listed as "generally regarded as safe" (21 CFR Section182.10, 182.20) by the Unites States Food and Drug Administration (FDA) [28]. Several biological activities have previously been reported from *A. officinarum* [28-32]. Both *A. galanga* and *A. officinarum* have similar appearance and the vernacular name, Lengkuas is applicable to both species. However, they can easily be distinguished based on their sizes; the former one appears bigger than the latter one. In addition, the skin and flesh of *A. galanga* is yellowish white while the skin and flesh of *A. officinarum* is reddish brown.

Antimicrobial and antioxidant activities of *A. galanga* and *A. officinarum* collected in various locations have previously been reported [16, 17, 29-31, 33-35]. However, our search showed that ethanolic and water extracts from these two plants are not documented well for their bioactivities. In our continuing research in molecular medicine, especially the interaction of CYP proteins with herbal extracts, we obtained ethanolic and water extracts from rhizomes of Malaysian species of *A. galanga* and *A. officinarum*. We envisioned that these extracts could also be subjected for their antimicrobial and DPPH radical scavenging activities. Therefore, we evaluated the inhibitory effect of ethanolic and water extracts from rhizomes of *A. galanga* on eight bacterial isolates *viz*. *Escherichia coli*, *Salmonella typhi*, *Bacillus thuringiensis*, *Pseudomonas aeroginosa*, *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas merobilis* and two fungal isolates *viz*. *Aspergillus niger* and *Candida albicans* and DPPH radical scavenging activity of ethanolic and water extracts from rhizomes of *A. officinarum*. The results obtained are communicated in this article.

Methods

Plant materials

10 kg of fresh rhizomes of *A. galanga* and 6 kg of dried rhizomes of *A. officinarum* were purchased from a local market in Penang, Malaysia in 2010 and voucher specimens, PillaiMK/MY-LengkuasG/06/2010

for *A. galanga* and PillaiMK/MY-LengkuasL/11/2010 for *A. officinarum* were deposited separately at School of Pharmaceutical Sciences, Univeristi Sains Malaysia, Penang, Malaysia and Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Penang, Malaysia.

Processing of plant materials

The fresh rhizomes of *A. galanga* were cut into small pieces by a chopper and 2 kg of this cut material was used to get water extract. The rest of the material was oven dried at 40-50°C for three weeks and then powdered using a grinding miller. The rhizomes of *A. officinarum* were powdered directly using a grinding miller since they were already purchased in dry condition.

Preparation of water extract from fresh materials of A. galanga

2 kg of fresh and cut rhizomes of *A. galanga* was taken in a glass jar and three litres of deionized water was added and digested on a water bath at 70-80°C for one week. The resulting solution was filtered using Whatman No.1 filter paper and kept aside. This process was repeated once again and the combined extracts were concentrated by two steps. Buchi rotavapour was used to remove as much as water possible and then Freeze dryer/Lyophiliser was used to remove the remaining water. A dark brown dry residue was obtained and stored in a refrigerator.

Preparation of water extract from dried materials of A. galanga and A. officinarum

About 500 g of the powdered *A. galanga* was taken in a glass jar and a litre of deionized water was added and digested on a water bath at 70-80°C for one week. The rest of the procedure was same as described in the previous paragraph. A dark brown dry residue was obtained and stored in a refrigerator. The same procedure was followed to obtain water extract from powdered *A. officinarum*. A dark brown dry residue was obtained and stored in a refrigerator.

Preparation of ethanol extracts from A. galanga and A. officinarum

The remaining powdered *A. galanga* was exhaustively extracted with ethanol using a Soxhlet's apparatus. The extract was filtered using Whatman No.1 filter paper and ethanol was completely removed using Buchi rotavapour. The same procedure was followed to obtain ethanolic extract from *A. officinarum*. A dark brown precipitate of ethanolic crude extract was obtained on both cases and kept in a cup-board at room temperature for further use.

Evaluation of antioxidant activity

DPPH radical scavenging assay and determination of IC_{50} values

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of ethanolic and water extracts of of *A. officinarum* was conducted according to the methods described in the literature [36]. Briefly, 2.0 mg of extract/mL of water was prepared and further dilutions *viz*. 1.0, 0.5, 0.125, 0.06 and 0.03 mg/mL were made. 50 µL of each one of them was separately mixed with 0.004 w/v % of DPPH solution in methanol (80% v/v). The mixture without extract sample was used as blank and just spiked with 50 µL of methanol. The commercial antioxidant butylhydroxytoluene (BHT, Sigma Aldrich) of the same concentration and further dilutions were prepared which served as positive controls. The mixture was incubated for 30 minutes and then its optical density was measured at 517 nm. The IC_{50} values were calculated from graph by plotting extract concentration (in abscissa) versus percentage inhibition of DPPH radical (in ordinate). The extract concentration that causes 50% reduction in DPPH initial concentration is defined as the IC_{50} value of extract which is important measure of potency for a given extract. Each experiment was carried out in triplicate and the averages of the three values were used to calculate IC_{50} values. Again, the plot of percentage radical scavenging activity against extract concentration was used to compare the radical scavenging activity at each concentration and standard deviation was calculated for each concentration from the three values of the experiment. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$

A_0 = optical density of DPPH radical + methanol; A_1 = optical density of radical + extract or BHT

Evaluation of antimicrobial activity

Disc diffusion assay

Antibacterial and antifungal activities of ethanolic and water extracts of *A. galanga* were evaluated by disc diffusion method as described in literature [37, 38]. Briefly, the MHA plates containing an inoculum size of 106 colony-forming units (CFU)/mL of bacteria or 2 x 10^5 CFU/mL yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L- shaped glass rod. Then discs impregnated with 25 µL of each extract at a concentration of 100 mg/mL was placed on the inoculated plates. Similarly, each plate carried a blank disc by adding solvent alone in the centre which served as negative control. Chloramphenicol (disc: 6.00 mm. dia.) and miconazole nitrate (disc: 6.00 mm. dia.) served as positive controls for bacteria and fungi, respectively. All the plates were incubated at 37°C for 18 to 24 hr. for bacteria and at 28°C for 48 to 96 hr. for fungi. The sensitivity of microorganisms to the ethanolic and water extracts of rhizomes of *A. galanga* was determined by measuring the sizes of inhibition zones on the agar surface around the discs. All experiments were performed in triplicate and the results are reported as the average of three experiments.

Statistical Analysis

Results were expressed as means of three determinations for antioxidant study. One way analysis of variance (ANOVA) was used to compare means at the significance level $p < 0.05$. All the analysis were performed by Microsoft Excel software.

Results

Antioxidant activity of A. officinarum

Ethanolic and water extracts from dried rhizome powder of *A. officinarum* were screened for their DPPH radical scavenging activity. Ethanolic extract from *A. officinarum* (AOEE) showed 22.59 ±5.69, 35.91±2.42, 44.44±1.31, 49.93±2.62, 59.59±0.26, 67.97±1.48 and 85.97±3.25% of scavenging activity at concentrations 0.03, 0.06, 0.125, 0.25, 0.50, 1.00 and 2.00 mg/mL, respectively. The positive control, butylhydroxytoluene (BHT) showed 38.04±3.40, 43.43±0.66, 52.17±3.94, 67.00±2.32, 71.42±1.09, 86.25±2.78 and 92.98±0.24% of scavenging activity at concentrations 0.03, 0.06, 0.125, 0.25, 0.50, 1.00 and 2.00 mg/mL, respectively. This result indicated that AOEE showed lower scavenging activity at all concentrations relative to positive control (BHT). Water extract from *A. officinarum* (AOWE) showed 41.58±4.39, 46.27±2.09, 51.04±3.76, 56.49±2.83, 62.71±2.64, 74.72±4.83 and 87.72±3.43% of scavenging activity at concentrations 0.03, 0.06, 0.125, 0.25, 0.50, 1.00 and 2.00 mg/mL, respectively. This result indicated that at low concentrations such as 0.03 and 0.06 mg/mL, AOWE showed slightly higher scavenging activity of 41.58±4.39 and 46.27±2.09%, respectively, and the positive control (BHT) showed scavenging activity of 38.04±3.40 and 43.43±0.66%, respectively. At a concentration of 0.125 mg/mL, both AOWE and BHT showed comparable scavenging activity of 51.04±3.76 and 52.17±3.94%, respectively. However, at higher concentrations such as 0.25, 0.50, 1.00 and 2.00 mg/mL, AOWE showed significantly lower scavenging activity of 56.49(±2.83), 62.71(±2.64), 74.72(±4.83) and 87.72(±0.24)%, respectively, and the positive control showed 67.00±2.32, 71.42±1.09, 86.25±2.78 and 92.98(±0.24)% of scavenging activity at the same concentrations (refer to Table 1). Figure 1 showed the comparison of percentage radical scavenging activities of AOEE, AOWE and the positive positive control (BHT) at various concentrations. The IC_{50} values AOEE, AOWE and BHT were also calculated by plotting percentage inhibition of DPPH radical against various concentrations and the values are listed in Table 2. The IC_{50} value is reciprocally related with scavenging activity; i.e. lower the IC_{50} value means higher its scavenging activity and vice versa. AOEE and AOWE showed IC_{50} values of ~ 0.18 and ~ 0.13 mg/mL, respectively. BHT showed an IC_{50} values of ~ 0.13 mg/mL. This result indicated that both AOWE and BHT have the same IC_{50} value of ~ 0.13 mg/mL. In other words, AOWE has same scavenging activity as that of BHT. AOEE showed slightly lower scavenging activity (~ 0.18 mg/mL) than BHT. It has also been noticed that although the percentage of radical scavenging

activity of positive control is slightly higher than AOWE at higher concentrations (refer to Table 1 and Figure 1), the IC_{50} value became the same for both AOWE and BHT.

Antimicrobial activity of A. galanga

Ethanolic extract from dried rhizome powder of *A. galanga* (AGEE), water extract from fresh rhizomes of *A. galanga* (AGWF) and water extract from dried rhizome powder of *A. galanga* (AGWD) were evaluated for their antibacterial activity against eight bacterial isolates viz. *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Pseudomonas merobilis* and two fungal isolates viz. *Aspergillus niger* and *Candida albicans*. The results are summarized in Table 3. AGEE showed inhibition zones of 22, 26 and 15 mm against *S. typhi*, *S. aureus* and *P. merobilis*, respectively. The positive control chloramphenicol showed inhibition zones of 27, 29 and 30 mm, respectively against the same bacteria. This result indicated that AGEE showed lower antibacterial activity against these three bacterial isolates relative to positive control. This result also indicated that AGEE did not show any visible inhibition zones against other five bacterial isolates.

AGWF showed an inhibition zone of 12 mm against *E. coli* while positive control showed an inhibition zone of 22 mm. against the same bacteria. This result indicated that AGWF showed lower antibacterial activity relative to positive control against *E. coli*. AGWF did not show any visible inhibition zones against other seven bacterial isolates. On the other hand, AGWD showed inhibitions zones of 15, 16, 17, 17 and 18 mm against *E. coli*, *M. luteus*, *P. aeruginosa*, *S. aureus* and *P. merobilis*, respectively. The positive control chloramphenicol showed inhibition zones of 22, 29, 28, 29 and 30 mm, respectively, against the same bacteria. This result indicated that AGWD also showed lower antibacterial activity relative to positive control against these five bacterial isolates and did not show any visible inhibition zones against other three bacterial isolates (refer to Table 3).

AGEE showed an inhibition zone of 25 mm against *A. niger* and showed no visible inhibition zone against *C. albicans*. The positive control miconazole nitrate showed inhibition zones of 29 and 27 mm against *A. niger* and *C. albicans* respectively. This results indicated that AGEE showed comparable activity as that of positive control miconazole nitrate against *A. niger*. AGWF showed an inhibition zone of 17 mm against *C. albicans* but did not show any visible inhibition zone against *A. niger*. This result indicated that AGWF showed lower antifungal activity against *C. albicans* relative to positive control miconazole nitrate (refer to Table 3). On the other hand, AGWD showed inhibition zones of 11 and 20 mm against *A. niger* and *C. albicans*, respectively. This result indicated that AGWD also showed lower antifungal activity against *A. niger* and *C. albicans* relative to positive control miconazole nitrate (refer to Table 3).

Discussion

The hot and cold aqueous ethanolic extracts from rhizomes of *A. officinarum* collected in India showed IC_{50} values 95.41 and 123.43 µg/mL, respectively [30] and the cold methanolic extract showed an IC_{50} value of 137.33 µg/mL [30]. Methanolic crude extract from rhizomes of *A. officinarum* collected in China and some fractions from this crude extract showed IC_{50} values ranging from 20.98 - 41.88 µg/mL [39]. Some phenylpropanoids isolated from methanolic extract of fresh rhizomes of *A. officinarum* exhibited antioxidative activities against autooxidation of methyl linoleate [41].

Ethanolic extract from flower of *A. galanga* collected in Gainsville, FL, USA showed an inhibition zone of 25 mm. against *S. aureus* but was inactive against *S. typhi* and *E. coli* [33]. Our study showed that AGEE has an inhibition zone of 26 mm against *S. aureus* and this value is in agreement with the reported value [33]. AGEE from our study did not show any invisible inhibition zone against in *E. coli* [33] but showed an inhibition zone of 22 mm. against *S. typhi*. The ethanolic extract of rhizomes of *A. galanga* from Nakhon Ratchasima, Thailand showed an inhibition zone of 22 mm. against *S. aureus* and did not show any visible inhibition zone against *E. coli* and *P. aeruginosa* [29]. Our study indicated that AGEE showed higher inhibition zone of 26 mm. against *S. aureus*. Water extract from rhizomes of *A. galanga* purchased from Selangor, Malaysia showed an inhibition zone of 8.4 and 14.3 mm, respectively, against *E. coli* and *S. aureus* [16]. However, AGWD from our study showed higher inhibition zones of 15

and 17 mm. respectively against the same bacterial isolates (refer to Table 3). Additionally, AGWF from our study also showed an inhibition zone of 12 mm. against *E. coli* but showed no visible inhibition zone against *S. aureus*. The water extract from dried rhizome powder of *A. galanga* from Selangor, Malaysia showed an inhibition zones of 8.7 mm. against *C. albicans* [16]. However, AGWD from our study showed significantly higher inhibition zone of 20 mm. against the same fungus. In addition, AGWF from our study also showed an inhibition zone of 17mm. against *C. albicans*. Petroleum ether, chloroform, methanol, acetone and ether extracts from various parts of *A. galanga* collected at various locations have previously been evaluated against various bacterial and fungal isolates. [17, 34].

Ethanolic and water extracts from Malaysian species of *A. officinarum* were evaluated for radical scavenging activity. They showed promising radical scavenging activity and have comparable IC_{50} values as that of positive control, BHT. Natural antioxidants are receiving increasing attention and very much sought after in industry owing to their safety. Since, *A. officinarum* has promising scavenging activity and has been listed as "generally regarded as safe" by FDA, it may replace the unsafe and undesirable synthetic antioxidants currently used in industry. Ethanolic and water extracts from Malaysian species of *A. galanga* were evaluated for their antimicrobial activities against eight bacterial and two fungal isolates. They showed disparate activities ranging from nil, week (< 12 mm), moderate (12 to ≤ 20 mm) and strong (≥ 20 mm) inhibitory effects. Since, both *A. officinarum* and *A. galanga* have widely been used in culinary in Asian continent, further research is required.

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Table 1. The percentage radical scavenging activity of ethanolic and water extracts of *A. officinarum* (AOEE and AOWE) and positive control (BHT) at various concentrations.

	Concentrations (mg/mL)						
Extracts	0.03	0.06	0.125	0.25	0.50	1.00	2.00
AOEE	22.59 (±5.69)	35.91 (±2.42)	44.44 (±1.31)	49.93 (±2.62)	59.59 (±0.26)	67.97 (±1.48)	85.97 (±3.25)
AOWE	41.58 (±4.39)	46.27 (±2.09)	51.04 (±3.76)	56.49 (±2.83)	62.71 (±2.64)	74.72 (±4.83)	87.72 (±3.43)
BHT	38.04 (±3.40)	43.43 (±0.66)	52.17 (±3.94)	67.00 (±2.32)	71.42 (±1.09)	86.25 (±2.78)	92.98 (±0.24)

AOEE = Ethanolic extract from dried rhizome powder of *A. officinarum*; AOWE = Water extract from dried rhizome powder of *A. officinarum*; BHT = Butylhydroxytoluene (positive control). All experiments were conducted in triplicate (n=3) and reported as mean of the three values along with standard deviation, ±SD.

Table 2. The IC_{50} values of ethanolic and water extracts from *A. officinarum* (AOEE and AOWE) and positive control (BHT).

S. No.	Extracts	IC_{50} values (mg/mL)
1	AOEE	~ 0.18
2	AOWE	~ 0.13
3	BHT	~ 0.13

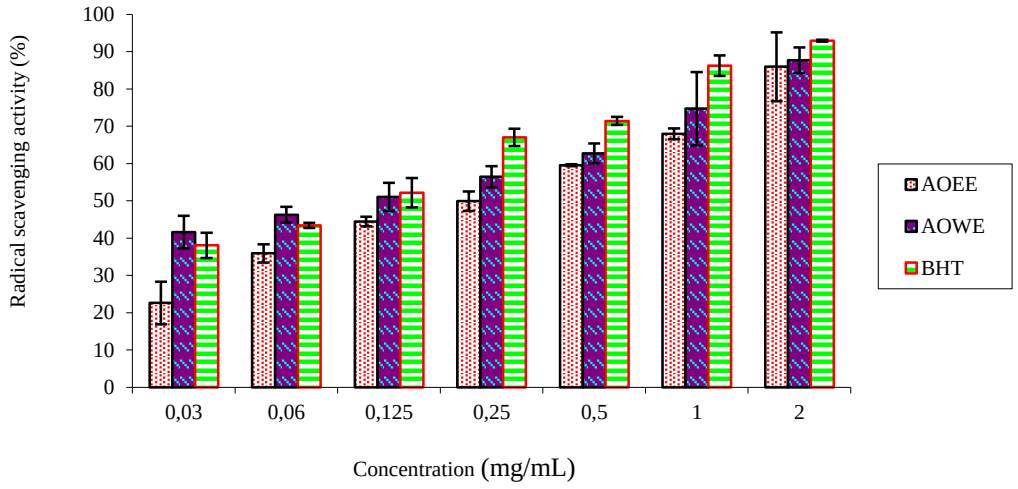
AOEE = Ethanolic extract obtained dried rhizome powder of *A. officinarum*; AOWE = Water extract obtained dried rhizome powder of *A. officinarum*; BHT = Butylhydroxytoluene (positive control).

Table 3. Inhibitory effect of ethanolic and water extracts of *A. galanga* (AGEE, AGWF and AGWD) on selected bacterial and fungal isolates.

S. No	Microorganisms (bacteria/fungi)	Zone of inhibition (mm)			Positive controls
		AGEE	AGWF	AGWD	
1	*E. coli*	-	12	15	22
2	*S. typhi*	22	-	-	27
3	*B. subtilis*	-	-	-	31
4	*B. thuringiensis*	-	-	-	27
5	*M. luteus*	-	-	16	29
6	*P. aeruginosa*	-	-	17	28
7	*S. aureus*	26	-	17	29
8	*P. merobilis*	15	-	18	30
9	*A. niger*	25	-	11	29
10	*C. albicans*	-	17	20	27

AGEE = Ethanolic extract from dried rhizome powder of *A. galanga*; AGWF = Water extract from fresh rhizomes of *A. galanga*; AGWD = Water extract from dried rhizome powder of *A. galanga*; Dashes (-) indicate no inhibition and the extract is devoid of antibacterial or antifungal activity; *E. coli* = *Escherichia coli; S. typhi* = *Salmonella typhi; B. subtilis* = *Bacillus subtilis; B.* thuringiensis = Bacillus *thuringiensis; M.* luteus = Micrococcus *luteus; P.* aeruginosa = Pseudomonas *aeruginosa; S.* aureus = Staphylococcus *aureus; P.* merobilis = Pseudomonas *merobilis; A.* niger = Aspergillus *niger; C.* albicans = Candida *albicans;* S. No. 1-8 are bacterial isolates; S. No. 9 and 10 are fungal isolates. Chloramphenicol served as positive control for bacteria and miconazole nitrate served as positive control for fungi. Inhibition zones of < 12 mm, 12 to ≤ 20 and ≥ 20 are, respectively, week, moderate and strong inhibitory effects [40].

Figure 1. The percentage radical scavenging activity of ethanolic and water extracts of *A. officinarum* (AOEE and AOWE) and positive control (BHT) at various concentrations.



AOEE = Ethanolic extract from dried rhizome powder of *A. officinarum*; AOWE = Water extract from dried rhizome powder of *A. officinarum*; BHT = Butylhydroxytoluene (positive control). All experiments were conducted in triplicate (n=3) and reported as mean of the three values together with standard deviation, ±SD.