Evaluation of Antioxidant, Total Phenols and Flavonoids Content and Antimicrobial Actvities of Artocarpus Altilis (Breadfruit) of Underutilized Tropical Fruit Extracts

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Abstract- Artocarpus altilis (breadfruit) pulp, peel and whole fruit were extracted with various solvents such as hexane, dichloromethane (DCM) and methanol. The antioxidant activity of these extracts were examined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test IC50 ranged from 55±5.89 to 4851±111.00 µg/ml. In the β-carotene bleaching assay the antioxidant activity was 90.02±1.51% for the positive control (Trolox) and 88.34±1.31% for the pulp part of the fruit methanol extract. The total phenolic content of the crude extracts was determined using the Folin-Ciocalteu procedure, methanol pulp part demonstrated the highest phenol content value of 6570.74±511.14 mg GAE/ g of dry sample. While the total flavonoid content was determined using the aluminium chloride colorimetric assay highest value of 5600.34±1000.91 mg QE/ g indicated by pulp part of the fruit methanol extract. The antimicrobial activity of the crude extracts was tested using disc diffusion method against pathogenic microorganisms: S. aureus, S. epidermidis, B. cereus, S. typhimurium, E. coli, K. pneumonia and C. albicans. Methanol extract of pulp part was recorded to have the highest zone of inhibition against Gram-positive and Gram-negative bacteria. The MIC and MBC/MFC for the extracts were also determined using the microdilution method ranged from 4000-63 µg/ml against pathogenic microbes. The MBC/MFC values varied from 250 to 4000 µg/ml. A correlation between antioxidant activity assays, antimicrobial activity and phenolic content was established. The results shows that the various parts of A. altilis fruit extracts promising antioxidant activities have a potential bioactivities due to high content of phenolic compounds.

***Key words**: Artocarpus altilis, antioxidants, DPPH, antimicrobial, MIC and MBC/MFC*

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I. INTRODUCTION

Medicinal plants play an important role in allopathic medicine, herbal medicine, homoeopathy and aromatherapy by being the sources of a variety of drugs in the modern world (Pradhan et al., 2013). The use of plant extract as therapeutic agents is cheaper and easily available to most people in the developing countries. Currently, the highest attention is given to the medicinal values including the antimicrobial properties of plants naturally occurring antioxidants of plant origin. Antioxidants have been reported to prevent oxidative damage by free radicals and reactive oxygen species (ROS), and prevent the occurrence of disease such as cancer and aging.

Antioxidant molecules interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (Halliwell, 1999; Seifried et al., 2007). Natural antioxidants, particularly in fruits, vegetables and beverages have gained interest among consumers. Medicinal plants are mostly used as an alternative treatment for some diseases by producing a variety of biologically active compounds of known therapeutic properties (Kumar et al., 2007).

Plant extract which is known to contain many phenolic compounds, including flavonoids, have attracted considerable attention because its antioxidant activity is more powerful than vitamins, C and E (Gazzani et al., 1998; Vinson et al., 1998). The use of plants and herbs as antioxidants in processed foods is importance in the food industry as an alternative to synthetic antioxidants (Charalampos et al., 2008; Sari et al., 2007).

Effective determination of biologically active compounds from plant material is basically dependent on solvents polarity. Traditional therapists use water for extraction but found out that plant extracts usinf organic solvents contained more constant biological activities compared to water extracts (Parekh et al., 2005). In this, study solvents of different polarities viz., hexane, dichloromethane (DCM) and methanol have been used for the extraction procedure. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarities (Green, 2004).

Microorganisms are common pathogens causing severe ailments and fatal infections in humans. Natural products of higher plants may provide a new source of antimicrobial agents with potentially novel mechanisms of action (Nostro et al., 2000).

Artocarpus altilis (Family-Moraceae) commonly known as breadfruit is originated from New Guinea grows extensively in the Southern parts of India. Breadfruit (*Artocarpus altilis)* is a multipurpose agroforestry tree crop which is primarily used for its nutritious, starchy fruit with rich source of carbohydrates, calcium and phosphorus (Ragone, 1997). The multifarious uses of breadfruit includes food, medicine, clothing and animal feed.

The main objectives of the present study are to: . determine the total phenolic and flavonoid contents: . determine antioxidant and antimicrobial activities of the crude extracts. Then, correlating the antioxidant assays with consideration of the parts of the fruit as well as the solvents (hexane, dichloromethane (DCM) and methanol) of the crude extracts.

II. MATERIALS AND METHODS

Collection of the plant material

The fresh fruits were collected in Taman pertanian in July 2013, Kuantan, Malaysia. After collection, the dried fruits were ground, then stored in cold room at 4 °C until further analyses.

Extraction

The ground plant material was extracted using Soxhlet apparatus according to Green, (2004). The pulp, peel and whole fruit of *Artocarpus altilis* were extracted using three solvent systems (hexane, dichloromethane and methanol) in order of increasing polarity.

Determination of Total Phenolic content

Total phenolic content was determined by Folin-Ciocalteu reagent (McDonald et al., 2001). 1 ml of each sample extract (4 mg/ml) were added to test tubes and made up to the volume of 10 ml by adding 5 ml of diluted Folin-Ciocalteu phenol reagent (1:10 distilled water) and 4 ml of sodium carbonate solution (7%) after four minutes sequentially. Soon after homogenizing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was recorded at 725 nm against a reagent blank. All tests were carried out in triplicate. Total content of phenolic compounds in extracts was expressed as Gallic Acid Equivalent (GAE) milligram per gram of dry sample extract. Gallic acid was used as standard for the calibration curve . Gallic acid of different concentrations viz., 0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml was prepared in 50% (v/v) methanol. The optical density was measured using ELISA Reader (Versa max, microplate reader).

Determination of Total Flavonoid content

Flavonoid content was determined according to the aluminium chloride colorimetric method with some modifications (Elija et al., 2010). The sample (4 mg/ml) solution (0.5 ml) was mixed with 0.1 ml of 5 % $C_4H_4O_6KNa.4H_2O$ (Potassium Sodium L-(+) - Tartrate Tetrahdrate). After 5 minutes, 0.1 ml of 10% aluminium chloride hexahydrate, was added to the mixture and make up to 3 ml using distilled water. After incubation at room temperature for 40 minutes the absorbance of the reaction mixture was measured at 430 nm. A blank was prepared substituting 0.1 ml of distilled water in place of 10% aluminum chloride in the above reaction mixture. All tests were performed in independent triplicates. The total flavonoid content was expressed in milligram Quercetin Equivalent (QE) per gram extract. Crude extracts that have been attuned to come under the linearity range and different dilution of standard solution of Quercetin (20-100 µg/ml).

DPPH (2, 2-diphenyl, 1-picryl hydrazyl) radical scavenging assay

The antioxidant effect of the extracts on DPPH radical was assayed using method described by Mensor et al. (2001) with some modifications 3 ml of DPPH at concentration of 0.004% (w/v) was added to 1 ml of the plant extracts at concentrations which are ranged from 4000-31µg/ml. DPPH solution in methanol was used as control and methanol alone acted as blank. After 30 minutes, the discoloration from deep violet to yellow colour was measured at 517 nm using spectrophotometer.

The percentage inhibition was calculated by the following formula:

Scavenging activity (% of inhibition) = (Ac – As/ Ac) x 100

Where,

Ac – Absorbance of DPPH radical as control.

As – Absorbance of DPPH radical in the presence of the sample of plant extract

β-carotene bleaching inhibition assay

The antioxidant activity of plant extract was also evaluated using β-carotene linoleic acid model system (Kabouche et al., 2007). β-carotene (0.5 mg) in 1 ml of chloroform was added to 25 µl of linoleic acid, and 200 mg of tween-80 (emulsifiedr mixture). Chloroform was evaporated at 40 °C using a rotary evaporator. 100 ml of distilled water was slowly added to the residue and the solution was vigorously agitated to form a stable emulsion. 4 ml of this mixture was added into the test tubes containing 200 µl of plant extracts (4 mg/ml). As soon as the emulsified solution was added to the tubes, zero time absorbance was recorded at 470 nm. Then, the tubes were incubated for 2 h at 50 °C. Trolox was used as standard. Antioxidant activity was calculated as percentage of inhibition (%) relative to the control using the following equation:

Inhition %= $[1-(A_{so}-A_{s120})/A_c-A_{c120}) \times 100]$

A_{so} was initial absorbance, A_{s120} was the absorbance of the sample after 120 min, Ac was initial absorbance of negative control and A_{c120} was the absorbance of the negative control after 120 min. The test was done in three replicates.

Antimicrobial activity tests

Microorganism

A total of seven microorganisms were selected for this study. Clinically isolated *Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis, Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae* and *Candida albicans* were obtained from medical microbiology laboratories, School of Medicine, Hospital Universiti Sains Malaysia.

Disc diffusion assay

The extracts were tested for their antimicrobial potential against the microorganisms using the disc diffusion method BSAC (BSAC, 2012). The experiments were carried out in triplicates. The diameter of the inhibition zone was measured and recorded for each organism. Prior to test, the preparation of standard suspension was carried out according to the British Society for Antimicrobial Chemotherapy.

Briefly, morphologically identical colonies of selected microorganisms were suspended from a plate of 18-24 h growth in sterile Mueller Hinton and Sabouraud dextrose broth (Oxoid) for bacteria and fungus respectively. Then, adjusted to a density of 0.5 McFarland standard unites using McFarland densitometer (DEN-1B, biosan) which correspond to 10^8 CFU/mL for bacteria and 10^6 CFU/mL for fungus (Sujatha and Rajan, 2014). Within 15 minutes 100 µl of inoculum preparation was then plated onto agar surface followed by impregnated disc application and incubation at 37 ºC for 24 h and 30 ºC for 48 h for bacteria and fungus respectively. The antimicrobial activity of *A. altilis* fruit extracts was indicated by measuring the diameter of the inhibition surrounding each disc. All tests were done in triplicate.

III. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERIAL CONCENTRATION (MBC)

The antimicrobial activity was carried out *in vitro* using microplate method (microwell dilution) according to the Wilkinson, (2007) procedure with some modifications. TTC (2, 3, 5-triphenyltetrazolium chloride) was used as visual indicator of microorganisms' growth. The extracts concentrations ranged from 4000-31 µg/ml by two fold dilution. The 96 well plates were set by dispensing the inoculum suspensions which was prepared as previously mentioned for disc diffusion assay. Into each well 95 µl of Mueller Hinton broth (OXDID, ENGLAND) and 5 µl of the suspensions was added and then made up to final volume of 200 µl for each well of by adding 100 µl of extract. The growth control was broth and suspensions. The plates were incubated at 37 ºC for 24 h for bacteria and 30 ºC for 48 h for fungus. The MIC value is considered as the lowest concentration of the sample extract which inhibits the growth of a microbe. To indicate the growth of microorganisms in well a red colouration would be formed after adding TTC (10 µl/well, 20 mg/ml, dissolved in distilled water) and incubated for 30 min in the dark whereas the clear well can be indicated as inhibition of the growth (Klancnik et al., 2010).

The MBC/MFC were determined by subculturing the clear wells from the MIC 96 well plates onto free microorganisms agar plates in order to determine the lowest concentration required to kill (≥ 99.9%) of the microorganisms by incubating the plates at 37 ºC for 24 h and 30 ºC for 48 h for bacteria and fungus respectively.

Statistical analysis

Data of replicates were analysed using a one way ANOVA analysis and were expressed as means ±SD (standard deviation). Statistical differences between the reference and the sample groups were evaluated by ANOVA (one way) with Duncan's multiple comparison tests $p < 0.05$ using (SPSS 20.0) software. Correlations among data were calculated using Pearson's correlation coefficient (R).

IV. RESULTS AND DISCUSSIONS

Determination of total phenols and total flavonoids content

All plants produce a remarkable range of secondary metabolites. One of the most important groups of these metabolites are phenolic compounds. Phenolic compounds can be simple, low molecular weight, single aromatic ringed compounds to large and complex polyphenols. The antioxidant action of phenolic compounds is due to to their high susceptibility to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind mostly iron and copper (Jung et al., 2003) and the concepet of antioxidant action of phenolic compounds is not new. Total phenol content per 0.004 g of each extract of pulp, peel and whole fruit of *Artocarpus altilis* results are shown in Table 1, they were quantified based on the linear equation obtained from Gallic acid standard calibration curve. Values of total phenol content of *A. altilis* fruit were in range from 678.70±1.20 to 6570.74±1.14 mg GAE/ g per g dry weight bases of extracts. Out of all extracts, methanol extract of pulp part had the highest content of phenols of 6570.74±1.14 mg GAE/ g exhibiting relatively high levels of polyphenols phytochemicals. This is Followed by methanol extract of peel part and whole of fruit of *A. altilis* (5606.18±1.19 and 4828.09±2.09 mg GAE/ g) respectively. Whereas the DCM (dichloromethane) extract of whole fruit had a phenol content of 2557.41±2.14 mg GAE/ g. Statistical analysis showed that these variations are significantly different. From the results, it was also found that methanol extracts of all parts had higher TPC content than the others (hexane and DCM) due the difference in solvents polarity. Methanol being a more polar solvent so during the extraction process dissolved phytochemicals with higher polarity (Zhang et al., 2007). Phenolic compounds also play an important role serve in defense mechanisms of plants by stabilizeng the reactive oxygen species and prevent molecular damage (Sengul et al., 2009).

The total flavonoids content of different parts of *A. altilis* are shown in Table 2. They were quantified based on the linear equation obtained from Querctin standard calibration curve. Total flavonoids (TFC) of *A. altilis* varied from 621.31±0.45 to 2546.01±0.91 in terms of mg Quercetin equivalents per gram of dried sample (mg QE/ g). Highest flavonoids found in content methanol extract of pulp part of *A. altilis* fruit (2546.01±0.91 mg QE/ g per dry weight of extract).

Antioxidant activity

Determination of DPPH and BCB

The crude extracts of *A. altilis* were analysed for their antioxidant activities using the DPPH radical test and the values are expressed as IC_{50} (Table 3).

One way (ANOVA) analysis showed significant differences between the values of methanol extract of the pulp part of the fruit (55±0.89 µg/ml) and the positive control (Ascorbic acid) (61±0.51 µg/ml). Methanol extract of pulp part (55±0.89 µg/ml) inhibited the the 50 % of DPPH free radicals, which is significantly lower than the standard (ascorbic acid). This indicated that maximum antioxidant activity is present in the pulp than other parts of the fruit.

Beside the DPPH test, β-carotene bleaching assay was also carried out and the results are shown in Figure 1. Trolox was used as a positive control which had highest antioxidant activity (90.02±1.51%) compared to all extracts.

Methanol extract of pulp part demonstrated antioxidant value of 88.34±1.31 % followed by methanol extracts of whole fruit and peel part with 78.83±1.38% and 76.93±1.36% respectively. When seen together with the values of total flavonoids content, it shows that non-phenolic flavonoids are contributing to a large extent to the antioxidant activity of methanolic extract of all parts of *A.altilis* fruit.

Antimicrobial activity tests:

Disc diffusion test

Natural product chemistry is a field that search for phytochemicals which could be synthesized and used as treatment for infectious ailments (Tanaka et al., 2006). Due to the development of drug-resistantance in microorganisms, the demand of more effective antimicrobial agents is increasing. Mathekaga and Meyer, (1998) reported that *in vitro* screening method of plant crude extracts could help to find the alternative of commercial antibiotics by further investigations. This study investigated the antimicrobial activities of *Artocapus altilis* fuirt extracts against seven human pathogenic microorganisms. Two methods viz. the disc diffusion method and microtitre plate method were used to determine the antimicrobial activity of the fruit extracts in terms of MIC and MBC/MFC. The results of antimicrobial activity of the crude extracts of *A. altilis* were shown in Table 4. In this study, it was found that the methanol extract of pulp part was the most active extract agaist Gram-positive and Gram-negative bacteria. In this experiment seven selective microorganisms were used. Methanol extracts of pulp part showed highest zone of inhibition (14.83±0.28 to 20.50±0.76mm) against all the species of microorganisms tested. The hexane and DCM extracts of both pulp and peel parts of *A. altilis* fruit showed antimicrobial activity only against Gram-positive bacteria such as *S. arureus*. Results in Table 4 indicated that methanol extracts of three parts of fruit exhibited highest activity against Gram-positive and Gram-negative bacteria among other extracts (hexane and DCM) and this, may be due to the correlation between the total phenolic content and the antimicrobial activity.

V. MIC AND MBC/MFC

Minimum Inhibitory Concentration (MIC) is defined as lowest concentration of the extracts that inhibit growth of microorganisms. It is an essential test in terms of confirming resistance of microorganism to an antimicrobial agent by observing the activities of the extracts (Langfield et al., 2004). Minimum Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC) was determined by subculturing the tested broth dilution (MIC) onto an agar plate and incubated further for 24 h and 48 h for bacteria and fungus, respectively. The concentration of plant extract that reduces the viability of the initial bacterial inoculum by ≥ 99.9% were taken as MBC and MFC, respectively. Moreover, Omar et al., 2010 reported that most of the antimicrobial properties in different plant part extractions shows, MBC/ MFC value that is almost two fold higher than the corresponding MIC.

Results in Table 5 show the MIC concentrations of the various parts of *A. altilis* fruit with various types of solvents which have been used in this study. Methanol extract of pulp part of fruit revealed the lowest concentration value of 63 µg/ml, whereas methanol extracts of peel and whole fruit showed a value of 125 µg/ml against *S. aureus*. Highest concentration of methanol extract of pulp part was 1000 µg/ml against *E.coli*. Whereby DCM extract of whole fruit showed comparatively efficient MIC value of 125, 250, 500 and 1000 a µg/ml against *S. arureus*, *S. epidermidis*, *S. typhimurium* and *C. albicans*, respectively. The lowest concentration against *C. albicans* among the extracts was 500 µg/ml demonstrated by methanol extract of pulp part. MBC and MFC results are shown in Table 6. The lowest concentration that killed 99.9% of pathogen was 250 µg/ml and 500 µg/ml of methanol extract of pulp part of the fruit against *S. arureus* and *S. typhimurium*, respectively. While the highest concentration was 4000 µg/ml by DCM extract of peel part against Gram-positive bacteria and fungus (*S. arureus*, *B. cereus* , *S. epidermidis* and *C. albicans*). The lowest MFC was 2000 µg/ml demonstrated by methanol extracts of pulp, peel and whole fruit and the DCM extract of whole fruit against *C.albicans*. It was also observed that extracts which have been tested via broth microdilution assay method demonstrated antimicrobial activity at lesser concentrations than that of disc diffusion methods and this refer to many factors such as the slow diffusion of extracts into agar, highly affected by temperature and pH. (Maltophilia et al., 2012).). It enables to use small quantities of extract which is important if the antimicrobial is limited as is case for many natural products; ability to distinguish between bacteriostatic and bactericidal effects; and quantitative determination of the MIC (Langfield et al., 2004). It is reported that disc diffusion and serial dilution methods do not always give parallel results while measuring their antimicrobial activity (Njenga et al., 2005).

As an overall insight of the study, the methanol extracts of various parts of *A. altilis* fruit showed highest antioxidant and antimicrobial activity compared to other extracts. This enhance the correlations between the antioxidants assays and with antimicrobial assays. Ncube et al., (2008) reported that the phenolics and polyphenols exhibited antimicrobial activity including an essential subclasses of this group such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and generally are soluble in polar solvents such as methanol (Houghton et al., 1998). There are also reports in literature that the most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol, and water (Parekh et al., 2005). Significant correlations were found between and within assays. The correlation coefficient of methanol extract of pulp part of the fruit for total phenol content and antioxidant activities using DPPH was 0.999, (p<0.05), and using BCB is was 0.997. Correlations between DPPH and BCB for methanol extract of pulp part was 0.999, (p<0.05). The total flavonoids of the pulp part extracts also showed strong correlations using DPPH (0.978, p<0.01), BCB (0.992, p<0.01). Methanol extract of whole fruit also showed a strong correlation for total phenols content using DPPH (0.998, p<0.05) and BCB (0.998, p<0.05), while for total flavonoids whole fruit extracts using DPPH showed a correlation coefficient of 0.876 and using BCB showed 0.910. These results are in concurrence with that of Yen et al., (2004) and Norshazila et al., (2010) who suggested strong correlations between the extracts with high amounts of total phenolic content and high antioxidant activity.

On other hand the correlations between the antioxidant content and the antimicrobial activity of methanol extract of pulp part against Gram-positive bacteria such as *S. arureus* (0.998,) and Gram-negative bacteria such as *S. typhimurium* (0.999, $p<0.05$), showed high correlation of 0.998 & 0.999 $p<0.05$) respectively. There were no significant correlations between the antioxidant assays of peel part of the fruit. Similar finding were reported by Akinmoladun et al., (2007) and Aboaba and Efuwape, (2001). Levy, (1994) also stated that flavonoid, phenolic compound, tannins and alkaloid are the most important antimicrobial agents and bioactive constituents in plant. In recent years, studies of *A. altilis* have increased due to its importance in pharmaceutical applications (Pradhan et al., 2013). It is important to bring underutilized plants to light not only for their potential bioactivities, but it may lead to discover new sources of economic resources such as tannis, oil, gums, flavonoids, saponins (Akrout et al., 2010) in line with current study, from the results which had been shown of *Artocarpus altilis* fruit extracts highly phenols and flavonoids been contented which make it valuable. Present study shows that *Artocarpus altilis* fruit contains high levels of phenols and flavonoids making it a potential source of antioxidants and antimicrobials

VI. CONCLUSION

Antioxidant and antimicrobial properties of various parts of plants extracts have recently been of abundant interest in both research and food industry, because of their possible use as natural additives. Based on the results which were obtained in this research, it was concluded that the pulp, peel parts and whole fruit of *Artocarpus altilis* extracts have a high antioxidant and antimicrobial activities. The methanol extracts of both pulp, peel and whole fruit showed considerably higher antioxidant and antimicrobial bioactivities, thus making it potential source of antioxidant and antimicrobial agents.

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Table 1: IC_{50} (mg/ml) values of the crude extracts

Plant parts	Solvents	IC_{50} values (µg/ml)
Pulp	*n*-hexane	4467 ± 110.57^{f}
	Dichloromethane	2615 ± 111.52^{e}
	Methanol	55 ± 5.89^{a}
Peel	*n*-hexane	4851 ± 131.00^{h}
	Dichloromethane	677 ± 71.10^{d}
	Methanol	123 ± 10.57^{c}
Whole fruit	*n*-hexane	4659 ± 111.00^{g}
	Dichloromethane	123 ± 13.00^{c}
	Methanol	123 ± 10.86^{c}
Standard	Ascorbic acid	61 ± 4.51^{b}

Value are presented as mean ± SD (n=3). Different letters are significant differences at $p < 0.05$ level compare to positive control (Ascorbic acid) and other varieties, as determined by s determined by Duncan's multiple range test. IC_{50}= the concentration of substrate that cause 50 % reduce of the DPPH colour.

Table 2: Antimicrobial activity of A. altilis fruit extracts (500mg/ml) showing zone of inhibitions.

Extracts of *A. altilis*	Inhibition zone against different microorganisms (mm)						
	S. arureus	**B. cereus**	**S. epidermidis**	**E. coli**	**S. typhimurium**	**K. pneumoniae**	**C. albicans**
HP	9.16 ± 0.57^{a}	8.66 ± 0.76^{a}	8.00 ± 0.50^{b}	NI	NI	NI	NI
DP	9.66 ± 0.57^{a}	8.83 ± 0.28^{a}	9.33±0.57a	NI	NI	NI	NI
MP	20.50 ± 0.76^{e}	16.00 ± 0.50^{d}	15.83 ± 0.50^{d}	15.16 ± 0.28^{c}	20.50 ± 1.00^{e}	14.83 ± 0.28^{c}	15.16 ± 0.57^{c}
HL	9.16 ± 0.76^{a}	9.33 ± 0.57^{a}	9.38 ± 0.57^{a}	NI	NI	NI	NI
DL	10.83 ± 0.28^{b}	11.83 ± 0.28^{b}	10.83 ± 0.57^{b}	NI	NZ	NZ	NI
ML	17.00 ± 0.50^{d}	15.16 ± 0.28^{c}	15.16 ± 0.28^{c}	14.16 ± 0.57^{c}	16.83 ± 0.57^{d}	13.66 ± 0.28^{c}	12.83 ± 0.28^{b}
HW	8.5 ± 0.50^{a}	9.33 ± 0.57^{a}	8.66 ± 0.28^{a}	NI	NI	NI	NI
DW	16.16 ± 0.57^{d}	14.83 ± 0.28^{b}	14.66 ± 0.28^{c}	14.13 ± 0.23^{c}	14.66 ± 0.28^{c}	13.33 ± 0.28^{b}	12.66 ± 0.28^{b}
MW	17.33 ± 0.28^{d}	15.16 ± 0.28^{c}	15.16 ± 0.28^{c}	14.46 ± 0.50^{c}	16.00 ± 0.50^{c}	14.16 ± 0.28^{c}	13.16 ± 0.57^{b}

Value are presented as mean ± SD (n=3) Different letters are significant differences and same letters are no significant differences at $p < 0.05$ level compare to other varieties determined by Duncan's multiple range test. NI= No inhibition zone. HP= Hexane pulp, DP= Dichloromethane pulp, MP= Methanol pulp, HL= Hexane peel, DL= Dichloromethane peel, ML= Methanol peel part, HW= Hexane whole fruit, DW= Dichloromethane whole fruit and MW= Methanol whole fruit. S. aureus = Staphylococcus aureus, B. cereus = Bacillus cereus, S. epidermidis= Staphylococcus epidermidis, E. coli = Escherichia coli, S. Typhimurium= Salmonella typhimurium, K. pneumoniae = Klebsiella pneumoniae and C. albicans = Candida albicans.

Tabl 3: 5: Minimum Inhibitory Concentration (MIC) (µg/ml) of *A. altilis* fruit extracts on different microorganisms.

Extracts of A. altilis	Microorganisms						
	S. arureus	B. cereus	S. epidermidis	E. coli	S. typhimurium	K. pneumoniae	C. albicans
HP	4000	4000	4000	4000	4000	4000	4000
DP	4000	4000	4000	4000	4000	4000	4000
MP	63	250	250	1000	250	500	500
HL	4000	4000	4000	4000	4000	4000	4000
DL	4000	4000	4000	4000	4000	4000	4000
ML	125	500	250	1000	500	1000	1000
HW	4000	4000	4000	1000	4000	4000	4000
DW	125	500	250	1000	500	1000	1000
MW	125	500	500	1000	500	500	1000

HP= Hexane pulp, DP= Dichloromethane pulp, MP= Methanol pulp, HL= Hexane peel, DL= Dichloromethane peel, ML= Methanol peel part, HW= Hexane whole fruit, DW= Dichloromethane whole fruit and MW= Methanol whole fruit. *S. aureus = Staphylococcus aureus*, *B. cereus = Bacillus cereus*, *S. epidermidis= Staphylococcus epidermidis*, *E. coli = Escherichia coli*, *S. Typhimuriu*m= *Salmonella typhimurium*, *K. pneumoniae = Klebsiella pneumoniae* and *C. albicans = Candida albicans.*

Table. 4 6: Minimum Bactericidal/Fungicidal (MBC)/ (MFC) Concentration (µg/ml) of *A. altilis* fruit extracts on different microorganisms

Extracts	Microorganisms						
	S. arureus	B. cereus	S. epidermidis	E. coli	S. typhimurium	K. pneumoniae	C. albicans
HP	NMBC	NMBC	NMBC	NMBC	NMBC	NMBC	NMFC
DP	NMBC	NMBC	NMBC	NMBC	NMBC	NMBC	NMFC
MP	250	500	250	1000	500	1000	2000
HL	NMBC	NMBC	NMBC	NMBC	NMBC	NMBC	NMFC
DL	4000	4000	4000	NMBC	NMBC	NMBC	4000
ML	500	1000	250	1000	1000	1000	2000
HW	NMBC	NMBC	NMBC	NMBC	NMBC	NMBC	NMFC
DW	500	1000	250	2000	1000	2000	2000
MW	500	1000	500	1000	1000	1000	2000

NMBC/NMFC= No minimum bactericidal/ fungicidal concentration observed. HP= Hexane pulp, DP= Dichloromethane pulp, MP= Methanol pulp, HL= Hexane peel, DL= Dichloromethane peel, ML= Methanol peel part, HW= Hexane whole fruit, DW= Dichloromethane of whole fruit and MW= Methanol whole fruit. *S. aureus = Staphylococcus aureus*, *B. cereus = Bacillus cereus*, *S. epidermidis= Staphylococcus epidermidis*, *E. coli = Escherichia coli*, *S. Typhimuriu*m= *Salmonella typhimurium*, *K. pneumoniae = Klebsiella pneumoniae* and *C. albicans = Candida albicans*.

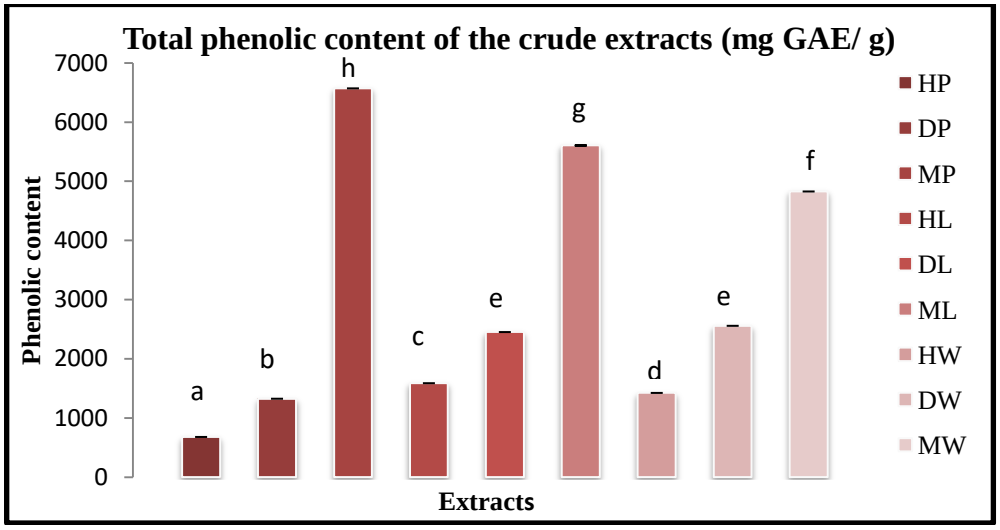

Figure 1.Total phenolic content of the crude extracts (mg GAE/ g) value are presented as mean ± SD (n=3). Different letters are significant differences at $p < 0.05$ level compare to other varieties s determined by Duncan's multiple range test. GAE – Gallic acid equivalents mg GAL/g dry sample.

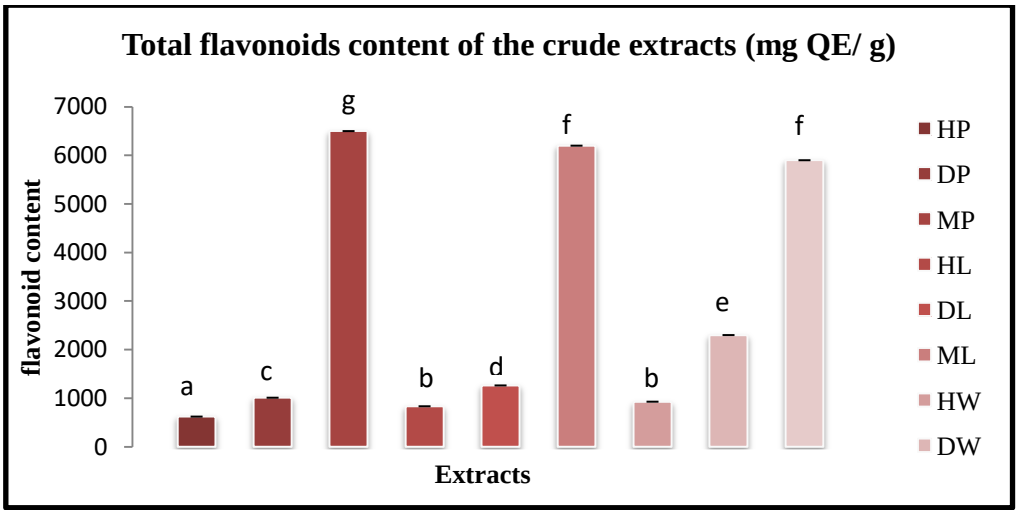

Figure 2. Total flavonoids content of the crude extracts (mg QE/ g) value are presented as mean ± SD (n=3). Different letters are significant differences at $p < 0.05$ level compare to other varieties s determined by Duncan's multiple range test. QE – Querctin equivalents mg QE/ g dry sample.

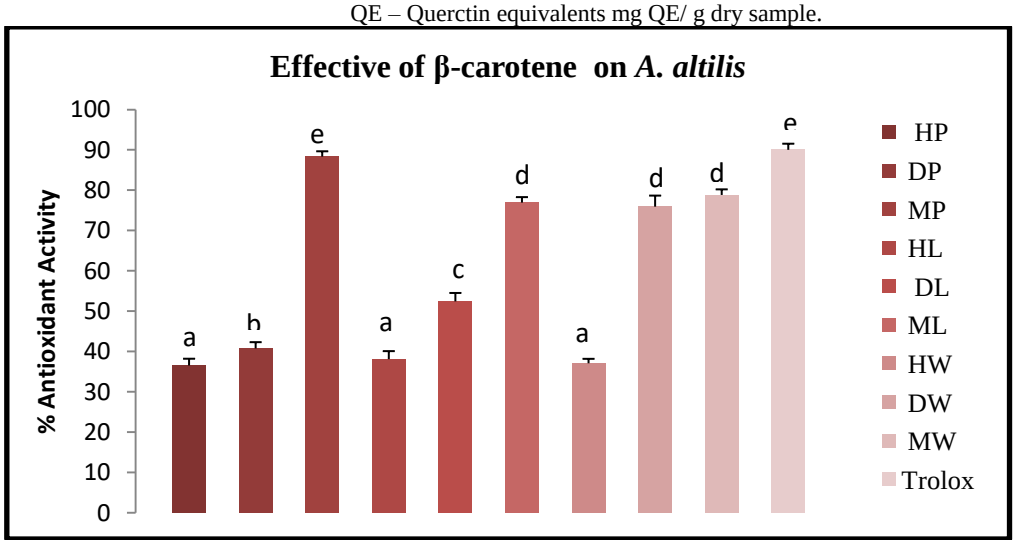

Figure 3: The end points total antioxidant activities of three parts of *A. altilis* fruit extracts against β-carotene oxidation by linoleate radical. Data were expressed as antioxidant activities (% AA), mean ± SD (n = 3). Different letters are showed significant difference compared to positive control (Trolox).