POTENTIAL ANTIOXIDATIVE AND ANTIFUNGAL ACTIVITIES FROM *Eugenia polyantha* WIGHT

POTENSI AKTIVITAS ANTIOKSIDAN DAN ANTI JAMUR DARI Eugenia polyantha WIGHT

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ABSTRAK

Aktivitas antioksidan ekstrak Eugenia polyantha Wight diuji secara in vitro dengan menggunakan uji radikal bebas 2,2-difenil–1-pikrilhidrazil, uji perlindungan β-karoten, dan uji peroksidase lemak TBARS. Hasil uji menunjukkan bahwa ekstrak kulit kayu E. polyantha memiliki aktivitas antioksidan. Ekstrak methanol-air memiliki kemampuan menangkal radikal bebas tertinggi dibandingkan dengan ekstrak lainnya ((ED_{50}) = 0,18 mg/ml), dan perlindungan terhadap pemudaran β-karoten (85,7% at 100 µg/ml). Ekstrak kulit batang E. polyantha juga mempunyai efek perlidungan terhadap peroksidasi lemak pada daging mentah dan matang. Ekstrak methanol-air memiliki kandungan total fenol tertinggi dibading ekstrak lainnya (856 mg (GAE)/g and 161 mg (CE)/g) dan kapasitas total antioksidan (449 mg (AAE)/g). Dari ketiga ekstrak E. polyantha, kapasitas total antioksidan memiliki hubungan dengan total fenol yang terkandung dalam masing-masing ekstrak tersebut. Minyak atsiri dari E. polyantha memiliki kemampuan menghambat pertumbuhan jamur perusak hasil pertanian, Pleurotus ostreatus (46,2%), Fusarium oxysporum (38,1%), dan Coreynespora cassicola (32,2%).

Kata kunci: Antioksidan, Eugenia polyantha, Minyak atsiri

ABSTRACT

Antioxidative activities of Eugenia polyantha Wight bark extracts grown in Indonesia were evaluated using 2,2-diphenyl–1-picrylhydrazyl radical-scavenging, β-carotene bleaching assay, and thiobarbituric acid reactive substance (TBARS) assay. The extracts also showed protection towards lipid peroxidation on cooked (68%) and raw meat (56%). The methanol-water extract showed the highest level of free radical-scavenging activity ((ED_{50}) = 0.18 mg/ml) and protection from β-carotene bleaching (85.7% at 100 µg/ml). The extracts showed the highest total phenolic content (856 mg gallic acid equivalent (GAE)/g and 161 mg catechin equivalent (CE)/g) and total antioxidative capacity (449 mg ascorbic acid equivalent (AAE)/g). A relationship between total antioxidative capacity and total phenolic content was recognized from E. polyantha bark extracts. Furthermore E. polyantha essential oil demonstrated inhibition growth activity of several agricultural pathogenic fungus i.e., Pleurotus ostreatus (46.2%), Fusarium oxysporum (38.1%), and Coreynespora cassicola (32.2%).

Keywords: Antioxidants, Eugenia polyantha, Essential oils

INTRODUCTION

Reactive oxygen species and several free radicals are well known inducers of cellular and tissue pathogenesis leading to diseases like cancer and inflammatory disorders, as well as the aging process.[1] Reactive oxygen species (ROS) cover a wide range of chemical components, including superoxide anion, hydrogen radicals, nitric oxide, and peroxynitrite, and have the potential to initiate

degenerative processes in the human body.[2] Many antioxidants, occurring naturally in plants, have been identified as scavengers of free radicals or active oxygen. Antioxidants provide protection to living organisms from damage caused by the uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breaks.[3]

The use of traditional medicines is widespread, and plants are still a major source of natural antioxidants that might serve as leads for the development of novel drugs. Several species belonging to the genus Eugenia of the Myrtaceae like *Eugenia jambolana* have been used traditionally for a wide variety of enthnomedical properties such as antidiabetes, antidiarrhea, antinematodes, and anti-inflammation. Among them is *E. polyantha* Wight, a deciduous tropical tree with spreading branches and simple leaves which can reach 60 to 90 feet and grows wild in the scrub forests of Indonesia. *E. polyantha* leaves have been widely used in Indonesian culinary as a food additive.[4] They have also been used for antiulcerous, antidiabetic, antiinflammatory, and antidiarrheal treatments, yet the antifungal properties is still understudy.

Fungi are one of the most harmful groups of plant pathogens, damaging agricultural crops, forest trees, woods and wood-based products. *Fusarium oxyporum* attacks roots and causing vascular wilt and root rot. *Corynespora cassiicola* can cause weeping fig, leaf spot, and stem rot, as well as rice blight. These pathogenic fungi cause considerable economic losses at least 20% of the yield of crops worldwide.[5]

In view of the wide spread ethnomedical uses of *E. polyantha*, this study was conducted to evaluate the antifungal activity of *E. polyantha* essential oils and antioxidative activities of its bark extracts using three procedures; 2,2-diphenyl–1-picrylhydrazyl (DPPH) radical-scavenging, β-carotene bleaching, and TBARS assays together with the total polyphenol content. The correlation between polyphenol content and the antioxidative activities was investigated in an attempt to clarify the antioxidative activities of *E. polyantha* bark extracts.

EXPERIMENTAL WORKS

E. polyantha bark was collected and air-dried at Indonesian Research Institute of Sciences, Serpong, Tangerang, Indonesia. The freshness of the leaves were maintained for the essential oil study. The bark was kept dry and away from direct sunlight during storage. The bark was cut into small pieces and air-dried prior to extraction. The dried bark (200 g) was successively extracted for 8 hours at 80°C with methanol, methanol-water (50%), and water. The extraction was repeated twice. Each solution was concentrated with a rotary evaporator under reduced pressure. *E. polyantha* essential oils were collected using a hydro distillation apparatus. Fresh leaves of *E. polyantha* were cut into small pieces and macerated in boiling water (2 x 8 hours), and the distilled oil was collected from the receiving chamber. The oil collected was then stored in a closed jar and placed in a refrigerator for the analysis.

In vitro 2,2-Diphenyl –1-picrylhydrazyl (DPPH) radical-scavenging assay

The free radical-scavenging activity of each extract was determined using free radical scavenging assay. Plant extracts were added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was measured under constant mixing at room temperature after 30 min and percent inhibitory activity was calculated from $[(A_0-A_1)/A_0]$ x100, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard.

Beta-carotene bleaching assay

β-carotene (10 mg) was dissolved in 10 ml of chloroform. The carotene-chloroform solution (0.2 ml) was pipetted into a flask containing 20 mg of linoleic acid and 200 mg of Tween40. Chloroform was removed using a rotary evaporator under reduced pressure at 40°C for 5 min, and 50 ml of distilled water was added slowly to the residue with vigorous agitation in order to form an emulsion. A 4.8 ml aliquot of the emulsion was added to a tube containing 0.2 ml of the sample solution, and the absorbance at 470 nm was immediately measured against a blank that was an emulsion without β-carotene. The tubes were

placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring the absorbance at 470 nm for 60 min. Control samples contained 0.2 ml of ethanol instead of the sample extracts. Ascorbic acid was used as the reference. The antioxidative activity was expressed as percent inhibition with reference to the control after 60 min of incubation using the following formula: $AA = 100(DR_C - DR_S)/DR_C$, where AA is the antioxidative activity; DR_C is the degradation rate of the control $\{DR_C = [\ln(a/b)/60]\}$; DR_S is the degradation rate in the presence of the sample $\{DR_S = [\ln(a/b)/60]\}$; where a is the absorbance at time 0; and b is the absorbance after 60 min.

Thiorabituric acid reactive substance lipid peroxidation assay (TBARS)

Freshly cut bovine meat samples were obtained from a local market and visible fat was removed. Samples from each type of meat were then divided into three treatment groups and homogenized with 3% (w/w) of sample extracts. Samples in the control treatment had no extracts added. The meat samples were then stored at 4°C for 24 h before the assays for antioxidative activity were performed. Consequently, each sample was split into 3 g portions one remaining in the raw state and the other receiving heat treatment (at 85°C for 30 min). The later is referred to herein as "cooked". Both the raw and cooked samples were subsequently stored at 4°C and evaluated until seven days of storage.

For the TBARS assay, a modified version of the method described by Ruberto *et.al*.[6] was used. In brief, 0.05 g of each meat sample was mixed with 1 ml of H_2O, 1.5 ml of 20% acetic acid, and 1.5 ml 0.8% (w/w) of TBA in 1.1% (w/w) SDS in a test tube, vortexed and heated to 100°C for 60 min in a water bath. After cooling, 5 ml of butan–1-ol was added and mixed in. Samples were then centrifuged at 10,000 rpm for 10 min. The absorbance of the upper layer was determined at 532 nm compared to butan–1-ol which was used as a blank. No free radical was used to induce oxidation. The results are expressed as absorbance and calculated from $[1-(A_1/A_0) \times 100]$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

The amounts of phenolic compounds, flavonoids and flavonols compounds

The total phenolic content of plant extracts was determined using Folin-Ciocalteu reagent. Aliquots (100 ul) of the plant extracts were mixed with the Folin-Ciocalteu reagent (500 ul) and 20% sodium carbonate (1.5 ml). Each mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h and absorbance was measured at 765 nm. The phenolic content was determined using a calibration curve obtained from various concentrations of gallic acid and catechin. Values are expressed in gallic acid equivalents (GAE) and catechin equivalents (CE).

The flavonoid content was determined with a calibration curve using rutin as a reference. One milliliter of plant extract in methanol (10 mg/ml) was mixed with 1 ml of aluminium trichloride in ethanol and made up to 25 ml. The absorption at 415 nm was read after 40 min. Blanks were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The absorption of a standard rutin solution in ethanol was measured under the same conditions. The amount of flavonoid in plant extracts in rutin equivalents (RE) was calculated by $X = (A.m_0)/(A_0.m)$, where X is the flavonoid content, mg/g plant extract in RE, A is the absorbance of the plant extract solution, A_0 is the absorbance of the standard rutin solution, m is the weight of the plant extract, and m_0 is the weight of rutin in the solution.

The amounts of flavonols were determined by rutin calibration curve. The rutin calibration curve was obtained from a mixture of 2 ml of various concentrations of rutin in ethanol solution, 2 ml of aluminium trichloride (20 mg/ml), and 6 ml of sodium acetate (50 mg/ml). The absorbance at 440 nm was recorded after 2.5 h. The same procedure was used for 2 ml of plant extract (10 mg/ml) instead of the rutin solution. The flavonol content was calculated using a calibration curve obtained from various concentration of rutin, and expressed as rutin equivalents (RE).

Antifungal assay and determination of *E. polyantha* essential oil composition

The dilution method was used for antifungal assays. Sterile potato dextrose agar and each extracts from selected plant were dissolved in acetone or water and placed in a 90-mm petri dish, whereas control plates contained medium and solvents only. Mycelial plugs were punched out with a 5-mm diameter cork borer from the test fungi grown on each medium and placed at the center of each petri dish. Inoculated agar plates were incubated at 25°C for 6 days in the dark. Antifungal activity was determined based on inhibition using the formula; Percent inhibition = (1-T/C) x 100; whereas T is hyphal extension in the treated plates and C is hyphal extension in the control.

E. polyantha essential oil was subjected to Gas Chromatography-Mass Spectra (GC-MS). The GC–MS apparatus was a GS Shimadzu QP2010 and coupled with mass spectrometer recorder, equipped with a split/splitless injector (maintained at 250°C) and an TC–1 capillary column. Helium was used as the carrier gas. The split ratio was 1:30 and the volume of each injected sample was 1.0 µl. Initial column temperature was set at 60°C and rate temperature was maintained at 2°C/minutes. The injection temperature was set at 280°C, the detector temperature being 280°C. Mass spectrum acquisition was performed in the mass range from 40 to 500 m/z. Ionization using 70 eV electrons (EI) was employed. The compounds were identified by comparing the experimental mass spectra with those found in the Wiley mass spectra library and by comparing their Kovats indices.

RESULTS AND DISCUSSION

DPPH radical-scavenging activity

The three successive bark extracts from *E. polyantha* showed strong DPPH free radical-scavenging activity. The scavenging effect of the extracts was as follows; methanol-water extract (ED_{50} = 0.181±0.04) mg/ml > methanol extract (ED_{50} = 0.335±0.16) mg/ml > water extract (ED_{50} = 0.354±0.11) mg/ml. *E. polyantha* extracts exhibited a dose-dependent effect on DPPH free radical-scavenging activity. All extracts showed similar activity at 0.001 mg/ml, and significantly increased activity at 0.1 mg/ml. The methanol-water extract exhibited higher free radical-scavenging activity than pyrogallol or ascorbic acid as a positive control at 1 mg/ml. The effect on DPPH radical-scavenging was thought due to their hydrogen-donating ability and radical scavenging activity.[7] As the concentration of phenolic compounds or the degree of hydroxylation of the phenolic compounds increases, so will the DPPH radical-scavenging activity. The DPPH radical-scavenging capacity of the extracts could be explained by the presence of phenolic components.[8]

Beta-carotene bleaching assays

In the β-carotene bleaching assay, the three extracts from *E. polyantha* were compared with a well known natural antioxidant, ascorbic acid. In antioxidative activities, the extracts ranked in the following order: methanol-water extract (85.72%±0.57) > methanol extract (76.43%± 0.91) > water extract (74.33%± 0.53). The water extract showed the highest level of activity at 1 mg/ml, and also demonstrated a significant increase in activity compared to the other extracts and positive control. In terms of β-carotene bleaching activity, ascorbic acid showed the lowest antioxidative activity. In spite that polar compounds like ascorbic acid are well known antioxidants, the β-carotene bleaching test did not show antioxidative properties. This interesting phenomenon has been described as the "polar paradox".[9] Polar antioxidants remaining in the aqueous phase of an emulsion are more diluted in the lipid phase and thus less effective in protecting linoleic acid. β-carotene undergoes rapid decolorization in the absence of an antioxidant. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing linoleate free radicals and other radicals formed in the system.[10]

Total phenolic, flavonoid, and flavonol contents

The total phenolic content of *E. polyantha* extracts was determined using the Folic-Ciocelteau assay,

and expressed as gallic acid equivalents (GAE mg/g) and catechin equivalents (CE mg/g), respectively, as shown in Table 1. The methanol-water extract had the greatest total phenolic content (856±28.2 mg GAE/g and 161±18.3 mg CE /g), and total antioxidative capacity expressed as ascorbic acid equivalents (449±23.5 mg AAE /g). The high level of phenolic content gives high antioxidant properties as observed in Fig 1. The total phenolic content (GAE and CE) and total antioxidative capacity (TOAC) of the methanol-water extract exhibited a positive correlation with DPPH free radical-scavenging activity and beta carotene bleaching activity. The DPPH free radical-scavenging activity and beta carotene bleaching activity were directly influenced by the total phenolic content and the total antioxidative capacity of each *E. polyantha* extract.

The antioxidative activity of phenolic compounds is mainly due to their redox properties, which play an important role in the adsorption and neutralization of free radicals, quenching of singlet and triplet oxygen, or decomposition of peroxides.[11] Phenolics are classified as simple phenols, which one hydroxyl group attached in an aromatic ring, and flavonoids are named polyphenols havening two subunits and higher phenol subunit attached subunits, called tannins.

Table 1. Total Phenolic Content of *E. polyantha* Bark Extracts.

Eugenia polyantha **bark extract**	**Total Polyphenol Content (mg/g extracts)**				
	Total Phenol (Gallic acid equivalents)	**Total Phenol (Catechin equivalents)**	**Total Flavonoid (Rutin equivalents)**	**Total Flavonol (Rutin equivalents)**	**Total Antioxidative Capacity (Ascorbic acid equivalents)**
Methanol extract	718 ± 26.4	113 ± 12.2	60.6 ± 15.2	19.4 ± 4.5	329 ± 21.1
Methanol water-extract	856 ± 28.2	161 ± 18.3	26.52 ± 7.1	18.9 ± 6.1	449 ± 23.5
Water extract	793 ± 34.9	141 ± 12.8	22.12 ± 4.8	16.7 ±3.5	398 ± 26.5

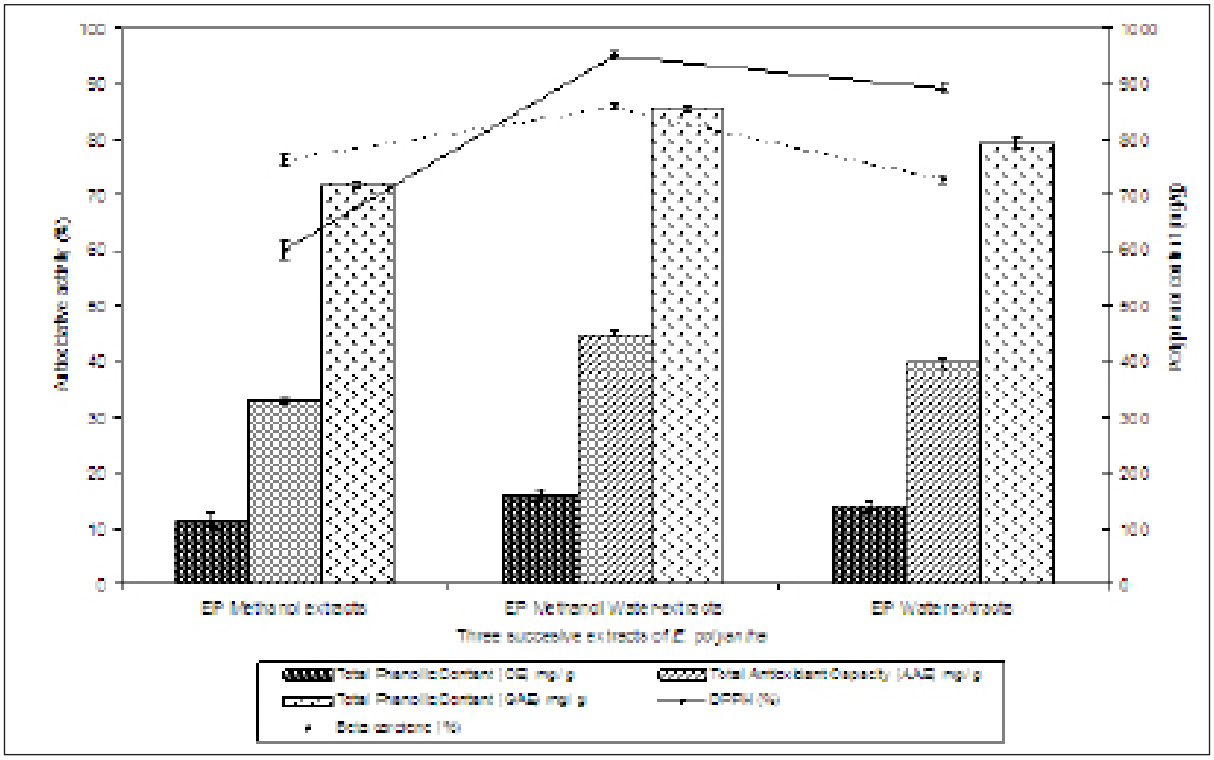

Figure 1. Correlation of antioxidative activities of the three *E. polyantha* bark extracts with total phenolic content and total antioxidative capacity.

Flavonoids, as one of the most diverse and widespread groups of natural compounds, are likely to be the most important natural phenolics. The key role of phenolic compounds as scavengers of free radicals has been emphasized in previous reports.[12]

The three methods of extraction gave significantly different levels of antioxidative activity. They also gave a significantly different GAE and CE and TOAC ($p<0.05$), but not total flavonoid or flavonol content. The total antioxidant activity of the methanol-water extract from *E. polyantha* bark exhibited a positive correlation with DPPH free radical-scavenging activity and protection from beta carotene bleaching. These results are consistent with those of Holosava *et.al.*[13] who found that total phenol content increased with antioxidative activity. Gheldof and Engeseth[14] also reported a linear correlation between phenolic content and antioxidative activity.

Thiobarbituric acid reactive substance lipid peroxidation assay (TBARS)

Lipid oxidation is one of the major problems encountered in meat processing, cooking, and refrigerated storage. It affects the quality of the product due to loss of desirable color, odor and flavor, and a reduced shelf –life.[15] Oxidation of lipids is assessed by the TBARS assay which is based on the reaction between thiobarbituric acid (TBA) and malonaldehyde (MDA) and the production of colored pigment, the concentration of which can be calculated by measuring absorbance at 532 nm.

Data obtained with the TBA assay are presented in Fig. 2. Each of the *E. polyantha* extracts provided protection against lipid peroxidation in both raw and cooked meat. The water soluble was most effective both in cooked and raw meat. All extracts showed diminished activity during 7 days storage under cool conditions. The water extract provided greater protection in the cooked meat (68%) than in raw meat (58%). The cooking process may contribute to the higher protection against lipid peroxidation. Some MDA is formed during the oxidation process; however, most is generated by the decomposition of lipid peroxides during the acid-heat treatment of the assay.[16] MDA is a metabolite derived from lipid peroxidation and has been widely used as an indicator of oxidative damage and meat quality. The TBARS test has been criticized for its low specificity and because it appeared to overestimate results. However, more recent studies show that despite the elevated levels, the TBARS formulation is similar to actual MDA formation.[6]

Antifungal assay and composition of *Eugenia polyantha* essential oil

Essential oil of *E. polyantha* at 10 ppm concentration, demonstrated antifungal activity towards three pathogenic fungi, *Pleurotus osteratus,*

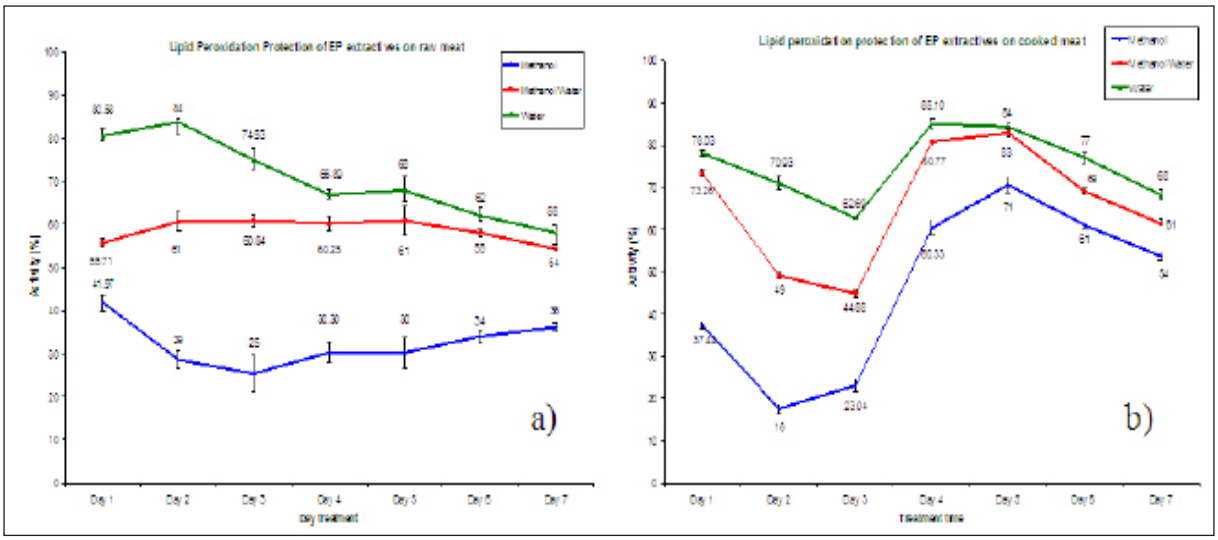

Figure 2. Protection from lipid peroxidation by *E. polyantha* extracts in raw meat [(a)] and coocked meat [(b)] for 7 days treatment.

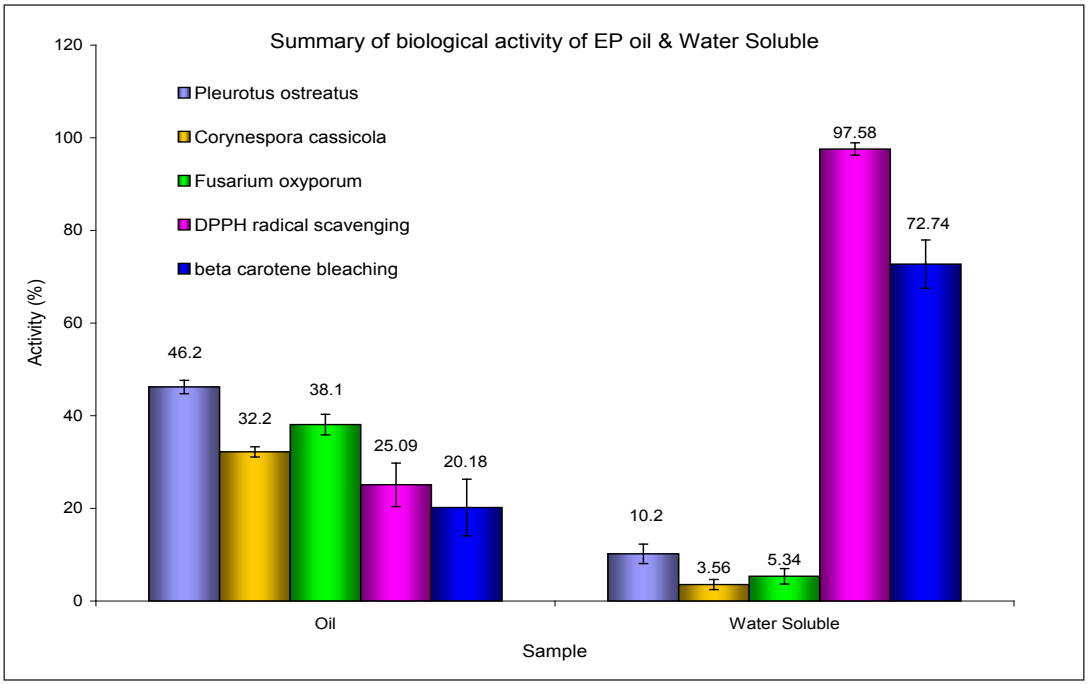

Figure 3. Antifungal activity of the *E. polyantha* essential oils and water extracts

Fusarium oxysporum, and *Coreynespora cassicola*. The oil was extracted from 500 g of fresh leaves in a yield of 670 mg of oil. The oil exhibited higher antifungal activity than the *E. polyantha* water extract, with up to 46% inhibition of *Pleurotus ostreatus*, 38% inhibition of *Fusarium oxysporum* and 32% inhibition of *Corynespora cassicola* as shown in Figure 3 The essential oil also scavenged the DPPH radical and protected against beta carotene bleaching to a small degree.

Identification of *E. polyantha* essential oil compositions were determined using kovats index retention time comparison from the standard. The low temperature rate in the GC was chosen to maximize the separation whereas in high temperature will cause low solubility in the stationary phase.

E. polyantha essential oil compositions are described in Table 2. Octanal (27%), nerolidol (26%), *trans*-undec–4-enal (9%), and *n*-decanoic acid (10%) were identified as the major components. Nerolidol is an important sesquiterpene in the Myerteceae family, which gives distinctive odour of the essential oils. However, it is known that terpene group act as inhibitory substance against several microorganisms due to the ability of the molecule to penetrate the microbial cell.[17] The variation of sensitivity due to varying permeability of the mycelia and spore wall of different fungi tested. In another mechanism, compounds had direct effect on cell membrane by modifying the structural-function properties.

CONCLUSION

The extracts of *E. polyantha* bark had significant antioxidative activity in vitro. The methanol-water extracts had twice radical scavenging activity than other extracts. Total antioxidative capacity and total phenolic content showed positive correlation with the antioxidative activities, i.e.: DPPH-scavenging and beta carotene bleaching assays. *E. polyantha* essential oil demonstrated activity against pathogenic fungi. The further isolation and identification of the individual phenolic compounds responsible for antioxidative activities are needed to understand their mechanisms of action.

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Tabel 2. *E. polyantha* Essential Oil Composition

RI	Compounds name	Composition (%)
704	heptanal	0.30
792	1-heptanol	0.26
831	octanal	27.03
899	2-decen–1-ol	0.08
1014	nonanal	0.36
1080	1-nonanol	0.53
1091	*trans*-undec–4-enal	9.41
1105	decenal	5.35
1182	*unidentified*	0.21
1198	2-undecanone	0.06
1303	*n-decanoic acid*	10.61
1336	*unidentifed*	0.19
1353	*unidentified*	0.47
1364	caryophyllene	0.57
1394	α guaiene	0.76
1402	1H-cycloprop[e]azulene, decahydro–1,1,7-trimethyl–4-methylene-	0.27
1405	longifolene-(V4)	0.35
1473	palustrol	6.09
1477	nerolidol	25.78
1485	α-bisabolol	0.44
1494	α- caryophyllene	0.69
1505	*caryophyllene oxide*	1.24
1541	*τ-cadinol*	1.70
1554	α-cadinol	1.13
1665	*2,6,10-dodecatrien–1-ol, 3,7,11-trimethyl-*	1.35
1867	pentadecanoic acid	1.54
2004	phytol	1.87

RI : retention indices on TC–1 column

REFFERENCES

[1]Aviram, M., Vuletich. J.L. and Osawa. Y. 2000. Enhanced lipid oxidation by oxidatively modified myoglobin: role of protein-bound heme. *Biochemical and Biophysical Research Communications*, 269: 647–651.

[2]Wang, S.Y., *et.al*. 2002. Profiling and characterization antioxidant activities in *Anoectochilus formosanus* Hayata. *Journal of Agricultural and Food Chemistry*, 50: 1859–1865.

[3]Zheng, W., and S.Y. Wang. 2001. Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, 49: 5165–5170.

[4]Dalimartha, S. 2001 Atlas Tumbuhan Obat Indonesia, Trubus Agriwidya. ISBN 979–661–050–7

[5]Punja, Z.K. and R.S. Ukhtede. 2003. Using fungi and yeast to manage vegetable crop diseases. *Trends in Biotechnology*, 21: 400–407.

[6]Ruberto, G., M.T. Baratta, S.G. Deans, and H.J.D Dorman. 2000. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Medica,* 66: 687–693.

[7]Oke, F., B. Aslim, S. Ozturk, and S. Altundag. 2009. Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. *Food Chemistry*, 112: 874–879

[8]Siddhuraju, P., P.S. Mohan, and K. Becker. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flower and fruit pulp. *Food Chemistry*, 79: 61–67.

[9]Kulisic, T., A. Radonic, V. Katalinic, and M. Milos. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, 85: 633–640

[10]Siramon, P and Y. Ohtani, 2007. Antioxidative and antiradical activities of *Eucalyptus camaldulensis* leaf oils from Thailand. *J. Wood of Sci*, 53: 498–504

[11]Osawa, T. 1994. Novel natural antioxidants for utilization in food and biological system. In I. Uritani, V.V. Gracia and E.M. Mendoza (Eds.), *Post harvest biochemistry of plant food-materials in the tropics* (pp. 241–251). Tokyo, Japan: Japan Scientific Societies Press.

[12]Tepe, B. and A. Sokmen. 2007 Screening of the antioxidative properties and total phenolic contents of three endemic *Tanacetum* subspecies from Turkish flora. *Bioresource Technology*, 98: *3076–3079.*

[13]Holasova, M., *et.al.* 2002. Buckwheat-the source of antioxidant activity in functional food. *Food Research International*, 35: 207–211

[14]Gheldof, N. and N.J. Engeseth. 2002. Antioxidant activity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, 50: 3050–3055.

[15]Buckley, D. J., P. A. Morrissey, and J.I. Gray. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal Animal Sci*ence, 73: 3122–3130.

[16]Guillén-Sans and Guzmán-Chozas. 1998. The thiobarbituric acid reaction in foods: a review. *Critical Reviews in Food Science and Nutrition* 38 (4): 315–330.

[17]Rai, M., D. Acharya, P. Wadegaonkar. 2003. Plant derived antimycotics: Potential of Asteraceous plants. *In Plant-Derived Antimycotics Current Trends and Future Prospects*. Food Products Press. pp. 165–195