Foliar application of selenium and humic acid changes yield, essential oil, and chemical composition of *Plectranthus amboinicus* (Lour.) plant and its antimicrobial effects

Ahmed E. El-Gohary[a], Heba M. Amer[a], Salah H. Salem[b], Mohamed S. Hussein[a]

Departments of [a]Medicinal and Aromatic Plants Research, [b]Botany, National Research Centre, Giza, Egypt

Correspondence to Heba M. Amer, PhD, Departments of Medicinal and Aromatic Plants Research, Dokki, Giza 12311, Egypt.
Tel: +20 238 339 394; fax: +20 333 70931;
e-mail: dr.heba_nrc@yahoo.com

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Background and objective
Plectranthus amboinicus is an indigenous vegetable that can be freshly eaten. This plant is used for medicine to cure common illnesses such as cough, stomachache, headache, and skin infection.
Materials and methods
This study was conducted to study the effect of both selenium (2, 4, 8, 12, and 16 g/l) and humic acid (1.5 and 3.00 g/l), in addition to control, which was sprayed with water.
Results and conclusion
Generally, mass production of *P. amboinicus* (Lour.) plants has significantly increased as a result of application of different levels of selenium and humic acid treatments, compared with the control treatment. Essential oil percentage and yield (ml/plant) increased significantly as a result of selenium and humic acid treatments compared with control (S0H0). For essential oil constituents, the results clear that carvacrol (5.96–15.45%) is the first main compound followed by γ-Terpinene (6.74–11.80 %). The third main component is Limonene (3.23–11.32%), whereas the fourth one is α-Muurolene. Moreover, these treatments had a positive effect on selenium, total carbohydrates, photosynthetic pigments, and total phenolic content. Based on scavenging the stable ATBS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical, all treatments increased significantly inhibition % especially S4H2 compared with untreated plants. Antibacterial and antifungal activities of *P. amboinicus* were studied.

Keywords:
ABTS, essential oil, antibacterial, antifungal, *Plectranthus amboinicus*, selenium, humic

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Introduction

Plectranthus amboinicus belongs to the family Lamiaceae. Some *Plectranthus* spp. are cultivated as ornamentals plants, whereas others are used as sources of traditional medicine and food flavorings [1]. Several investigators [2–5] evaluated the quantity and quality of essential oil of *P. amboinicus* plants growing under different locations and conditions, and Pino *et al*. [2] found that the quality and quantity among the oil samples was greatly influenced by extraction methods of the oils, as well as the locality of the plant [3]. Essential oil of *P. amboinicus* possesses antimicrobial [4,6–8], insecticidal [3], and antileptospiral [9] activities.

Selenium is an essential trace element for humans, animals, and some species of microorganisms [10]. The selenium element acts to prevent cancer [11], such as colon and mammary tumors [12] as well as prostate cancer [13]. It has an important protective role in immunity, arthritis, and atherosclerosis, as well as improving fertility [14]. Although it replaces the sulfur in the amino acids, it seems that Se is not confirmed to be required by higher plants. In this connection, several authors [15,16] reported that low concentration of selenium element plays an important role in hormone balance and antioxidative reactions in plant cells through enhancing glutathione peroxidase activity. Selenium as foliar application was shown to have more effect than application of fertilizers [17]. In tea leaves and rice, Xu *et al*. [18] as well as Xu and Hu [19] found that supplementation of Se to plants increases antioxidant activity of the plants, which improves the production and quality of edible plant products. Humic acid has numerous benefits for crop production. Humic acid is used for plant nutrition, for improving plant growth by improving absorption of elements, and making them available to plant [20]. Humic acid has great positive effects on cell membrane

Table 1 Mechanical and chemical properties of the soil

Texture	pH	E.Ce (ds/m)	Available nutrients (mg/100 g)			Water-soluble ions (meq/l) in the soil paste				
			N	P	K	Ca	Mg	Fe	Zn	Mn
Clay loam	7.0	6.9	12.4	0.463	3.3	9.4	12.2	2.6	4.1	1.8

functions by biosynthesis of nucleic acid, respiration, promoting nutrient uptake, ion absorption, and enzyme because they are hormone-like substances [21]. Humic acid improves plant hormones and responsiveness, because it inhibits indole acetic acid oxidase activity, leading to increased IAA hormone activity, which encourages plant growth [22,23].

So, this investigation was carried out to evaluate the response of *P. amboinicus* plant to selenium and humic acid. Moreover, this study included the antimicrobial effects of this plant.

Materials and methods

This study was conducted to examine the effect of selenium and humic acid on yield and essential oil production of *P. amboinicus* plant.

Plant materials

Uniform seedlings of *P. amboinicus* were obtained from the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt. On February 3, 2017 in the first season and February 7, 2018 in the second season, the seedlings were placed in plastic pots (30 cm height×25 cm diameter) filled with 12 kg of soil. The pots were kept outdoor under natural environmental conditions. The seedlings were thinned twice, leaving one plant per pot. The seedlings were irrigated, and the soil was kept moist.

Site description and experimental design

This study was carried out at the National Research Centre, Dokki, Cairo, Egypt, during the two successive seasons of 2017 and 2018. The experimental sites were situated at 30° 0' 47.0016" N DMS Lat., 31° 12' 31.8708" E DMS long, and elevation of 24 m. Experiments were carried out according to one-way randomized blocks design, with three replications.

Weather data

Giza experiences a hot desert climate like arid climate. Its climate is similar to Cairo, owing to its proximity. Wind storms can be frequent across Egypt in spring, bringing Saharan dust into the city during the months of March and April. High temperatures in winter range from 16 to 20°C, whereas night time lows drop to below 7C. In summer, the highs are 40C, and the lows can drop to about 20C. Rain is infrequent in Giza; snow and freezing temperatures are extremely rare.

Table 2 Guaranteed analysis and physical data of humic acid total

Guaranteed analysis	
Humic acid	80%
Potassium (K_2O)	10–12%
Zn, Fe, Mn, etc	100 ppm
Physical data	
Appearance	Black powder
pH	9–10%
Water solubility	>98%

Based on the climatic conditions, the plants were irrigated, where they were irrigated with water to maintain near field capacity. Physical and chemical properties of the soil used in this study were determined and are presented in Table 1.

Treatments

Three weeks later after transplanting, the plants were sprayed with aqueous solution of the test nutrient compounds, selenium and humic acid. Foliar application was repeated after 2 weeks from first cut. Humic acid was produced by Leili Agrochemistry Co. Ltd (Beijing, China), and its properties are shown in Table 2. Selenium was supplied as sodium selenate in this experiment.

Treatments that were carried out can be summarized as follows:

S0H0 (Foliar application with water).
S1H1 (Foliar application with selenium at 2 g^{-1}+humic acid at 1.50 g^{-1}).
S1H2 (Foliar application with selenium at 2 g^{-1}+humic acid at 3.00 g^{-1}).
S2H1 (Foliar application with selenium at 4 g^{-1}+humic acid at 1.50 g^{-1}).
S2H2 (Foliar application with selenium at 4 g^{-1}+humic acid at 3.00 g^{-1}).
S3H1 (Foliar application with selenium at 8 g^{-1}+humic acid at 1.50 g^{-1}).
S3H2 (Foliar application with selenium at 8 g^{-1}+humic acid at 3.00 g^{-1}).
S4H1 (Foliar application with selenium at 12 g^{-1}+humic acid at 1.50 g^{-1}).
S4H2 (Foliar application with selenium at 12 g^{-1}+humic acid at 3.00 g^{-1}).
S5H1 (Foliar application with selenium at 16 g^{-1}+humic acid at 1.50 g^{-1}).

S5H2 (Foliar application with selenium at 16 g^{-1}+humic acid at 3.00 g^{-1}).

Harvesting and Sampling procedure

Two cuts (harvests) were carried out during the two successive seasons of study. The first cut was carried out after 3 months of transplanting, and the second cut was done after 2 months from the first one.

Data recorded

The following data were recorded:

(1) Growth and yield characteristics: such as herb fresh and dry weights.
(2) Percentage and yield of oil: the percentages of volatile oil were determined in the fresh herb using 100-g samples for each cut per plant. The volatile oil of air dried herb was extracted by water distillation method according to Guenther *et al*. [24]. The extracted essential oil was dehydrated over anhydrous sodium sulfate and stored in the freezer till use for gas chromatography-mass spectrometry analysis. Essential oil yield was calculated and expressed as ml/plant.
(3) Gas liquid chromatographic analysis of essential oil constituents:
The identification of the components of essential oil constituents was carried out using gas liquid chromatography on a Hewlett Packard Model 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped under the following conditions:
   (a) Separation was done on an INNO wax polyethylene glycol, Model No. 19095 N-123, 240°C maximum, capillary column 30.0 m×530 µm×1.0 µm, nominal flow 15 ml/min, with average velocity 89 cm/s and pressure 8.2 psi. Column temperature was 240°C with temperature programming as follows: initial temperature 100–240°C maximum, with 10°C rising for each minute, and then hold at 240°C for 10 min.
   (b) Injection temperature 280°C, back inlet, with split ratio 8 : 1, split flow 120 ml/min., and gas saver 20 ml/min.
   (c) Carrier gas was nitrogen with flow rate 15 ml/min.
   (d) Flame ionization detector temperature 280°C.
   (e) Hydrogen flow rate 30 ml/min.
   (f) Air flow rate 300 ml/min.
(4) Total Se determination:

Before Se determination, all samples were digested, where it is an acceptable matrix for consistent recovery of Se, which is compatible with the analytical method [25]. Se analyses were performed on Agilent 5100 Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) with Synchronous Vertical Dual View (SVDV) using hydride generation, Agilent Vapor Generation Accessory VGA 77. For each series of measurements, intensity calibration curve was constructed composed of a blank and three or more standards from Merck company (Germany). Accuracy and precision of the Se measurements were confirmed using external reference standards from Merck, and standard reference materials for trace elements in water and quality control sample from National Institute of Standards and Technology (NIST) were used to confirm the instrument reading.

Determination of photosynthetic pigments

Extraction of pigments was carried out in stoppered tubes according to Costache *et al*. [26]. Vegetable samples were prepared with a laboratory homogenizer using about 0.5 g sample in 20 ml 90% aqueous methanol solution. Homogenized mixture was separated by centrifugation at 3000 rpm for 10 min. The analytical determination was performed with Helios α spectrophotometer at the following wavelengths: 645, 653, 662 and 664 nm for chlorophyll a and b (according to each extraction solvent) and 470 nm for carotene. Equations used for calculation are as follows: Chlorophyll a=15.65 A666–7.340 A653,

Chlorophyll b=27.05 A653–11.21 A666, and

Carotene=1000 A470–2.860 Chl a–129.2 Chl b/245.

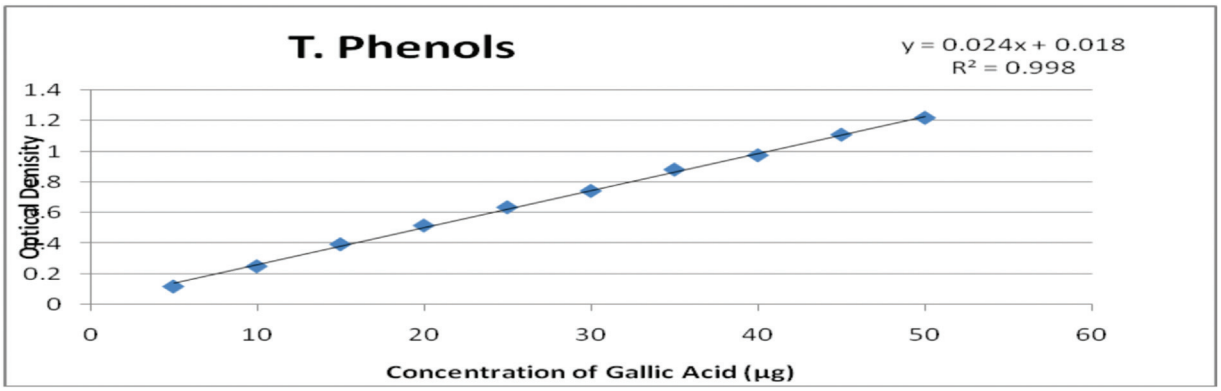
Determination of total carbohydrates (%)

Total carbohydrates in the dried herb were determined according to Dubois *et al*. [27]. Overall, 5 ml of 67% sulfuric acid was added to 0.03 g of dry plant in a test tube. After 1 h, the volume was completed to 100 ml with distilled water, and the solution was filtered. A volume of 1 ml of the filtrate was pipetted into a test tube and aqueous phenol solution (5%) was added to the solution, followed by 5 ml of concentrated H_2SO_4. The color intensity was recorded using Roy colorimeter (model Spectronic 21 D) at 490 nm. The total carbohydrate content was determined by using the standard curve of glucose.

Determination of total phenolic content

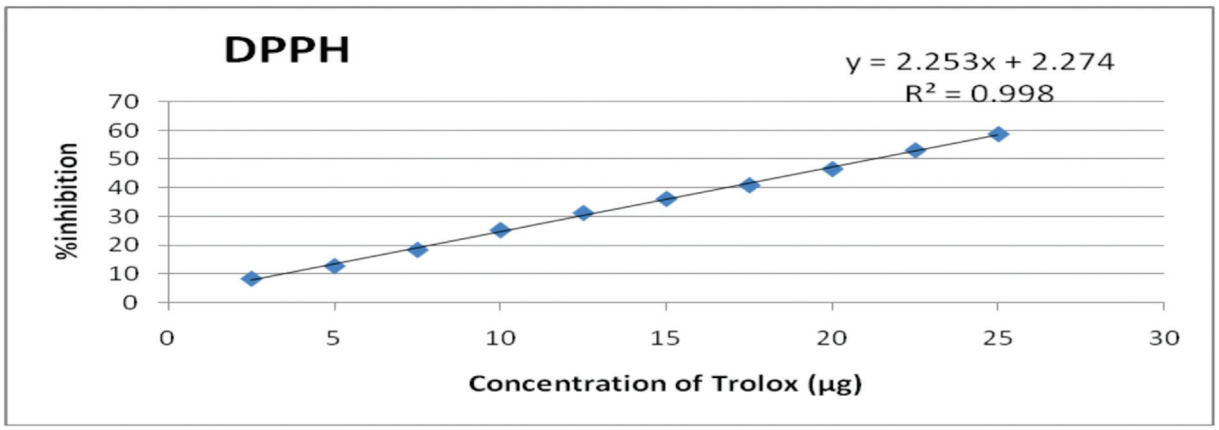
The total phenolic content was determined according to the Folin-Ciocalteu procedure [28]. In brief, the extract (100 µl) was transferred into a test tube, and the volume was adjusted to 3.5 ml with distilled water and oxidized with the addition of 250 µl of Folin-Ciocalteu

Figure 1



Calibration curve constructed from known concentrations of gallic acid.

Figure 2



Calibration curve constructed from known concentrations of trolox.

reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous sodium carbonate (Na_2CO_3) solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid (Fig. 1) and expressed as µg of gallic acid equivalent (mg GAE) per g of sample.

Determination of radical DPPH scavenging activity

Free radical scavenging capacity of extracts was determined using the stable DPPH* according to Hwang and Do Thin [29]. The final concentration was 200 µmol/l for DPPH*, and the final reaction volume was 3.0 ml. The absorbance was measured at 517 nm against a blank of pure methanol after 60 min of incubation in a dark condition. Percent inhibition of the DPPH free radical was calculated by the following equation: inhibition (%)=100×[(acontrol−asample)/acontrol]

where acontrol is the absorbance of the control reaction (containing all reagents except the test compound), and asample is the absorbance with the test compound.

The standard curve was prepared using Trolox (Fig. 2). Results were expressed as µg Trolox equivalents (TE)/g sample.

Antimicrobial effect

Antimicrobial activity was carried out according to the methods described by Bauer *et al.* [30] and EUCAST [31]. Five pathogenic bacteria, comprising *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* as gram-negative organisms as well as *Staphylococcus aureus* and *Bacillus cereus* as a gram-positive ones, were used for antibacterial assay. The tested bacterial species were grown on Tryptic soya broth tubes and incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually after four hours of incubation). A uniform bacterial layer was developed on the surface of solidified nutrient agar plates using the adjusted bacterial suspension and sterile cotton swabs and left to dry. Whatman filter

paper no. 1 is used to prepare discs of 6 mm, which are impregnated with tested oil from different treatments. The impregnated discs were applied on the surface of streaked nutrient agar plates. Dimethyl sulfoxide was used as a negative control, whereas 1 mg/ml Ceftriaxone was used as a positive control. The plates were inverted and incubated at 35°C for 16–18 h.

Moreover, five fungal species, comprising *Aspergillus flavus* NRRL 3357, *Aspergillus carbonarius* ITAL 204, *Aspergillus ochraceus* ITAL 14, *Fusarium proliferatum* MPVP 328, and *Penicillium verrucosum* BFE 500, were used for antifungal assay. The antifungal assay was conducted using disc diffusion technique and potato dextrose agar (PDA) media. The tested fungal species were subculture and grown into PDA for 5–7 days and the spore suspension was prepared by transferring a loopful of grown tested fungi to test tube containing 10 ml of 0.01% tween 80 solution. From spore suspension, 100 µl was spread on the solidified PDA plates using glass rod, and the plates were left to dry for half an hour. The oil-impregnated discs were applied onto the surface of the dry plates. Dimethyl sulfoxide was used as a negative control, whereas Miconazole (Sigma-Aldrich) with concentration of 1.0 mg/ml was used as a positive control. The plates were inverted and incubated at 25°C for 24–48 h.

After incubation, the inhibition zones were measured including the diameter of the disc. Zones are measured to the nearest millimeter using a ruler which is held on the back of the inverted petri plates. All treatments consisted of three replicates, and the results were expressed as mean±SE.

Statistical analysis

Data were combined over the two seasons for statistical analysis. All recorded data was subjected to analysis of variance procedures, and treatment means were compared using least significant differences at 5%, as described by Snedecor and Cochran [32].

All data were subjected to analysis of variance and significant means were compared with Duncan multiple range test method, performed using SPSS package.

Results and discussion

Results of variance analysis showed that selenium and humic acid treatments had significant effect (P=0.05) on different traits of *P. amboinicus* (Lour.) under study (Tables 3–5).

Table 3 Summary analysis of variance at 5% for yield traits and essential oil of *Plectranthus amboinicus* (Lour.) plant (first cut), mean values of two seasons

Source	*d.f.*	Herb fresh weight (g/plant)	Herb dry weight (g/plant)	Essential oil %	Essential oil yield (ml/plant)
Replication	2	1.73	5.48	0.00007	0.0000008
Treatments	10	20 064.87***	300.84***	0.00044***	0.00098***
Error	20	4.32	6.38	0.000045	0.000001
CV%		0.27	3.13	6.27	1.15

CV%, coefficient of variation %.

Table 4 Summary analysis of variance at 5% for yield traits and essential oil of *Plectranthus amboinicus* (Lour.) plant (seocnd cut) mean values of two seasons

Source	*d.f.*	Herb fresh weight (g/plant)	Herb dry weight (g/plant)	Essential oil %	Essential oil yield (ml/plant)
Replication	2	47.73	2.27	0.0000044	0.000013
Treatments	10	23 903.78***	388.10***	0.00025***	0.000941***
Error	20	47.43	3.74	0.0000055	0.0000027
CV%		0.98	2.49	2.19	2.04

CV%, coefficient of variation %.

Table 5 Summary analysis of variance at 5% for total herb fresh and dry yield as well as total essential oil yield of *Plectranthus amboinicus* (Lour.) plant (first+second cuts) mean values of two seasons

Source	*d.f.*	Herb fresh weight (g/plant)	Herb dry weight (g/plant)	Essential oil yield (ml/plant)
Replication	2	37.18	12.75	0.000020
Treatments	10	82 607.20***	1281.85***	0.00358***
Error	20	42.98	6.2538212	0.000006
CV%		0.44	1.61	1.47

CV%, coefficient of variation %.

Table 6 Herb fresh and dry weights of *Plectranthus amboinicus* (Lour.) at different levels of selenium and humic acid (mean values of two successive seasons)

Treatments	First cut		Seocnd cut		Total herb fresh weight (g/plant)	Total herb dry weight (g/plant)
	Herb fresh weight (g/plant)	Herb dry weight (g/plant)	Herb fresh weight (g/plant)	Herb dry weight (g/plant)		
S0H0	553.00^{f}	55.54^{d}	514.68^{h}	54.04^{h}	1067.68^{j}	109.58^{h}
S1H1	709.25^{e}	72.34^{c}	612.05^{g}	62.43^{g}	1321.3^{i}	134.77^{g}
S1H2	793.55^{c}	79.67^{b}	712.33^{d}	80.49^{c}	1505.88^{f}	160.16^{d}
S2H1	784.40^{d}	78.44^{b}	638.63^{f}	63.86^{g}	1423.03^{h}	142.3^{f}
S2H2	796.80^{c}	79.68^{b}	733.08^{c}	73.31^{e}	1529.88^{e}	152.99^{e}
S3H1	797.70^{c}	81.98^{b}	679.75^{e}	69.86^{f}	1477.45^{g}	151.51^{e}
S3H2	826.50^{a}	87.32^{a}	790.00^{b}	83.97^{a}	1616.5^{b}	176.29^{a}
S4H1	822.00^{b}	87.95^{a}	719.20^{d}	76.95^{d}	1541.2^{d}	164.90^{c}
S4H2	829.40^{a}	88.05^{a}	803.00^{a}	88.33^{a}	1632.4^{a}	176.38^{a}
S5H1	821.80^{b}	89.24^{a}	738.00^{c}	81.18^{c}	1559.8^{c}	170.42^{b}
S5H2	818.75^{b}	88.98^{a}	809.00^{a}	84.91^{b}	1627.75^{a}	173.88^{b}
CV%	0.268	3.127	0.978	2.487	0.44	1.61

CV%, coefficient of variation %. Means with the same letters in each column indicate no significant difference between treatments at 5% level of probability.

Yield characters

Data presented in Table 6 clear that the herb fresh and dry weights of *P. amboinicus* (Lour.) plants were increased significantly as a result of different treatments of selenium and humic acid application in first and second cuts comparing with untreated plants. During the first cut, the highest values of herb fresh weight (829.40 g/plant) and herb dry weight (89.24 g/plant) were noticed with S4H2 and S5H1 treatments, respectively. S5H2 and S4H2 treatments caused the maximum mean values of fresh weight (809.00 g/plant) and dry weight (88.33 g/plant) during the second cut, respectively. Concerning the effect of these treatments on total yield (first cut +second cut), it is clear that S4H2 treatment gave the highest mean values of herb fresh weight (1632.40 g/plant) and herb dry weight (176.38 g/plant). Several authors reported that selenium has a stimulating effect on fresh and dry weights of plants [33–36]. The results obtained by Hartikainen *et al.* [37] asserted that selenium interaction with plants depends on its concentration. On *Brassica napus* plants, Bansal *et al.* [38] found that different concentrations of Se had a pronounced effect on vegetative and reproductive growth compared with untreated plants (control), which caused difference in the content of dry matter. The positive effect of humic acid treatment on *P. amboinicus* (Lour.) plants growth characteristics may be owing to increasing the content of the soil nutrients that are available for the growth and stimulate meristem tissue growth by increasing the physiological processes related to photosynthesis, which is reflected in the increase in plant herb fresh and dry weight of the plant. The results are in harmony with those obtained by Pizzeghello *et al.* [39] and Nikbakht *et al.* [40] on *Fagus sylvaticae* and gerbera, respectively.

Essential oil percentage and yield (ml/l)

Essential oil percentage and yield (ml/plant) increased significantly as a result of selenium and humic acid treatments compared with control (S0H0) (Table 7). For instance, essential oil percentage and yield in the control were recorded 0.085 and 0.095%, and 0.047 and 0.051 ml/plant for first and second cuts, respectively. All treatments tended to increase essential oil percentage and yield (ml/plant) as compared with the control treatment (S0H0) in both cuts. The highest mean values of essential percentage and yield were obtained from plants treated with S4H2 treatment during both cuts. The total yield of essential oil (first cut+second cut) reached to its maximum mean value (0.212 ml/plant) as a result of S4H2 treatment. Misra *et al.* [41] reported that the increment of essential oil percentage as a result of Se treatments may be because Se can accelerate the secondary product metabolism in plants, especially essential oil. The same author found that Se had a pronounced effect on CO_2 assimilation level, photosynthetic pigments content, and ultimately the accumulation of geranium essential oil. The aforementioned results are in agreement with those obtained by Lee *et al.* [42], who revealed that Se caused an increment in essential oil of basil and lemon balm from two to three folds compared with untreated plants. The increment of essential oil yield as a result of Se and humic acid treatments may be owing to enhancement of herb weight and/or essential oil percentage. Burbott and Loomis [43] reported that humic acid may accelerate metabolic reactions and stimulate enzymatic systems responsible for the biosynthesis of essential oil and its constituents. Similar results were obtained by several

Table 7 Essential oil percentage and yield (ml/plant) of *Plectranthus amboinicus* (Lour.) at different levels of selenium and humic acid (mean values of two successive seasons)

Treatments	First cut		second cut		Total essential oil yield (ml/plant)
	Essential oil %	Essential oil yield (ml/plant)	Essential oil %	Essential oil yield (ml/plant)	
S0H0	0.085^{c}	0.047^{i}	0.095^{d}	0.051^{i}	0.098^{h}
S1H1	0.085^{c}	0.061^{h}	0.095^{d}	0.059^{h}	0.120^{g}
S1H2	0.100^{b}	0.080^{g}	0.095^{d}	0.076^{e}	0.156^{f}
S2H1	0.115^{b}	0.090^{f}	0.102^{c}	0.065^{g}	0.155^{f}
S2H2	0.115^{b}	0.092^{e}	0.105^{c}	0.077^{e}	0.169^{e}
S3H1	0. 115^{b}	0.094^{d}	0.105^{c}	0.073^{f}	0.167^{e}
S3H2	0. 115^{b}	0.100^{b}	0.110^{b}	0.098^{b}	0.198^{b}
S4H1	0.110^{b}	0.097^{c}	0.115^{a,b}	0.088^{d}	0.185^{d}
S4H2	0.120^{a}	0.106^{a}	0.120^{a}	0.106^{a}	0.212^{a}
S5H1	0.110a^{b}	0.098^{b,c}	0.115^{a,b}	0.093^{c}	0.191^{c}
S5H2	0.110a^{b}	0.098^{b,c}	0.115^{a,b}	0.098^{b}	0.196^{b}
CV%	6.271	1.153	2.192	2.041	1.49

CV%, coefficient of variation %. Means with the same letters in each column indicate no significant difference between treatments at 5 % level of probability.

authors, that is, Vafa *et al.* [44] on savory, El-Sayed *et al.* [45] on basil, and Said-Al Ahl Hah *et al.* [46] on fennel.

Essential oil constituents

Data presented in Table 8 show the effect of selenium and humic acid treatments on the essential oil constituents during the second cut. The number of identified constituents in the essential oil samples ranged from 24 to 27 components, constituting from 75.12 to 98.35% of the total oil composition. Data show that total mono-oxygenated compounds formed a minor fraction. It can be noticed that total hydrocarbon compounds ranged from 49.53 to 66.61%, whereas total oxygenated compounds ranged from 25.59 to 37.62%. Carvacrol (5.96–15.45%) is the first main compound followed by γ-Terpinene (6.74–11.80%). The third main component is Limonene (3.23–11.32%), whereas the fourth one is α-Muurolene. The maximum relative percentage of Carvacrol (15.45%) was obtained from plants treated with S4H1 whereas S0H0 gave the lowest one. Murthy *et al.* [6] recorded carvacrol was the major (70%) component, whereas Selvakumar *et al.* [47] reported much lower carvacrol composition (13–14%). In this respect, Swamy *et al.* [48] reported that essential oil components vary according to many factors, such as climate, geographical features, and date of collection. In India, Senthilkumar *et al.* [49] found that carvacrol (28.65%), thymol (21.66%), a-humulene (9.67%), undecanal (8.29%), and c-terpinene (7.76%) were the main components of *P. amboinicus,* whereas in Brazil, the main constituents of *P. amboinicus* essential oil were carvacrol and thymol [50]. In Malaysia, 3-carene (20.78%), a-terpinene (6.04%), o-cymene (5.06%) c-terpinene (8.94%), camphor (17.96%), and carvacrol (19.29%) were reported to be the major constituents [9]. Moreover, in Morocco, Hassani *et al.* [51] found that the main essential oil constituents were carvacrol (23.0%) and camphor (22.2%).

Selenium content (mg/kg)

Data presented in Table 9 indicate that selenium content increased continuously with the increasing rate of foliar applications of Se and humic acid. Foliar application with S5H2 had more positive effect on Se content (91.25 mg/kg) compared other treatments, followed by S5H1 (90.25 mg/kg).

Photosynthetic pigments (mg/g)

Some selenium and humic acid treatments had a positive significant effect on chlorophyll A and B content (mg/g) compared with untreated plants (Table 9). Plants treated with S5H1 gave the highest mean values of chlorophyll A (1.20 mg/g) and B (1.00 mg/g). The lowest mean values of chlorophyll A (0.18 mg/g) and B (0.27 mg/g) were from untreated plants. Moreover, all selenium and humic acid treatments increased significantly total carotenoid content (mg/g) carotenoids, as shown in Table 10. Total carotenoid content reached its maximum mean values (114.5 mg/g) from plants treated with S5H2. Similar results were obtained by Nancy and Arulselvi [52] as well as Mozafariyan and Pessarakliand [53] who revealed that Se treatments increased chlorophyll content. This may be owing to the repression membrane protein transporters.

Carbohydrates content (%)

Application of S5H2 significantly enhanced the accumulation of carbohydrate content (%), as shown

Table 8 Essential oil constituents of *Plectranthus amboinicus* (Lour.) at different levels of selenium and humic acid during the second cut

No	Components	KI	Formula	S0H0	S1H1	S1H2	S2H1	S2H2	S3H1	S3H2	S4H1	S4H2	S5H1	S5H2
1	β-Pinene	980	C10H16	0.98	1.11	0.87	1.53	0.49	1.34	1.27	1.17	1.31	1.36	1.29
2	α-Terpinene	1017	C10H16	0.22	1.13	0.81	0.76	0.75	1.16	0.88	0.74	1.21	1.26	1.08
3	Cymene	1024	C10H14	2.55	8.39	6.13	6.07	4.69	8.41	6.39	4.95	8.36	8.86	7.38
4	Limonene	1932	C10H16	8.03	9.13	6.92	6.44	3.23	11.32	8.24	5.32	8.30	8.46	6.05
5	1,8 Cineol	1035	C10H18O	–	0.30	0.26	0.22	–	0.18	0.39	0.23	0.37	0.36	–
6	Trans-β Ocimene	1050	C10H16	0.57	1.00	0.72	1.15	0.50	1.15	0.83	0.68	0.91	1.02	0.98
7	γ-Terpinene	1066	C10H16	8.64	11.80	9.69	8.78	6.74	10.51	9.74	8.72	11.07	11.46	10.67
8	Terpinolene	1096	C10H16	0.63	0.66	0.63	–	–	0.62	0.73	0.69	0.60	0.55	0.57
9	Linalool	1103	C10H18O	0.16	1.53	1.42	2.19	0.76	1.59	1.99	1.70	1.47	1.36	1.02
10	Terpine-4-ol	1187	C10H18O	2.93	0.65	0.65	0.86	0.45	0.66	1.00	0.80	0.71	0.61	0.51
11	Carvacrol	1310	C10H14O	5.96	11.54	12.67	15.33	13.21	11.19	11.88	15.45	11.76	11.87	11.90
12	β-Bourbonene	1392	C15H24	2.12	3.46	4.19	4.31	4.42	4.00	3.83	4.07	3.40	3.70	3.40
13	β-Caryophellene	1407	C15H24	0.71	0.77	0.95	0.77	0.69	0.80	0.78	0.94	0.80	0.81	0.79
14	β-Copaene	1429	C15H24	0.39	0.35	0.44	0.46	0.48	0.41	0.40	0.43	0.36	0.39	0.38
15	Aroma dendrene	1441	C15H24	5.21	5.44	6.52	5.39	6.75	5.20	5.76	6.57	5.23	5.55	5.40
16	y-Muurolene	1475	C15H24	5.51	5.68	6.58	6.33	7.74	5.98	5.79	6.71	5.37	5.64	6.01
17	β-Selenine	1493	C15H24	0.29	0.40	0.27	0.34	0.42	0.31	0.25	0.32	0.40	0.42	0.39
18	α-Muurolene	1502	C15H24	7.31	7.74	9.78	10.47	8.56	7.78	8.15	9.48	8.77	8.56	8.51
19	γ-Cadinene	1508	C15H24	0.35	0.32	0.40	0.37	0.43	0.35	0.37	0.38	0.35	0.36	0.36
20	Transcycloisolongifol-5-ol	1516	C15H24O	1.11	1.40	1.68	1.40	1.45	1.02	1.46	1.35	1.50	1.49	1.47
21	δ-Cadinene	1541	C15H24	6.02	6.26	7.76	8.00	9.25	7.27	6.95	7.85	6.49	6.66	6.65
22	Caryophellene oxide	1599	C15H24O	0.48	0.62	0.69	0.68	0.45	0.76	0.82	0.64	0.66	0.57	0.61
23	Cis-asarone	1607	C12H16O3	–	0.34	0.38	–	–	0.46	0.45	0.40	0.35	0.32	0.36
	Epi-α-Muurolol	1650	C15H26O	0.69	0.73	0.68	0.89	1.53	0.95	0.92	1.13	0.71	0.72	0.71
24	α-Cadinol	1663	C15H26O	5.67	4.03	3.97	3.63	5.62	3.25	4.53	4.93	4.16	3.46	4.09
25	δ-Cadinol	1678	C15H26O	6.44	6.33	6.34	8.78	9.53	7.76	6.61	7.94	6.59	5.46	6.19
26	oplupanone	1738	C15H26O	0.98	0.94	0.90	1.21	1.42	0.98	0.66	1.07	0.83	0.81	0.82
27	α-Muurolene-4-hydroxy		C15H24O	1.17	1.56	1.72	1.99	2.56	1.72	1.40	1.98	1.60	1.55	1.51
Total identified compounds				75.12	93.61	94.02	98.35	92.12	97.13	92.47	96.64	93.64	93.64	89.10
Total hydrocarbons compounds				49.53	63.64	62.66	61.17	55.14	66.61	60.36	59.02	62.93	65.06	59.91
Total oxygenated compounds				25.59	29.97	31.36	37.18	36.98	30.52	32.11	37.62	30.71	28.58	29.91
Total monohydrocarbons compounds				21.62	33.22	25.77	24.73	16.4	34.51	28.08	22.27	31.76	32.97	28.02
Total monooxygenated compounds				9.05	14.02	15	18.6	14.42	13.62	15.26	18.18	14.31	14.2	13.79
Total sesquiterpene hydrocabon compounds				27.91	30.42	36.89	36.44	38.74	32.1	32.28	36.75	31.17	32.09	30.89
Total oxygenated sesquiterpene compounds				16.54	15.95	16.36	18.58	22.56	16.9	16.85	19.44	16.4	14.38	16.12

in Table 9. Moreover, increasing levels of Se and humic acid increased carbohydrate percentage compared with untreated plants. The increment of carbohydrates content could be explained by increasing the content of the soil nutrients which resulted in the increased activity of microorganisms, which increased plant nutrients that improved the efficiency of photosynthesis. All these effects positively reflected on the plant yield and also increased the active ingredient characteristics.

Total phenolic content

Total phenol compounds were reported as gallic acid equivalents by reference to standard curve (y=0.024x +0.018,r^2=0.998). The effect of selenium and humic acid treatments on total phenolic compounds was significant ($P \leq 0.05$), as shown in Table 9. Generally, selenium and humic acid treatments increased total phenolic compounds compared with untreated plants. The maximum mean values of phenolic compounds of leaves (8.2 mg/g) were obtained from plants treated with S4H2 followed by S4H1, which recorded 8.2 mg/g, whereas S3H1 gave the highest mean values of total phenolic compounds (5.5 mg/g) for leaves followed by plants treated with S2H2, which gave 5.4 mg/g. From these data, it can be noticed that total phenolic compounds of leaves gave great values compared with those obtained from stem.

Regarding phenolic content, Lewis *et al.* [54] reported that total phenolic compound content plays an important role for mechanism in the regulation of plant metabolism and consequently of overall plant growth. Moreover, Khattab [55] found that phenolic compounds act as a substrate for many antioxidant enzymes. Similar results were found by other authors

Table 9 Pigments, carbohydrates, total phenols, and ABTS of *Plectranthus amboinicus* (Lour.) at different levels of selinium and humic acid (mean values of two successive seasons)

Treatments	Pigments (mg/g)			Carbohydrates (%)	Total phenols (μg GAE/g)		ABTS %inhibition	
	A	B	C		Leave	Stem	Leave	Stem
S0H0	0.2^{d}	0.3^{d}	35.2^{i}	16.5^{i}	3.0^{d}	1.5^{b}	4.0^{d}	2.7^{d}
S1H1	0.2^{d}	0.3^{d}	39.1^{h}	18.67^{h}	$3.6^{c,d}$	$3.0^{a,b}$	4.6^{d}	4.3^{d}
S1H2	0.3^{d}	0.3^{d}	47.1^{g}	$20.0^{g,h}$	$3.7^{c,d}$	$3.0^{a,b}$	$5.6^{c,d}$	4.6^{d}
S2H1	0.3^{d}	0.3^{d}	47.0^{g}	$21.7^{f,g}$	$4.7^{b,c,d}$	$3.4^{a,b}$	$5.7^{c,d}$	4.4^{d}
S2H2	0.3^{d}	0.3^{d}	49.5^{g}	23.0^{f}	$5.0^{b,c,d}$	5.4^{a}	$5.6^{c,d}$	6.5^{c}
S3H1	0.5^{c}	0.3^{d}	54.3^{f}	$25.7^{6,e}$	$4.3^{c,d}$	5.5^{a}	6.6^{c}	10.3^{b}
S3H2	0.5^{c}	0.6^{b}	73.5^{e}	27.67^{d}	$4.8^{b,c,d}$	5.1^{a}	$11.9^{a,b}$	10.9^{b}
S4H1	$1.1^{a,b}$	0.5^{c}	92.8^{d}	30.00^{c}	8.1^{a}	5.3^{a}	$11.9^{a,b}$	11.2^{b}
S4H2	1.0^{b}	0.5^{c}	100.3^{c}	32.0^{b}	8.2^{a}	$4.0^{a,b}$	13.1^{a}	13.3^{a}
S5H1	1.2^{a}	1.0^{a}	108.2^{b}	33.0^{b}	$6.0^{a,b,c}$	$3.9^{a,b}$	10.4^{b}	1.03^{b}
S5H2	$1.1^{a,b}$	0.6^{b}	114.5^{a}	35.7^{a}	$6.9^{a,b}$	$4.2^{a,b}$	11.1^{b}	10.9^{b}
CV%	10.8	7.2	1.8	3.9	18.9	8.6	25.8	10.5

CV%, coefficient of variation %.

Table 10 Antibacterial activity of *Plectranthus amboinicus* (150 mg/ml (w/v) in dimethyl sulfoxide

	Treatment	Inhibition zone diameter (mm) (mean±SE)				
		Bacillus cereus	*Staphylococcus aureus*	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	S0H0	$16.0\pm2.00^{a,b}$	$14.0\pm1.00^{a,b}$	$9.33\pm0.67^{d,e,f}$	8.00 ± 0.00^{d}	0.0 ± 0.00^{b}
2	S1H1	17.67 ± 1.45^{a}	$14.33\pm0.33^{a,b}$	$10.0\pm1.15^{c,d,e}$	10.67 ± 1.33^{b}	0.0 ± 0.00^{b}
3	S1H2	$15.0\pm0.58^{a,b,c}$	$14.00\pm0.58^{a,b}$	$10.0\pm0.00^{c,d,e}$	$9.33\pm0.67^{b,c,d}$	0.0 ± 0.00^{b}
4	S2H1	$15.0\pm0.58^{a,b,c}$	$13.67\pm0.33^{a,b}$	$9.33\pm0.67^{d,e,f}$	8.00 ± 0.00^{d}	0.0 ± 0.00^{b}
5	S2H2	$15.67\pm1.30^{a,b}$	$14.67\pm0.33^{a,b}$	$9.67\pm0.67^{c,d,e,f}$	8.00 ± 0.00^{d}	0.0 ± 0.00^{b}
6	S3H1	$12.67\pm0.33^{c,d}$	13.00 ± 0.00^{b}	8.00 ± 0.00^{f}	$8.67\pm0.67^{c,d}$	0.0 ± 0.00^{b}
7	S3H2	$13.67\pm0.88^{b,c,d}$	$13.67\pm0.88^{a,b}$	$8.67\pm0.67^{e,f}$	$9.33\pm0.67^{b,c,d}$	0.0 ± 0.00^{b}
8	S4H1	$14.00\pm0.58^{b,c,d}$	$13.67\pm0.88^{a,b}$	$10.0\pm0.58^{c,d,e}$	$8.67\pm0.67^{c,d}$	0.0 ± 0.00^{b}
9	S4H2	$14.67\pm0.88^{b,c}$	15.00 ± 0.00^{a}	$11.0\pm0.58^{b,c,d}$	10.67 ± 0.33^{b}	0.0 ± 0.00^{b}
10	S5H1	$14.33\pm0.33^{b,c,d}$	$13.67\pm0.88^{a,b}$	12.0 ± 0.00^{b}	$10.00\pm0.00^{b,c}$	0.0 ± 0.00^{b}
11	S5H2	$14.00\pm0.00^{b,c,d}$	$13.67\pm0.33^{a,b}$	$11.33\pm0.67^{b,c}$	10.67 ± 0.67^{b}	0.0 ± 0.00^{b}
12	Ceftriaxone	11.67 ± 0.33^{d}	$14.67\pm0.67^{a,b}$	24.33 ± 0.33^{a}	24.67 ± 0.67^{a}	10.67 ± 0.67^{a}
	LSD	2.85	1.79	1.75	1.79	0.56

LSD, least significant differences.

[56,57], suggesting that selenium plays an important role for increasing the phenolic content in plants.

Total antioxidant activity

The lowest inhibition values were recorded in samples of S0H0, whereas the highest inhibition effect was obtained as a result of S4H2 for both leaves and stem (Table 9). Generally, all treatments increased significantly inhibition % compared with untreated plants. The same results were obtained by Poldma and colleagues [58–60]. In this respect, literature surveys reported that total phenolic compound is one of the major groups of compounds acting as primary antioxidants [25,61].

Antimicrobial effect

The antibacterial activity results of *P. amboinicus* essential oil have been presented in Table 10. Essential oil of *P. amboinicus* showed antibacterial activity against gram-positive and gram-negative bacteria. The tested gram-positive bacteria were more sensitive to *P. amboinicus* essential oil than gram-negative ones. The most sensitive organism was *B. cereus* followed by *S. aureus*, with inhibition zones ranged from 12.67 to 17.67 mm and from 13.0 to 15.0 mm for *B. cereus* and *S. aureus*, respectively. However, the gram-negative bacteria were more resistant to *P. amboinicus* essential oil than gram-positive bacteria. The obtained inhibition zones for *S. typhi* and *E. coli* ranged from 8.0 to 12.0 mm and from 8.0 to 10.67 mm, respectively, which significantly decreased than that obtained by control positive ceftriaxone, with 24.33 and 24.67 mm respectively. However, *P. aeruginosa* was the most resistant organism to all tested treatments, recording no inhibition zone. Among the examined essential oil treatments, the treatment S1H1 showed the higher inhibition zone against *B. cereus*, with 17.67 mm, which significantly increased over that obtained by ceftriaxone, with

Table 11 Antifungal activity of *Plectranthus amboinicus* (150 mg/ml (w/v) in dimethyl sulfoxide)

	Treatments	Inhibition zone diameter (mm) (mean±SE)				
		Aspergillus flavus	*Aspergillus carbonarius*	*Aspergillus ochraceus*	*Fusarium proliferatum*	*Penicilliumverrucosum*
1	S0H0	10.0±0.0^{b,c}	10.67±0.67^{e,f}	12.67±0.67^{c,d}	11.33±0.67^{b,c}	12.33±1.20^{c}
2	S1H1	10.67±0.67^{b,c}	14.67±1.33^{b}	13.67±0.67^{b,c,d}	12.33±0.33^{a,b}	15.0±0.00^{b}
3	S1H2	9.33±1.33 ^{c,d}	14.33±0.67^{b}	12.67±0.33^{c,d}	10.67±0.67^{c,d}	14.33±0.33^{b}
4	S2H1	8.0±0.0^{d}	13.0±0.58^{b,c,d}	13.67±0.67^{b,c,d}	10.0±0.00^{c,d,e}	13.67±0.67^{b,c}
5	S2H2	9.33±0.67^{c,d}	12.33±0.67^{c,d,e}	12.33±0.33^{d}	9.33±0.67^{d,e}	13.67±0.33^{b,c}
6	S3H1	9.33±0.67^{c,d}	13.33±0.33^{b,c}	13.0±0.58^{b,c,d}	8.67±0.67^{e}	12.33±0.33^{c}
7	S3H2	9.33±0.67 ^{c,d}	11.33±0.67^{e,d}	12.67±0.67^{c,d}	11.33±0.67^{b,c}	14.0±0.00^{b,c}
8	S4H1	10.0±0.0^{bc}	10.67±0.67^{e,f}	14.0±0.00^{b,c}	10.67±0.67^{c,d}	14.0±0.58^{b,c}
9	S4H2	11.33±0.67^{b}	9.33±0.67 ^{f}	14.33±0.33^{b}	10.67±0.67^{c,d}	13.67±0.88^{b,c}
10	S5H1	9.33±0.33^{c,d}	13.67±0.33^{b,c}	13.33±0.33^{b,c,d}	12.33±0.33^{a,b}	14.33±0.33^{b}
11	S5H2	9.67±0.33^{b,c,d}	14.0±0.00^{b,c}	13.67±0.33^{b,c,d}	12.67±0.67^{a,b}	14.33±0.33^{b}
12	Miconazole	21.3±0.67^{a}	19.33±0.67^{a}	18.67±0.67^{a}	13.67±0.33^{a}	21.33±0.67^{a}
	LSD	1.82	1.97	1.40	1.66	1.69

LSD, least significant differences.

11.67 mm. Moreover, the treatment S1H1 showed higher activity against *S. aureus* as well as *E. coli* with inhibition zones 14.33 and 10.67 mm, respectively. In general, all tested treatments of *P. amboinicus* essential oil have antibacterial activity toward all tested bacteria except *P. aeruginosa,* with different extent of effectiveness, and Table 10 illustrated the significant differences among them. Erny *et al.* [8] reported the antibacterial activity of *P. amboinicus* essential oil against gram-positive and gram-negative bacteria, with inhibition zones ranged from 10.0 to 22.0 mm. Our findings were in agreement with that reported by El-Hawary and colleagues [5,8,62], as they reported no activity of *P. amboinicus* essential oil toward *P. aeruginosa.* Aqueous and acetone extracts of *P. amboinicus* leaves also have no activity against *P. aeruginosa, as reported* by Sathasivam and Elangovan [63].

The antifungal activity of *P. amboinicus* essential oil against five mycotoxigenic fungi is presented in Table 11. The obtained results indicated the antifungal activity of all treatments of *P. amboinicus* essential oil against all tested fungi with different extent of effectiveness. The treatment S1H1 showed the highest inhibition zones against *A. carbonarius* and *P. verrucosum*, with inhibition zones 14.67 and 15.0 mm, respectively. Moreover, the treatment S4H2 recorded the highest activity against *A. flavus* and *A. ochraceus,* with inhibition zones 11.33 and 14.33 mm, respectively. The obtained inhibition zones from all essential oil treatments were below or close to that developed by control positive Miconazole 1 mg/ml, with significant differences among them, except in the case of *F. proliferatum.*

The antifungal activity of *P. amboinicus* essential oil was reported by many researchers [5,62]. Antifungal activity of the *P. amboinicus* volatile oil was studied against various fungi by an agar well diffusion susceptibility test. In that, growth of *Aspergillus ochraceus*, *Aspergillus niger*, and *Penicillium* spp. was inhibited by 60, 64, and 60%, respectively, with 10 µl of volatile oil [8].

Conclusion

Application of different levels of selenium and humic acid treatments had a pronounced effect on growth characteristics of essential oil (percentage and different chemical composition under study.

Moreover, the results indicated that *P. amboinicus* had a great antifungal and antibacterial effect.

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Conflicts of interest

There are no conflicts of interest.

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