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Chemical Composition, Antioxidant, Antitumor and Antifungal Activities of Methanolic Extracts of *Coleus blumei*, *Plectranthus amboinicus* and *Salvia splendens* (Lamiaceae)

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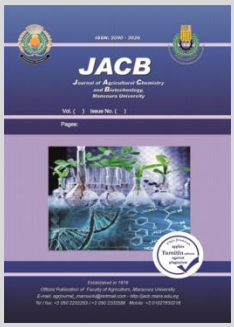

ABSTRACT

The present study aims to elucidate the chemical characterization of the methanolic extracts of *Coleus blumei*, *Plectranthus amboinicus*, and *Salvia splendens* with emphasis on their antioxidant, antifungal, and cytotoxic activities. Among all methanolic extracts, *P. amboinicus* extract contains the highest contents of the total polyphenols and total flavonoids by 122.2 g GAE Kg^{-1} and 41.4 g QE Kg^{-1}, respectively. Rosmarinic acid (1.021 g Kg^{-1}) was the predominant phenol in *P. amboinicus* according to HPLC analysis. Carvacrol was the predominant component (7.59%) of *P. amboinicus* extract by GC-MS analysis. The extract of *P. amboinicus* recorded the lowest IC_{50} and EC_{50} values for DPPH and reducing power by 0.042 g L^{-1}, and 0.327 g L^{-1}, respectively, indicating the greatest antioxidant potential. Also, *P. amboinicus* showed the highest antitumor activity using A549, MCF7, and HEPG cell lines with IC_{50} values by 0.041, 0.047 and 0.043 g L^{-1}, respectively. The extract of *S. splendens* showed a remarkable selectivity toward MCF7 cell line with IC_{50} value by 0.040 g L^{-1}. The methanol extract of *P. amboinicus* displayed the greatest inhibition on the mycelial growth of *Sclerotinia sclerotiorum* (83.0 %), *Cochliobolus cynodontis* (82.1 %), *Botrytis cinerea* (77.8 %), *Bipolaris hawaiensis* (74.1 %), *B. spicifera* (73.9 %), and *Alternaria alternata* (73.7 %) at the concentration of 32 g L^{-1}.

***Keywords*:** DPPH, flavonoids content, GC-MS, HPLC, polyphenols content, reducing power (RP)

INTRODUCTION

Plant preparations have been used by humans since ancient times for therapeutic purposes (Newman and Cragg, 2012). Those preparations are very important for healthcare in developing countries as a substitute for expensive pharmaceutics. The *Plectranthus* genus belongs to the Lamiaceae family, including more than 300 species dispersed through tropical and subtropical regions of Asia, Africa, South America, and Oceania. *Plectranthus* plants are frequently used for medicinal purposes such as dermatologic, infectious, and gastrointestinal pathologies (Lukhoba *et al.*, 2006). Their pharmacological properties have frequently been attributed to the presence of bioactive oxygenated diterpenes from the abietane, kaurane, phyllocladane, labdane, neoclerodane, and halimane classes (Matias *et al.*, 2019). In this context, the solvent extracts and essential oil of *P. amboinicus* have anti-inflammatory, analgesic, and antimicrobial properties as well as antihyperglycemic and antihyperlipidemic activities (Viswanathaswamy *et al.*, 2011; Chiu *et al.*, 2012; Hassani *et al.*, 2012). The genus *Plectranthus* is related to the genera *Salvia*, *Rabdosia*, *Coleus*, and *Isodon*. The name 'Coleus' is extensively used by horticulturalists and gardeners, denotes a defunct genus, and considered as a common name for the genus *Solenostemon*. The current taxonomic generally follow Harley *et al.* (2004) by including the previously documented *Coleus* Lour. and *Solenostemon* Thonn. within *Plectranthus*.

The leaves extract of *P. scutellarioides* (= *C. blumei*) contain many bioactive compounds, including polyphenols, flavonoids, monoterpenoids, sesquiterpenoids, triterpenoids, and steroids (Levita *et al.* 2016). The identification of novel diterpenoids having the skeleton of abietane from the aerial parts of the plant has been reported (Cretton *et al.*, 2018; Ito *et al.*, 2018; Jurkaninová *et al.*, 2019). A recent study has identified phenolics of luteolin 5-O-β-D-glucoside, caffeic acid, rosmarinic acid, apigenin 5-O-(3″-O-acetyl)-β-D-glucuronide, and apigenin 7-O-(3″-O-acetyl)-β-D-glucuronide as the key constituents of the ethyl acetate fraction of *P. scutellarioides* (Kubínová *et al.*, 2019). Furthermore, the antimicrobial, antidiabetic, cytotoxic, and anti-inflammatory properties of *P. scutellarioides* extracts have been reported (Cretton *et al.*, 2018; Ito *et al.*, 2018; Salaeh *et al.*, 2018; Jurkaninová *et al.*, 2019; Tungmunnithum *et al.*, 2019).

Genus *Salvia*, frequently identified as sage, is a wide genus that belongs to the family Lamiaceae, which has about 252 genera and 7200 species (Harley *et al.*, 2004). Several plant species of *Salvia* are cultivated for their aromatic features and are usually used as flavoring agents, cosmetics, food additives, and perfume ingredient (Firdous *et al.*,1999). Moreover, the essential oils and extracts of *Salvia* species have normally been extensively used in traditional medicines as anti-inflammatory, antitumor, antibacterial, antiviral, antioxidant, and in treatments of gastrointestinal conditions (Lu and Foo ,2002; Shaheen *et al.*,2011; Moharram *et al.*, 2012; Boukhary *et al.*, 2016). Besides, many researchers have

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separated different diterpenoids, triterpenes, coumarins, polysaccharides, sterols, phenolic acids, flavonoids, and anthocyanins from the plants of this genus (Ibrahim, 2012). Likewise, the extract of the aerial parts of *S. splendens* contains numerous compounds that act as antihyperglycemic, neuroprotective, antioxidants, and cytotoxicity (Kumar *et al.*, 2010; Shaheen *et al.*, 2011; El-Sawi *et al.*, 2020).

Up to now, no comprehensive study was done to evaluate the chemical composition and the biological activities of Lamiaceae family plants from *Coleus*, *Plectranthus*, and *Salvia* genera. Therefore, the present study aims to 1) identify the phenolic profiles, 2) describe the GC-MS fingerprints, and 3) evaluate the antioxidant, antitumor, and antifungal activities of leaves methanolic extracts of *C. blumei*, *P. amboinicus*, and *S. splendens* in a comparative style.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu reagent, sulfuric acid, Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), aluminum chloride, gallic acid, quercetin, butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO), RPMI1640-medium, trypan blue, Fetal Bovine Serum, Penicillin Streptomycin antibiotic, and Trypsin-EDTA were purchased from Sigma Aldrich Chemical Co. (USA). AppliChem (Germany) was the source of Tris buffer. Ascorbic acid (AA) and ammonium molybdate were obtained from El-Nasr Company for pharmaceuticals and chemicals (Egypt).

Preparation of methanolic extracts

Green leaves of *C. blumei*, *P. amboinicus* and *S. splendens* were collected from the Faculty of Agriculture Farm, Mansoura University, Mansoura, Egypt. Leaves of each plant were air-dried and ground separately. Extracts were obtained from the ground dry leaves of the plants according to the protocol previously described by Sanad *et al.* (2012). In detail, one kilogram of powdered leaves of each plant was soaked separately with the appropriate volume of methanol overnight. The extracts were then filtered, and the residues were re-extracted twice in the same manner. The combined extracts were evaporated to dryness using a rotary evaporator.

Determination of total polyphenols content

Total polyphenols (TPs) of tested extracts were determined according to the Folin-Ciocalteu method (Singleton *et al.*,1999). Gallic acid was used as a standard ranged from 0.025 to 0.2 g L^{-1}. Total polyphenols content was expressed as grams gallic acid equivalents per kilogram dry weight extract (g GAE Kg^{-1}).

Determination of total flavonoids content

The total flavonoids content (TFs) of each methanolic extract was determined using aluminum chloride colorimetric technique as described by Lin and Tang (2007). Quercetin (QE) at the concentrations ranged from 0.005 to 0.1 g L^{-1} was used to prepare the standard curve. TFs content was expressed as grams quercetin equivalents per kilogram dry weight (g QE Kg^{-1}).

HPLC analysis

The identification of the phenolics was done using Agilent 1260 infinity high-performance liquid chromatography (Agilent, USA) equipped with a quaternary pump. Firstly, the methanolic extract was passed through 0.45 µm filters and then injected in a volume of 20 µL. The separation route was performed on a kintex-R 5µM EVO C_{18} (4.6 mm × 100 mm, Phenomenex, USA), at a temperature of 30 °C. A binary solvent mixture involves water acidified with 0.2% H_3PO_4 (solvent A) and acetonitrile/methanol 1:1 (solvent B) was used as a gradient elution system in a constant flow rate (1 mL min^{-1}). The linear mode of the mobile phase was done according to Ghomari *et al.* (2019). All phenolic compounds were recognized by matching their retention times with those of reference phenolic ingredients.

GC-MS analysis

The analysis of the methanolic extracts was performed using an Agilent 7890A GC equipped with 5975 Inert MS with triple Axis Detector, using a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). GC oven temperature was programmed to hold at 50 ºC for 5 min, then heated to 280 ºC with a rate of 6 ºC per min. Injector and detector temperatures were 250 ºC and 230 ºC, respectively. The MS was fixed at 70 eV ionization energy with a mass electron (m/z) range of 50-550. Spitless mode was used in the injection of 1 sample solution and helium was used as a carrier gas with a flow rate of 1 mL min^{-1}. The compounds were identified by matching their mass spectral fragmentation patterns with those of the WILEY/NIST mass spectral database.

Antioxidant activity

DPPH assay

The scavenging potentials of methanolic extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated according to Taher *et al.* (2020). Ascorbic acid (AA) was used as a reference antioxidant in the same range of the extracts (0.012-0.15 g L^{-1}).

Reducing power

Reducing power (RP) of methanolic extracts was estimated according to Oyaizu (1986). The methanolic extracts were tested at concentrations between 0.125 to 1 g L^{-1}. Butylated hydroxytoluene (BHT) was chosen as a reference antioxidant. The absorbance of each sample was recorded at 700 nm by T80 UV/VIS spectrophotometer (PG Instruments Ltd, Leicestershire, England).

Phosphomolybdenum assay

The total antioxidant capacity (TAC) of the extracts was measured in triplicate by the method of Prieto *et al.* (1999). A volume of 0.6 mL of each methanolic extract (1 g L^{-1}) was added to 2 mL of reagent solution comprises of (28 mM sodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate). The reaction mixture was held in a water bath at 65 ºC for 90 min, then the tubes were left for cooled at room temperature. The absorbance of each sample was measured by T80 UV/VIS spectrophotometer at 765 nm. Blank was prepared as termed before but differ in the addition of 0.6 mL methanol instead of the extract. BHT was chosen as a reference antioxidant. TAC was calculated using a standard curve of ascorbic acid (AA). The antioxidant capacity was expressed as grams of ascorbic acid equivalents per kilogram (g AAE Kg^{-1}).

Antitumor activity

A549 (human lung adenocarcinoma), MCF7 (breast adenocarcinoma), HEPG2 (human liver carcinoma), and HCT (colon carcinoma) cell lines were obtained from the American Type Culture Collection (ATCC, Minnesota, USA). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial subculturing. Cell proliferation and viability was determined colorimetrically by using sulforhodamine B (SRB) according the method of Vichai and

Kirtikara (2006). In brief, cells were cultured in RPMI-1640 medium supplemented with 1% penicillin/ streptomycin and 10% fetal bovine serum. Also, different concentrations of the methanolic extracts and 5-fluorouracil were prepared (0.012, 0.025, 0.05, 0.1 and 0.2 g L^{-1}) in the culture medium. The cells were seeded in 96-well microtiter plates at initial concentration of 3×10^3 cell/well in a 150 µL fresh medium and left for 24 h to attach to the plates. Then, a volume of 100 µL of each concentration of tested samples was added to every well except control wells in which the same volume of 0.1% DMSO was added instead of prepared extracts. After 48 h, the cells were fixed with ice-cold trichloroacetic acid (50 µL/well, 10% w/v) for 1 h at 4°C. To the washed and dried plates, 50 µL SRB (0.4% w/v in 1% aqueous acetic acid) solution was added and kept at room temperature for 30 min. The unbound SRB solution was removed by washing the plates four times with 1% acetic acid (v/v) followed by drying. A volume of 200 µL of 10 mM Tris Base (pH 10.5) was added to each well to solubilize the bound SRB. Plates ware shaked for 5-10 min., then read in a 96-well plate reader with working wavelength of 570 nm and optical density was noted.

Antifungal activity

The fungal strains used were obtained from Assiut University Mycological Centre (AUMC), Assiut ,Egypt, including *Alternaria alternata* AUMC 10301, *Aspergillus parasiticus* AUMC 8947, *Bipolaris hawaiensis* AUMC 1120, *B. spicifera* AUMC 459, *Botrytis cinerea* AUMC 6095, *Cochliobolus cynodontis* AUMC 2393, *Fusarium oxysporum* AUMC 9704, *F. sambucinum* AUMC 1266, *Macrophomina phaseolina* AUMC 10204, and *Sclerotinia sclerotiorum* AUMC 5400. The methanol extracts were dissolved in Tween 80 (0.5 % v/v) and mixed with molten potato dextrose agar (PDA), then added to the Petri plates (90 mm diameter) to obtain the final concentrations of 8, 16, and 32 g L^{-1}. PDA plates without methanol extracts were served as a control. A mycelial disk of each fungal inoculum (5 mm diameter) was taken from the edge of actively growing cultures and placed in the center of the plates. All plates were incubated at 24±2 °C until the growth in the control reached the edge of the plates. All treatments were conducted in triplicate, and the experiment was repeated twice. The inhibition of mycelial growth in each treatment against control was calculated using the following equation:-

Inhibition of mycelia growth (%) = [(C - T) / C] × 100

Where C and T represented the mycelial growth (mm) in the control and treated plates, respectively.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SAS (version 9.1, SAS Institute, NC, USA). The significant difference of means was compared using the Tukey's HSD test at $P < 0.05$. The values of EC_{50} (concentrations of the methanolic extracts which recording 0.5 absorbances at 700 nm for the antioxidant activity, and IC_{50} (concentrations of the extracts that producing 50 % inhibition on both DPPH and the viability of the cells) were calculated by probit analysis using SAS (version 9.1, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Total polyphenols and total flavonoids content

P. amboinicus methanol extract contained the highest value of total polyphenols (TPs), and total flavonoids (TFs) contents by 122.2 g GAE Kg^{-1} extract and 41.4 g QE Kg^{-1} extract, respectively (Fig. 1). On the contrary, the TPs content in *C. blumei* methanolic extract was only 46.4 g GAE Kg^{-1} extract, while *S. splendens* methanolic extract contained the lowest value of TFs content (13.1 g QE Kg^{-1} extract) with the considerable value of TPs by 93.3 g GAE Kg^{-1} extract.

In the case of *P. amboinicus* extract, the quantities of TPs and TFs in the present study were slightly higher than those obtained by Swamy *et al.* (2017), who recorded the values of 94.37 g GAE Kg^{-1} and 26.90 g RE Kg^{-1} for TPs and TFs, respectively. The results generally disagree with the results obtained by (El-hawary *et al.*,2012), who stated that *P. amboinicus* methanol extracts of stems, leaves, and roots had much lower values of TPs contents which ranged between 5.4 to 9.6 g GAE Kg^{-1}. Using of other solvents such as acetone and ethyl acetate, for extracting *P. amboinicus* as reported by Gupta *et al.*, 2013, showed considerable amounts of TPs with the values of 85.15 and 67.83 g GAE Kg^{-1}, respectively Recently, it has been stated by (Terto *et al.*, 2020) that *P. amboinicus* extract contained a slightly higher level of TPs content as 142.39 g GAE Kg^{-1} of the extract. Overall, compared with previous reports, the methanolic extract of *P. amboinicus* was found to show a high value of TPs, indicating that the amount found in this extract is promising. Furthermore, the butanol fraction of *S. splendens* had significant amounts of TPs and TFs contents by 92.4 g GAE Kg^{-1} and 36.2 g CEE Kg^{-1}, respectively (El-Sawi *et al.*, 2020). The quantity of TFs in *S. splendens* butanol fraction in the previous study is much higher than the obtained value in this work for the methanol extract. This may indicate that the *S. splendens* flavonoid fraction preferentially to be extracted in polar solvents such as butanol. To the best of our knowledge, no information available about *C. blumei* extract for the colorimetric determination of the total polyphenols and flavonoids contents.

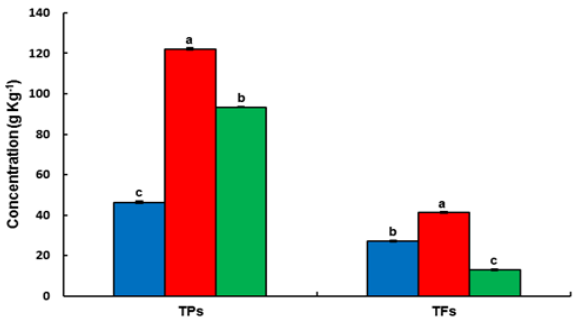

Figure 1. Total polyphenols (TPs) and total flavonoids (TFs) content of the methanolic extracts. All values represent mean ± standard error (SE). Bars with different letters are significantly different according to Tukey's HSD test at P < 0.05 for each treatment point. ( ) Coleus blumei; ( ) Plectranthus amboinicus; ( ) Salvia splendens.

HPLC analysis

The phenolic profile of all methanolic extracts involved twelve phenolic acids, two simple phenols, and five flavonoids with different concentrations (Table 1). Rosmarinic acid was identified in a high concentration as the predominant phenolic constituent in *P. amboinicus* extract by 1.021 g Kg^{-1}. Naringin, catechol, syringic acid, ellagic acid, and myricetin were also quantified in *P. amboinicus* extract in moderate amounts ranged between 0.972 to 0.606 g Kg^{-1}.

The richness of *P. amboinicus* extract with rosmarinic acid, in this study, was in accordance with the findings obtained by Terto *et al.* (2020), who mentioned that the rosmarinic acid was one of the key fingerprints of this extract. Also, the HPLC data of *P. amboinicus* extract agreed to some extent with those obtained by Sulaiman *et al.* (2018) using HPLC coupled tandem mass spectroscopic analysis (LC-MS/MS). In detail, they identified two flavonoids (vicenin 1 and gallocatechin) three phenolic acid derivatives (methyl gallate, sinapic acid hexose, and salicylic acid glucoside), and seven phenolic acids (protocatechuic, caffeoylquinic, syringic, ellagic, vanillic, ferulic, and caffeic acids).

Syringic acid was the major phenol in *S. splendens* methanol extract (0.268 g Kg^{-1}). Other phenols like kaempferol, myricetin, quercitin, rutin, and catechol were detected in *S. splendens* in various concentrations ranged between 0.251 to 0.105 g Kg^{-1} (Table 1). Generally, it could be noted that obtained results concerning *S. splendens* extract conflicted with those in literature. For instance, the rosmarinic acid and its derivative methyl rosmarinate were detected in considerable amounts in the methanol extract of *S. splendens* (Moharram *et al.*,2012). However, HPLC chromatogram of *S. splendens* extract in the current study did not contain any signal for rosmarinic acid. Also, butanol fraction of *S. splendens* had other constituents rather than those identified in the present study as the major phenolics such as rosmarinic acid, caffeic acid, and chrysoeriol (El-Sawi *et al.*, 2020). These variations in the phenolic profiles of plants depend on several factors, including environmental conditions, genetic properties, and different chemotypes (Elsherbiny *et al.*, 2017).

Table 1. HPLC analysis of methanolic extracts of *Coleus blumei, Plectranthus amboinicus,* and *Salvia splendens.*

Compound	Chemical class	Concentration (g Kg^{-1})		
		Coleus blumei	*Plectranthus amboinicus*	*Salvia splendens*
p-Hydroxybenzoic acid	Hydroxybenzoic acids	0.181	0.144	0.066
Chlorogenic acid	Hydroxycinnamic acids	0.058	0.102	0.012
Vanillic acid	Hydroxybenzoic acids	0.163	0.389	0.063
Caffeic acid	Hydroxycinnamic acids	0.023	0.051	0.007
Syringic acid	Hydroxybenzoic acids	0.465	0.946	0.268
p-Coumaric acid	Hydroxycinnamic acids	0.021	0.080	0.008
Ferulic acid	Hydroxycinnamic acids	0.019	0.049	0.014
Salicylic acid	Hydroxybenzoic acids	0.268	0.043	0.161
Rosmarinic acid	Hydroxycinnamic acids	-	1.021	-
Gallic acid	Hydroxybenzoic acids	0.022	-	0.009
Ellagic acid	Hexahydroxydiphenic acids	0.177	0.721	0.036
α-Coumaric acid	Hydroxycinnamic acids	0.034	0.049	0.038
Pyrogallol	Hydroxylated phenols	0.027	-	0.019
Catechol	Hydroxylated phenols	0.240	0.881	0.105
kaempferol	Flavonols	1.797	-	0.251
Quercitin	Flavonols	0.873	-	0.193
Myricetin	Flavonols	0.408	0.606	0.230
Naringin	Flavanone glycoside	0.609	0.972	-
Rutin	Flavonol glycoside	2.653	0.110	0.177

Rutin, kaempferol, quercetin, and naringin as flavonoids were identified in *C. blumei* extract by 2.653, 1.797, 0.873, and 0.609 g Kg^{-1}, respectively (Table 1). While , three other flavonoid glycosides namely luteolin hexoside, luteolin glucuronide, and apigenin-X have been reported to be the major phenolic constituents in *C. blumei* (Dörr *et al.*,2019). HPLC analysis of *C. blumei* extract showed also the presence of syringic acid (0.465 g Kg^{-1}), myricetin (0.408 g Kg^{-1}) and salicylic acid (0.268 g Kg^{-1},table 1). Remarkably , the obtained concentration of syringic acid in the leaf extract of *C. blumei* was much lower than that obtained in an earlier report as 4.086 g Kg^{-1} DW (Sytar *et al.*, 2018).

GC-MS analysis

The GC-MS analysis of *P. amboinicus* extract displayed the presence of 37 compounds in the form of sesquiterpenes (20.48%), monoterpenes (11.78%), fatty acids (7.48%), diterpenes (3.67%), phenols (2.88%), sterols (2.87 %), pyrans (2.45%), furans (1.33%), hydrocarbons, and naphthalenes (Table 2). Carvacrol was the predominant component (7.59%) of *P. amboinicus* extract with palmitic acid (4.41%) as the other major constituent (Table 2). Besides, the main components in the extract detected were phytol (3.67%), δ-cadinene (3.64%), and *trans*-calamenene (3.24%). According to the results obtained, the methanolic extract of *P. amboinicus* is a rich source of volatile constituents in the form of mono and sesquiterpenes. In concordance with this study, carvacrol, an isomeric form of thymol, was the major constituent in the petroleum ether extract of *P. amboinicus* by 37.70% (Swamy *et al.*,2017). In addition, four compounds identified in this study namely eugenol, 4-methoxy-2,3,6-trimethylphenol, 2-methoxy-4-vinylphenol, and palmitic acid were also detected in the butanol fraction of *P. amboinicus* by GC-MS analysis (Rajesh and Gayathri, 2015). On the contrary, Swamy *et al.* (2017) established the diminished level of the volatile terpenes simultaneously with high contents of solid acyclic alkanes such as tetracontane (16.67%), tetrapentacontane (11.32%), and pentacosane (7.88%) as the predominant compounds in the methanolic extract of *P. amboinicus*. Furthermore, the thymol isomers particularly the carvacrol monoterpene had been previously described in the essential oil of *P. amboinicus* in different percentages between 28 to 70% (Hassani *et al.*, 2012; Hsu and Ho, 2019). Although the monoterpene carvacrol was the major compound in *P. amboinicus* methanolic extract, the higher percentage of total sesquiterpenes than that of monoterpenes was in accordance with the previous work by Bandeira *et al.* (2011). They recognized 14 chemical components mostly sesquiterpenes in the essential oil of four species of *Plectranthus* with an abundance of *trans*-caryophyllene using GC-MS analysis.

Fatty acids (38.05%), diterpenes (7.91%), triterpenes (7.09%), esters (6.22%), and phenols (6.01%), were the characteristic constituents of the methanolic extract of *S. splendens*. The chemical analyses by CG-MS revealed that the main compounds of *S. splendens* extract were the fatty acids of palmitic (15.51%), α-linolenic (11.74%), and stearic (7.04%) (Table 2). Similarly, the petroleum ether extract of *S. bicolor* had 21fatty acids especially α-linolenic acid (21.65%), and erucic acid (16.65%) as well as considerable values of β-amyrin and phytol (Ibrahim 2012). The chemical composition of *S. bicolor* grown in Egypt displayed high content of sterols particularly β-sitosterol as 24.75% (Ibrahim, 2012). However, the present study revealed much lower contents of phytosterols mostly in the form of campesterol in the extract of *S. splendens*.

Analyzed *C. blumei* extract mainly consisted of pyrans and furans (48.61%), and fatty acids (20.67%). As shown in table (2), the major component was 5-Hydroxymethylfurfural (36.94%) followed by palmitic acid (8.12%). Similarly, the identification of 5HMF or any of its derivatives in high percentage as the predominant compound in different plant

extracts has been previously reported (Rani and Kapoor, 2019; Ni *et al.* 2020). HMF might occur naturally in plants or be generated during high-temperature processes in sugar-containing food such as drying or during GC-MS analysis (Abu-Bakar *et al*., 2015). The GC-MS results of *C. blumei* extract in the present study were entirely in conflict with those obtained in the previous work by Surahmaida and Umarudin (2019), who identified eight different compounds.

Table 2. GC-MS analysis of methanolic extracts of *Coleus blumei*, *Plectranthus amboinicus*, and *Salvia splendens*.

Retention Time (min)	Compound	Area (%)			Molecular formula
		Coleus blumei	*Plectranthus amboinicus*	*Salvia splendens*	
4.09	Furfural	4.16	0.80	-	$C_5H_4O_2$
4.56	2-Furanmethanol	0.55	-	-	$C_5H_6O_2$
5.90	1,2-Cyclopentanedione	0.67	-	-	$C_5H_6O_2$
6.68	2-Furancarboxaldehyde, 5-methyl-	0.55	-	-	$C_6H_6O_2$
7.00	1-Octen-3-ol	0.35	-	-	$C_8H_{16}O$
7.06	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.70	-	-	$C_6H_8O_4$
8.14	*p*-Cymene	-	0.83	-	$C_{10}H_{14}$
9.79	Furyl hydroxymethyl ketone	0.53	-	-	$C_6H_6O_3$
9.87	Benzene, 1-methyl-4-(1-methylethenyl)	-	0.49	-	$C_{10}H_{12}$
10.61	3-Hydroxy-2-methyl-4*H*-pyran-4-one	-	1.06	-	$C_6H_6O_3$
11.49	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxyl -6-methyl-	3.96	1.39	2.93	$C_6H_8O_4$
13.68	Benzofuran, 2,3-dihydro-	1.22	-	-	C_8H_8O
14.31	5-Hydroxymethylfurfural	36.94	0.53	-	$C_6H_6O_3$
15.77	Carvacrol	-	7.59	-	$C_{10}H_{14}O$
16.07	2-Methoxy-4-vinylphenol	0.50	0.40	0.66	$C_9H_{10}O_2$
16.98	α-Cubebene	-	0.22	-	$C_{15}H_{24}$
17.04	Glycerin triacetate	0.42	-	-	$C_9H_{14}O_6$
17.20	Eugenol	-	0.59	-	$C_{10}H_{12}O_2$
17.68	α-Copaene	-	1.66	-	$C_{15}H_{24}$
18.82	Caryophyllen	-	0.95	-	$C_{15}H_{24}$
19.68	Humulene	-	1.25	-	$C_{15}H_{24}$
20.35	Germacrene D	-	0.46	-	$C_{15}H_{24}$
20.69	γ-Gurjunene	-	0.44	-	$C_{15}H_{24}$
20.80	α-Muurolene	-	0.80	-	$C_{15}H_{24}$
21.15	γ-Muurolene	-	1.21	-	$C_{15}H_{24}$
21.36	δ-Cadinene	-	3.64	-	$C_{15}H_{24}$
21.84	1,1,5-Trimethyl-1,2-dihydronaphthalene	-	1.14	-	$C_{13}H_{16}$
22.32	4-Methoxy-2,3,6-trimethylphenol	-	1.48	-	$C_{10}H_{14}O_2$
23.48	*cis*-α-Copaene-8-ol	-	1.20	-	$C_{15}H_{24}O$
23.78	Megastigmatrienone	0.48	-	-	$C_{13}H_{18}O$
24.03	*cis*-Carveol		-	0.62	$C_{10}H_{16}O$
24.09	Bicyclo [4.4.0] dec-1-ene, 2-isopropyl-5-methyl-9-methylene	-	2.25	-	$C_{15}H_{24}$
24.44	α-Cadinol	-	1.47	-	$C_{15}H_{26}O$
25.74	Cyclohexene, 4-methyl-1-(1-methylethyl)-	-	1.02	-	$C_{10}H_{18}$
26.70	Tetradecanoic acid	1.00	-	1.00	$C_{14}H_{28}O_2$
27.31	*trans*-Calamenene	-	3.24	-	$C_{15}H_{22}$
27.49	Salicin	1.22	-	-	$C_{13}H_{18}O_7$
27.86	β-Spathulenol	-	1.69	-	$C_{15}H_{24}O$
28.24	1-Methoxy-3-(2-hydroxyethyl) nonane	0.50	-	1.56	$C_{12}H_{26}O_2$
28.38	2-Pentadecanone, 6,10,14-trimethyl	0.57	-	1.84	$C_{18}H_{36}O$
28.73	1, E-11, Z-13-Hexadecatriene	-	1.13	-	$C_{16}H_{28}$
28.80	1,1,2-Trimethyl-3,5-bis(1-methylethenyl) cyclohexane	-	1.26	-	$C_{15}H_{26}$
29.91	1-Hexadecanol, 2-methyl-	-	-	0.72	$C_{17}H_{36}O$
29.95	7,9-Di-tert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione	1.57	-	-	$C_{17}H_{24}O_3$
29.99	Hexadecanoic acid, methyl ester	-	-	1.90	$C_{17}H_{34}O_2$
30.38	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	3.44	-	-	$C_{18}H_{28}O_3$
30.81	Palmitic acid	8.12	4.41	15.51	$C_{16}H_{32}O_2$
33.31	Linolenic acid, methyl ester		-	1.15	$C_{19}H_{32}O_2$
33.52	Phytol	2.76	3.67	6.68	$C_{20}H_{40}O$
33.76	Octadecanoic acid, methyl ester		-	0.65	$C_{19}H_{38}O_2$
33.92	Linoleic acid	3.09	0.46	1.70	$C_{18}H_{32}O_2$
34.05	α-Linolenic acid	4.67	1.62	11.74	$C_{18}H_{30}O_2$
34.43	Stearic acid	2.73	0.99	7.04	$C_{18}H_{36}O_2$
34.84	Stigmasterol	-	-	0.26	$C_{29}H_{48}O$
37.68	4,8,12,16-Tetramethylheptadecan-4- olide	-	-	1.23	$C_{21}H_{40}O_2$
37.85	Eicosanoic acid	1.06	-	1.06	$C_{20}H_{40}O_2$
38.78	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	5.35	1.00	5.35	$C_{23}H_{32}O_2$
39.94	Pentacosane	-	1.01		$C_{25}H_{52}$
40.14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.79	-	2.52	$C_{19}H_{38}O_4$
41.02	β-Sitosterol	-	1.56	0.09	$C_{29}H_{50}O$
42.13	β-Amyrin	-	-	1.70	$C_{30}H_{50}O$
43.22	Octadecanoic acid, 2,3-dihydroxypropyl ester	0.83	-	-	$C_{21}H_{42}O_4$
43.56	dl-α-Tocopherol	0.92	-	-	$C_{90}H_{50}O_2$
44.26	α-Amyrin	-	-	3.12	$C_{30}H_{50}O$
44.78	Squalene	-	-	2.27	$C_{30}H_{50}$
45.71	Campesterol	-	1.31	2.74	$C_{28}H_{48}O$

Antioxidant activity

DPPH radical is usually used to estimate the antioxidant capacity as a substrate. It is a stable free radical to become a stable molecule that can accept electron or the radical of hydrogen from antioxidants found in the examined extract (Taher *et al.*, 2018). In the present study, scavenging potential of *C. blumei*, *P. amboinicus*, and *S. splendens* extracts on DPPH radical increased in a dose-dependent manner (Fig. 2A). At the concentration of 0.05 g L^{-1}, the scavenging activity of *P amboinicus* extract was 60.84% with 27.3, and 13.67% inhibition of DPPH radical for the extracts of S. *splendens*, and C. *blumei*. Also, the scavenging activity of *P amboinicus*, S. *splendens*, and C. *blumei* extracts were 90.55%, 64.73, and 55.31%, respectively at the concentration of 0.15 g L^{-1}. The antiradical activity was highest for the extract of *P. amboinicus* (IC_{50} = 0.042 g L^{-1}) followed by *S. splendens* extract (IC_{50} = 0.097 g L^{-1}), and *C. blumei* extract (IC_{50} = 0.138 g L^{-1}). Moreover, the IC_{50} value of *P. amboinicus* extract was much lower than that of AA (IC_{50} = 0.1 g L^{-1}).

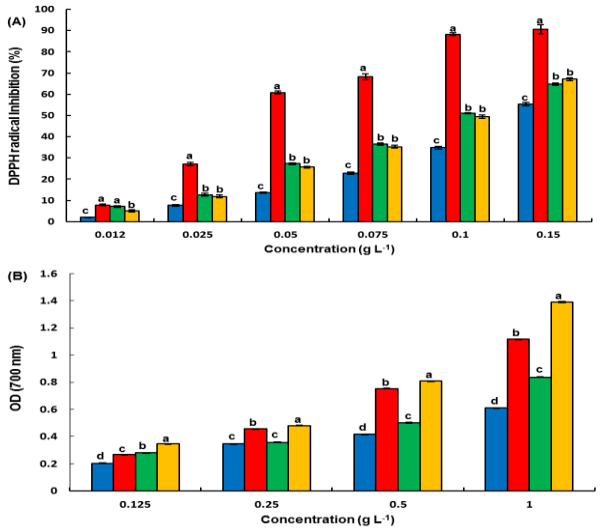

Figure 2. Radical scavenged activity of the methanolic extracts at different concentrations (A). Reducing power assay as OD at 700 nm of the tested extracts (B). All values represent mean ± standard error (SE). Bars with different letters are significantly different according to Tukey's HSD test at $P < 0.05$ for each concentration point. (DPPH) = 1,1-diphenyl-2-picrylhydrazyl, (AA) = Ascorbic acid, and (BHT) = Butylated hydroxytoluene. ( ) *Coleus blumei*; ( ) *Plectranthus amboinicus*; ( ) *Salvia splendens*; ( ) AA or BHT.

Data showed that the extract of *P. amboinicus* had the highest antiradical activity when compared to the other extracts. The strong antiradical activity of *P. amboinicus* extract in the current work agreed to a large extent with the results of the previous work using different solvent extracts (Swamy *et al.*, 2017). Besides, the anti-DPPH ability of leaves aqueous methanol extract of *S. splendens* has been previously reported (Moharram *et al.* 2012).

Plant bioactive metabolites are able to donate electrons and this capability reflects the equivalent RP (Ak and Gülçin, 2008). Metabolites possessing antioxidant ability reducing Fe^{3+}/ferricyanide complex to Fe^{++} form. High absorbance value at 700 nm in RP assay indicates a high reductive potential of the tested sample. In this study, the antioxidant capacity of *P. amboinicus, S. splendens, and* C. *blumei* methanolic extracts relatively increased with graded concentrations. At the low concentration (0.125 g L^{-1}), the methanolic extracts were significantly ($P < 0.05$) effective as BHT in reducing Fe^{3+}/ferricyanide complex to Fe^{++} form (Fig. 2B). Remarkably, the absorbance values of all tested extracts at the maximum concentration (1 g L^{-1}) were significantly ($P < 0.05$) lower than that of BHT in reducing Fe^{3+}/ferricyanide complex to Fe^{++} form(Fig. 2B).In other words, the absorbance value of *P. amboinicus* (1.11) was comparable to BHT (1.39), whereas those of *S. splendens* (0.836), and *C. blumei* (0.609) extracts were much lower than that of reference antioxidant at the concentration of 1 g L^{-1}. Among all tested extracts, the methanolic extract of *C. blumei* recorded the highest EC_{50} value by 0.762 g L^{-1}. While, EC_{50} values of BHT, *P. amboinicus* and *S. splendens* extracts were 0.256, 0.327 and 0.508 g L^{-1}, respectively. Based on the present results, the extract of *P. amboinicus* exhibited the highest RP compared with the other extracts. Furthermore, this is the first study regarding the conduct of RP assay for the three tested extracts.

Phosphomolybdenum antioxidant assay (TAC) is a better method of the depiction of the combined influence of phenolic constituents and other reducing ingredients in the plant extracts. Total antioxidant capacity has been measured on the basis that the reactant antioxidants might afford to the reduction of phosphate-molybdenum (VI) to green molybdenum complex (V) (Abdalla *et al.*, 2019). In the present study, total antioxidant activity (TAC) of BHT, *P. amboinicus, S. splendens,* and *C. blumei* were 353, 306.9, 254.5, and 103.1 g AAE Kg^{-1}, respectively. On other words, the TAC activity of the synthetic antioxidant (BHT) was 1.14, 1.38, and 3.3-fold higher than the methanolic extracts of *P. amboinicus, S. splendens,* and *C. blumei*, respectively.

The antioxidant potential of the tested organic extracts is often attributed to polyphenolic compounds (Taher *et al.*,2018). According to the results, the methanolic extract of *P. amboinicus* was found to be highly rich in the contents of TPs and TFs compared with the other tested extracts. So, the TPs of the tested extracts have an evident effect on the scavenging of free radicals or reducibility. Numerous studies have confirmed the powerful antioxidant properties of carvacrol (Yanishlieva *et al.*, 1999; Ramos *et al.*, 2014). Consequently, the strong antioxidant activity of *P. amboinicus* extract might be mainly attributed to its high TPs content that synergistically acts with other bioactive non-phenolics compounds like the predominant compound of carvacrol.

Antitumor activity

The effect of methanolic extracts at different concentrations (0.012 – 0.2 g L^{-1}) on A549, MCF7, HEPG2, and HCT cell lines were studied. Most of the tested extracts significantly ($P < 0.05$) inhibited the viability of all cell lines used in a concentration-dependent manner (Fig. 3). Only *P. amboinicus* extract inhibited A549, MCF7, and HEPG2 cells by around 50% viability at the concentration of 0.05 g L^{-1}, and caused 80% inhibition on A549 and MCF7 cell lines at 0.1 g L^{-1}. The extract of *C. blumei* exhibited a marginal inhibitory

effect or might be not at all effective. The lowermost IC_{50} values of *P. amboinicus* were found ranged from 0.041 to 0.1 g L^{-1} (Table 3). The methanolic extract of *S. splendens* showed the lowest IC_{50} value against MCF7 cells by 0.04 g L^{-1}. The *C. blumei* extract showed the weakest antiproliferative activity, reflected by high IC_{50} values.

Table 3. IC_{50} values of the methanolic extracts for the antitumor activity.

Parameters	IC_{50} (g L^{-1})			
	Coleus blumei	*Plectranthus amboinicus*	*Salvia splendens*	5-FU
A549	> 0.2	0.041 ± 1.16	0.160 ± 1.99	0.012 ± 0.58
MCF7	0.129 ± 2.17	0.047 ± 1.03	0.040 ± 0.08	0.019 ± 0.58
HEPG2	> 0.2	0.043 ± 1.06	> 0.2	0.018 ± 1.01
HCT	> 0.2	0.1 ± 1.72	> 0.2	0.022 ± 0.71

Results represent mean ± standard error (SE).

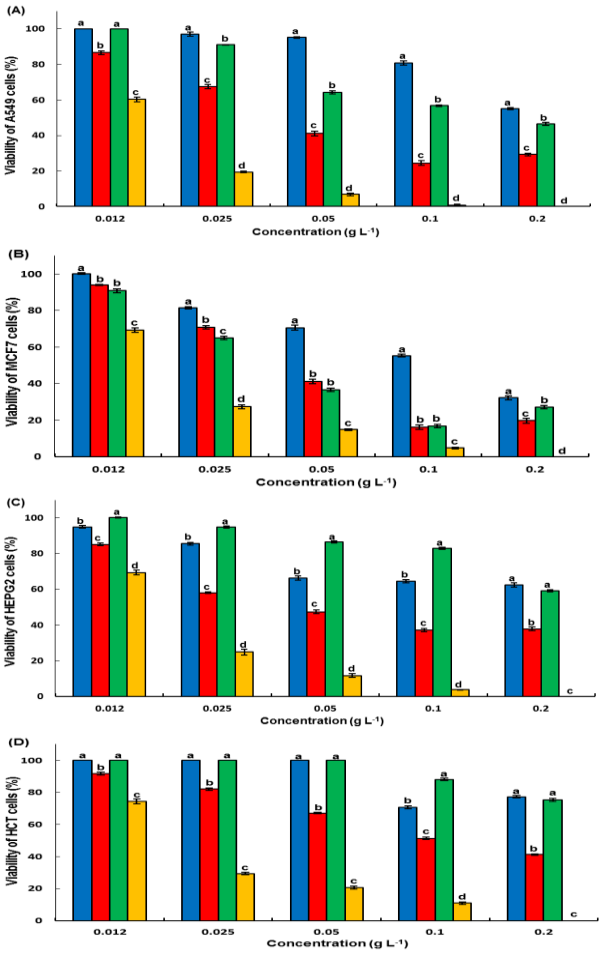

Figure 3. Antitumor activity of the methanolic extracts at different concentrations on viability of the cells of (A) human lung adenocarcinoma-A549, (B) breast adenocarcinoma-MCF7, (C) human liver carcinoma-HEPG2, and (D) colon carcinoma-HCT. All values represent mean ± standard error (SE). Bars with different letters are significantly different according to Tukey's HSD test at $P < 0.05$ for each concentration point. ( ) *Coleus blumei*; ( ) *Plectranthus amboinicus*; ( ) *Salvia splendens*; ( ) 5-Fluorouracil.

The carvacrol nanoemulsion showed excellent antitumor activity *in vitro* and *in vivo* against human lung adenocarcinoma A549 cells via mitochondrial-mediated apoptosis (Khan *et al.*, 2018). Also, Horvathova *et al.* (2007) stated that carvacrol had cytotoxic properties against HEPG2, K562, and colonic Caco-2 cells and significantly decreased the level of DNA damage induced in these cells by the strong oxidant H_2O_2. Also, Arunasree *et al.* (2010) examined the mechanism of carvacrol-inhibitory activity against MDA-MB 231 human metastatic breast cancer cells and found that this compound induced apoptosis in a concentration-dependent pattern. The mechanism of action of carvacrol may in fact be associated with its antioxidant potential and not related to a DNA-damaging effect. Consequently, the strong antitumor activity of *P. amboinicus* extract in this study could be partially explained by the presence of a significant level of the bioactive carvacrol. Furthermore, the antitumor activity of other main constituents in *P. amboinicus* extract such as δ-cadinene, phytol, β-spathulenol, α-copaene and β-sitosterol has been reported (Pejin *et al.*,2014; Türkez *et al.*, 2014; Hui *et al.*,2015; Allam *et al.*, 2018; Robey *et al.*, 2018).

Many studies suggest that polyunsaturated fatty acids (PUFA) particularly n-3 PUFA may have anticancer effects. For instance, Song *et al.* (2014) stated that saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA) are known to proliferate cancer risk, while PUFA, particularly n-3, is recognized to have cytotoxic activity in many cancer types. Correspondingly, α-linolenic acid has documented to adjust the growth of cervical and breast cancer cell lines through the regulation of NO release and initiation of lipid peroxidation (Deshpande *et al.*,2013). So, the considerably high percentage of α-linolenic acid in *S. splendens* extract in this study can partially contribute to the proper anticancer activity. The cytotoxic potential of syringic acid, the main phenol in *S. splendens* extract, against human colorectal cancer cells has been previously reported (Abaza *et al.*, 2013). The extract of C. *blumei* showed the lowest antitumor activities toward all examined cell lines. However, the notable cytotoxicity of this extract against A549 and MCF7 cell lines could be explained by the presence of a high amount of 5-HMF as the major compound. Besides, Zhao *et al.* (2013) found that 5-HMF had antiproliferative activity on human A549 and MCF7 cell lines higher than those of other tested cell lines. Total fatty acids in *C. blumei* extract were notably high, however, it recorded the weakest antitumor activity. This could be elucidated by the minimized content of n-3 fatty acids that owing cytotoxic effects.

As tumors are categorized by uncontrolled cellular proliferation, there is considerable interest in treatment-induced apoptosis. In this respect, the further increases in cell viabilities of most of the tested cell lines at the highest concentration (0.2 g L^{-1}) might be explained by the induction of late apoptosis. Similarly, Jiménez *et al.* (2016) found that the higher tested concentration of *Rosa canina* fractions were able to induce late apoptosis causing a probable increase in the viability of Caco2 cells.

Antifungal activity

Methanolic extracts of all samples were very different in terms of their antifungal activities against all tested fungi (Table 4). The methanol extract of *P. amboinicus* exhibited the strongest inhibition on mycelial growth of many tested fungi compared to other plant extracts, including *S. sclerotiorum*, and *C. cynodontis* by 83.0, and 82.1%, respectively, followed by *B. cinerea*, *B. hawaiensis*, *B. spicifera*, and *A. alternata* by 77.8, 74.1,

73.9, and 73.7%, respectively, at the concentration of 32 g L^{-1}. Also, *P. amboinicus* extract displayed remarkable antifungal activity against *F. sambucinum* (68.1%), *A. parasiticus* (66.7%), and *F. oxysporum* (55.2%) at the same concentration. The greatest effect of the methanolic extract of *S. splendens* was on *B. hawaiensis*, and *F. oxysporum* by 32.0% inhibition on the fungal growth at 32 g L^{-1}. Besides, the methanol extract of *C. blumei* showed a weak inhibitory effect against all tested fungi even with the highest concentration.

It could be represented that , *P. amboinicus* extract caused the higher inhibition on the mycelial growth of most tested fungi particularly at the concentration of 32 g L^{-1}. On the contrary, Sivaranjani *et al.* (2019) found the diminished antifungal activity of methanolic extract of *P. amboinicus* grown in India against postharvest pathogens simultaneously with high antibacterial activity at concentrations higher than those tested in the present study (50-100 g L^{-1}). This discrepancy of the prospective antifungal activity of *P. amboinicus* extract might be due to the presence of great variations in its main components particularly the essential oil, carvacrol, and/or thymol have always been among the key constituents. Similarly, Tadros *et al.* (2017) declared that parsley essential oil which was widely varied worldwide as a result of environmental and/or genetic factors predominantly affected its antimicrobial activity. The remarkable antifungal activity of *P. amboinicus* extract might be partially due to the presence of a significant amount of carvacrol as well as other monoterpenes and sesquiterpenes. In this context, several reports have documented the strong antifungal activity of carvacrol against oral candidiasis, food-relevant fungi, and postharvest pathogens *(*Chami *et al.*,2004; Abbaszadeh *et al.*, 2014; Zhang *et al.*,2019). Also, the antifungal activity of the other major compounds in the extract of *P. amboinicus* such as δ-cadinene, phytol, β-sitosterol, and rosemarinic acid has been reported (Kundu *et al.*,2013; Ghaneian *et al.*, 2015; Fialová *et al.*,2019; Moosavi *et al.*,2020). Thus, the application of *P. amboinicus* extract and its components might be considered as a good alternative to reduce the use of synthetic fungicides.

Table 4. Effect of methanolic plant extracts on mycelial growth of fungal pathogens at different concentrations.

Fungi	Mycelial growth inhibition (%)								
	Coleus blumei			*Plectranthus amboinicus*			*Salvia splendens*		
	8 g L^{-1}	16 g L^{-1}	32 g L^{-1}	8 g L^{-1}	16 g L^{-1}	32 g L^{-1}	8 g L^{-1}	16 g L^{-1}	32 g L^{-1}
Alternaria alternata	0 ± 0.0	0 ± 0.0	12.5 ± 1.2	0 ± 0.0	0 ± 0.0	73.7 ± 0.9	0 ± 0.0	15.6 ± 0.8	15.6 ± 0.9
Aspergillus parasiticus	0 ± 0.0	0 ± 0.0	0 ± 0.0	1.4 ± 0.8	15.6 ± 0.6	66.7 ± 1.2	0 ± 0.0	0 ± 0.0	0 ± 0.0
Bipolaris hawaiensis	0 ± 0.0	0 ± 0.0	10.2 ± 1.4	0 ± 0.0	0 ± 0.0	74.1 ± 0.8	0 ± 0.0	0 ± 0.0	32.0 ± 1.2
Bipolaris spicifera	0 ± 0.0	0 ± 0.0	22.2 ± 1.2	0 ± 0.0	0 ± 0.0	73.9 ± 1.1	0 ± 0.0	0 ± 0.0	0 ± 0.0
Botrytis cinerea	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	77.8 ± 1.2	0 ± 0.0	0 ± 0.0	22.4 ± 0.7
Cochliobolus cynodontis	0 ± 0.0	6.2 ± 0.6	16.6 ± 0.9	5.2 ± 0.8	39.3 ± 0.9	82.1 ± 0.6	0 ± 0.0	0 ± 0.0	0 ± 0.0
Fusarium oxysporum	0 ± 0.0	17.5 ± 0.6	24.8 ± 1.2	0 ± 0.0	3.0 ± 0.8	55.2 ± 0.8	0 ± 0.0	7.0 ± 1.3	32.0 ± 1.0
Fusarium sambucinum	0 ± 0.0	9.7 ± 0.9	31.1 ± 1.2	0 ± 0.0	17.8 ± 1.2	68.1 ± 0.8	0 ± 0.0	0 ± 0.0	0 ± 0.0
Macrophomina phaseolina	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	10.0 ± 0.6	0 ± 0.0	0 ± 0.0	0 ± 0.0
Sclerotinia sclerotiorum	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	83.0 ± 0.6	0 ± 0.0	0 ± 0.0	0 ± 0.0

Results represent mean ± standard error (SE).

CONCLUSION

The present study indicated that the methanolic extract of *P. amboinicus* containing the highest values of the total polyphenols content and total flavonoids content among all tested extracts. Rosmarinic acid, naringin, catechol, syringic acid, ellagic acid, and myricetin were detected in the extract of *P. amboinicus* as major phenolic compounds by HPLC analysis as well as carvacrol, the predominant constituent by GC-MS analysis. *P. amboinicus* extract showed the greatest antioxidant potential with a remarkable antitumor activity on A549, MCF7, and HEPG cell lines. Besides, this extract caused a significant inhibition on the mycelial growth of some human and plant pathogenic fungi. Overall, the ornamental plants of different genera belonging to the family of Lamiaceae have therapeutic properties with various levels. Therefore, the points of future researches depend on the application of the essential oil nanoemulsions of these plants as effective agents for the treatment of cancer as well as in biofungicides.

REFERANCES

Abaza, M.S., Al-Attiyah, R., Bhardwaj, R., Abbadi, G., Koyippally, M., and Afzal, M. (2013). Syringic acid from *Tamarix aucheriana* possesses antimitogenic and chemo-sensitizing activities in human colorectal cancer cells. Pharmaceutical Biology 51: 1110-1124.

Abbaszadeh, S. , Sharifzadeh, A. , Shokri, H. , Khosravi, A.R. , and Abbaszadeh, A. (2014). Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. Journal de Mycologie Médicale 24: e51-e56.

Abdalla, M.E. , Taher, M.A. , Sanad, M.I., and Tadros, L.K. (2019). Chemical properties, phenolic profiles and antioxidant activities of pepper fruits. Journal of Agricultural Chemistry and Biotechnology 10: 133-140.

Abu-Bakar, M.F. , Ahmad , N.E. , Suleiman, M., Rahmat, A., and Isha, A. (2015). Garcinia dulcis fruit extract induced cytotoxicity and apoptosis in HepG2 liver cancer cell line. Biomed Research International 2015, 916902. doi: 10.1155/2015/916902

Ak, T., and Gülçin, I. (2008). Antioxidant and radical scavenging properties of curcumin. Chemico-Biological Interactions 174: 27-37.

Allam, P. , Al-Yousef, H. , Siddiqui, N., Alhowiriny, T., Alqasoumi, S. , Amina, M ., Hassan, W. , Hassan, B. , Abdelaziz, S., and Abdalla, R. (2018). Anticancer activity and concurrent analysis of ursolic acid, β-sitosterol and lupeol in three different *Hibiscus species* (aerial parts) by validated HPTLC method. Saudi Pharmaceutical Journal 26: 1060-1067.

Arunasree, M. (2010). Anti-proliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB 231. Phytomedicine 17: 581-588.

Bandeira, M., Barbosa, F. Barbosa, M., Rodrigues, C., Bacarin, A., Peters, A., and Braga, J. (2011). Composição do óleo essencial de quatro espécies do gênero Plectranthus. Revista Brasileira de Plantas Medicinais. 13: 157-164.

Boukhary, R., Raafat, K., Ghoneim, I., Aboul-Ela, M., and El-Lakany, A. (2016). Anti-inflammatory and antioxidant activities of *Salvia fruticosa*: An HPLC determination of phenolic contents. Evidence-Based Complementary and Alternative Medicine 2016: 7178105

Chami, N., Chami, F., Bennis, S. , Trouillas, J. , and Remmal, A. (2004). Antifungal treatment with carvacrol and eugenol of oral candidiasis in immunosuppressed rats. Brazilian Journal of Infectious Diseases 8: 217-226.

Chiu, J. , Huang, H. , Chiu, S. , Lu, T. , Chen , Y. , Peng, W., and Chen, C.(2012). Analgesic and Anti-inflammatory activities of the aqueous extract from *Plectranthus amboinicus* (Lour.) Spreng. both in vitro and in vivo. Evidence-Based Complementary and Alternative Medicine 2012: 08137.

Cretton, S. , Saraux, N. , Monteillier, A. , Righi, D. , Marcourt ,L. , Genta-Jouve, G. , Wolfender , L. , Cuendet, M. , Christen, P. (2018). Anti-inflammatory and antiproliferative diterpenoids from *Plectranthus scutellarioides*. Phytochemistry 154: 39-46.

Deshpande, R., Mansara, P., Suryavanshi, S., Kaul-Ghanekar, R. (2013). Alpha-linolenic acid regulates the growth of breast and cervical cancer cell lines through regulation of NO release and induction of lipid peroxidation. Journal of Molecular Biochemistry 2:6-17.

Dörr, S., Zimmermann, F., Kögler, S. , Mibus, H. (2019). Influence of leaf temperature and blue light on the accumulation of rosmarinic acid and other phenolic compounds in *Plectranthus scutellarioides* (L.) Environmental and Experimental Botany 167:103830.

El-hawary, S. , El-sofany, H., Abdel-Monem, R. , Ashour, S., and Sleem, A.(2012). Polyphenolics content and biological activity of *Plectranthus amboinicus* (Lour.) spreng growing in Egypt (Lamiaceae). Pharmacognosy Journal 4: 45-54.

El-Sawi , A. , Ezzat, M. , Aly, F. , Merghany, M., and Meselhy, R.(2020). Neuroprotective effect of *Salvia splendens* extract and its constituents against $AlCl_3$-induced Alzheimer's disease in rats. Advances in Traditional Medicine 20:381-393.

Elsherbiny, E., Safwat, N., and Elaasser, M. (2017). Fungitoxicity of organic extracts of *Ocimum basilicum* on growth and morphogenesis of *Bipolaris* species (teleomorph *Cochliobolus*). Journal of Applied Microbiology 123: 841-852.

Fialová, B., Kello, M. , Čoma, M. , Slobodníková, L. , Drobná, E., Holková , I. , Garajová, M. , Mrva, M ., Zachar, V. , and Lukáč, M.(2019).Derivatization of rosmarinic acid enhances its in vitro antitumor, antimicrobial and antiprotozoal properties. Molecules 24:1078. https://doi.org/10.3390/molecules24061078

Firdous, S. , Dadass, A. , Khan, K. , Usmani, S.,and Ahmad. V. (1999). A new triterpenoid from the leaves of *Salvia triloba*. Fitoterapia 70:326-327.

Ghaneian, M., Ehrampoush, M., Jebali, A., Hekmatimoghaddam, S. , and Mahmoudi, M.(2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2: 13-16.

Ghomari, O. , Sounni, F., Massaoudi, Y., Ghanam, J., Batsoule, L. , Kaitouni, D. , Merzouki, M.,and Benlemlih, M.(2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnology Reports 23: e00347.

Gupta, S., Bhatt, P., Joseph, G., Negi, P., and Varadaraj, M. (2013). Phenolic constituents and biological activities of leaf extracts of traditional medicinal plant *Plectranthus amboinicus* Benth (Lamiaceae). TANG 3, e32. DOI:10.5667/tang.2013.0027

Harley, R., Atkins, S., Budantsev, A. , Cantino, P., Conn, B., Grayer, R., Harley, M. , De Kok, R. , Krestovskaja, T., and Morales, R.(2004). Labiatae. In: Kadereit J.W., editor. The Families and Genera of Vascular Plants. Springer-Verlag; Berlin, Germany, pp. 167-282.

Hassani, M. , Zainati, I. , Zrira ,S. , Mahdi , S. , and Oukessou, M. (2012). Chemical composition and antimicrobial activity of *Plectranthus amboinicus* (Lour) Spring. essential oil from Archipelago of Comoros. Journal of Essential Oil Bearing Plants15:637-644.

Horvathova, E. , Turcaniova, V. , and Slamenova, D. (2007).Comparative study of DNA-damaging and DNA-protective effects of selected components of essential plant oils in human leukemic cells K562. Neoplasma 54:478-483.

Hsu, K., and Ho, C. (2019). Antimildew effects of *Plectranthus amboinicus* leaf essential oil on Paper. Natural Product Communications 14:1-6.

Hui ,L., Zhao, G., and Zhao, J.(2015). δ-Cadinene inhibits the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest. International Journal of Clinical and Experimental Pathology 8: 6046-6056.

Ibrahim, T.(2012). Chemical composition and biological activity of extracts from *Salvia bicolor* Desf. growing in Egypt. Molecules 17: 11315-11334.

Ito, T. , Kartika, S. , Rakainsa, Nisa, K. , Morita, H.(2018).Three new abietane-type diterpenoids from the leaves of Indonesian *Plectranthus scutellarioides*. Fitoterapia 127: 146-150.

Jiménez, S., Gascón, S., Luquin, A., Laguna, M., Ancin-Azpilicueta, C., and Rodríguez-Yoldi, M.J.(2016) *Rosa canina* Extracts Have Antiproliferative and Antioxidant Effects on Caco-2 Human Colon Cancer. PLoS ONE 11(7): e0159136. https://doi.org/ 10.1371/journal.pone.0159136

Jurkaninová, S. , Kubínová, R. , Nejezchlebová, M., Gazdová, M. , Hanáková, Z. ,and Dall'Acqua. (2019). Anti-MRSA activity of abietane diterpenes from *Coleus blumei* Benth. Natural Product Research. DOI: 10.1080/14786419.2019.1686371

Khan , I. , Bahuguna, A. , and Kumar, P. (2018). In vitro and in vivo antitumor potential of carvacrol nanoemulsion against human lung adenocarcinoma A549 cells via mitochondrial mediated apoptosis. Scientific Reports 8: 144. DOI: 10.1038/s41598-017-18644-9

Kubínová, R. , Gazdová ,M. , Hanáková, Z. , Jurkaninová, S. , Dall'Acqua, S. , Cvačka. J. , and Humpa, O. (2019). New diterpenoid glucoside and flavonoids from *Plectranthus scutellarioides* (L.) R. Br. South African Journal of Botany 120:286-290.

Kumar, P. , Sasmal, D. , and Mazumder, P. (2010).The antihyperglycemic effect of aerial parts of *Salvia splendens* (scarlet sage) in streptozotocin-induced diabetic-rats. Pharmacognosy Research 2: 190-194.

Kundu, A. , Saha, S. , Walia , S. , Shakil, N., Kumar, J. , and Annapurna , K. (2013). Cadinene sesquiterpenes from *Eupatorium adenophorum* and their antifungal activity. Journal of Environmental Science and Health, Part B 48: 516-522.

Levita, J. , Sumiwi , S., Pratiw, T., Ilham, E., Sidiq, S.,and Moektiwardoyo, M.(2016).Pharmacological activities of *Plectranthus scutellarioides* (L.) R.Br. leaves extract on cyclooxygenase and xanthine oxidase enzymes. Journal of Medicinal Plants Research 10: 261-269.

Lin, J. ,and Tang, C.(2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry 101: 140-147.

Lu, Y. , and Foo , L.(2002). Polyphenolics of Salvia. A review. Phytochemistry 59:117-140.

Lukhoba, C. , Simmonds, M., and Paton, A. (2006). Plectranthus: A review of ethnobotanical uses. Journal of Ethnopharmacology 103: 1-24.

Matias, D. , Nicolai, M. , Fernandes, A., Saraiva, N. , Almeida, J ., Saraiva, L. , Faustino, C. , Díaz-Lanza, A., Reis, C., and Rijo, P.(2019).Comparison study of different extracts of *Plectranthus madagascariensis*, *P. neochilus* and the rare *P. porcatus* (Lamiaceae): chemical characterization, antioxidant, antimicrobial and cytotoxic activities. Biomolecules 9: 179.

Moharram, F., Marzouk, M., El-Shenawy, S., Gaara, A.,and El Kady, W.(2012).Polyphenolic profile and biological activity of *Salvia splendens* leaves. Journal of Pharmacy and Pharmacology 64: 1678-1687.

Moosavi, B., Liu, S., Wang, N. , Zhu, X., and Yang,G. (2020). The anti-fungal β-sitosterol targets the yeast oxysterol-binding protein Osh4. Pest Management Science 76:704-711.

Newman, D., and Cragg, G.(2012).Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products 75: 311-335.

Ni, J., Ren, Q. , Luo, J. , Chen, Z ., Xu, X. , Guo, J. , Tan, Y. , Liu , W. , Qu, Z. , Wu , Z. , Wang, J. , Li .Y , Guan , G. , Luo, J. , Yin , H ., and Liu, G. (2020). Ultrasound-assisted extraction extracts from *Stemona japonica* (Blume) Miq. and *Cnidium monnieri* (L.) Cuss. could be used as potential *Rhipicephalus sanguineus* control agents. Experimental Parasitology 217: 107955.

Oyaizu, M.(1986).Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics 44:307-315.

Pejin, B., Kojic, V. , and Bogdanovic, G. (2014).An insight into the cytotoxic activity of phytol at in vitro conditions. Natural Product Research 28: 2053-2056.

Prieto, P., Pineda, M., and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 269:337-341.

Rajesh, V. , and Gayathri, K.(2015). Angiogenesis modulation by *Plectranthus amboinicus* leaf extract and its fractions on chorioallantoic membrane and tumor induced angiogenesis. Oriental Pharmacy and Experimental Medicine 15: 257-276.

Ramos, M., Beltrán, A. , Peltzer, M. , Valente, A.,and Garrigós,M.(2014).Release and antioxidant activity of carvacrol and thymol from polypropylene active packaging films. LWT - Food Science and Technology 58: 470-477.

Rani, J., and Kapoor, M. (2019).Gas chromatography-mass spectrometric analysis and identification of bioactive constituents of *Catharanthus roses* and its antioxidant activity. Asian Journal of Pharmaceutical and Clinical Research 12:461-465.

Robey, R. W. , Pluchino, K. ,Hall, M., Fojo, A., Bates ,S., and Gottesman, M.(2018) Revisiting the role of ABC transporters in multidrug-resistant cancer. Nature Reviews Cancer 18: 452-464.

Salaeh, A. , Augusti , R., Susilawati , Y., Sumiwi, S., and Moektiwardojo, M. (2018).Antidiabetic activity of fractions and sub fraction of Iler [*Plectranthus scutellarioides* (L.) R. Br.] Leaves on diabetic mice induced by Alloxan. Research Journal of Chemistry and Environment 22: 5-10.

Sanad, M. ,El-Rafey, H. , Hassan, R. ,and Taher, M. (2012). Evaluation of antidiabetic, antihyperlipidemic and antioxidant activities of the methanolic extracts of *Bauhinia variegata* and *Enterolobium cyclocarpum* leaves in streptozotocin diabetic rats. Journal of Agricultural Chemistry and Biotechnology 3: 261-275.

Shaheen, U., Hussain, M.,and Ammar, H. (2011). Cytotoxicity and antioxidant activity of new biologically active constituents from *Salvia lanigra* and *Salvia splendens*. Pharmacognosy Journal 3: 36-48.

Singleton, V., Orthofer, R., and Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology 299:152-178.

Sivaranjani, D. , Saranraj, P. , Manigandan, M. , and Amala, K.(2019). Antimicrobial activity of *Plectranthus amboinicus* solvent extracts against human pathogenic bacteria and fungi, Journal of Drug Delivery and Therapeutics 9: 36-39.

Song, M. , Chan, A. , Fuchs, C., Ogino, S ., Hu, F., Mozaffarian, D. , Ma, J. , Willett, W., Giovannucci, E. , and Wu, K. (2014). Dietary intake of fish, ω-3 and ω-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women. International Journal of Cancer 135: 2413-2423.

Sulaiman, C., Deepak, M., and Balachandran, I. (2018). Spectrophotometric and tandem mass spectroscopic analysis of Indian borage (*Plectranthus amboinicus* (Lour.) Spreng.) for its polyphenolics characterization, Beni-Suef University Journal of Basic and Applied Sciences 7: 471-473.

Surahmaida, S. , and Umarudin, U.(2019). Toxicity of miana leaf (*Coleus* blumei) extract against houseflies (Musca domestica). Biosaintifika 11: 249-255.

Swamy, M., Arumugam, G. , Kaur, R. , Ghasemzadeh ,A. , Yusoff , M. , and Sinniah , U.(2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* leaves. Evidence-Based Complementary and Alternative Medicine 2017, 1517683.

Sytar, O. , Hemmerich, I. Zivcak, M.,Rauh, C., and Brestic, M.(2018). Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi Journal of Biological Sciences 25:631-641.

Tadros, L. , El-Rafey, H. , Elfadaly, H. , Taher, M. , and Elhafny, M .(2017). Phenolic profile, essential oil composition, purification of kaempferol 3-arabinofuranoside and antimicrobial activity of parsley cultivated in Dakhalia governorate. Journal of Agricultural Chemistry and Biotechnology 8:183-189.

Taher, M., Tadros, L., and Dawood, D. (2018). Phytochemical constituents, antioxidant activity and safety evaluation of Kei-apple fruit (*Dovyalis caffra*). Food Chemistry 265:144-151.

Taher, M., MennatAllah, E., Tadros, L., and Sanad, M.(2020) The effects of new formulations based on Gum Arabic on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. Journal of Food Measurement and Characterization 14:2489-2502.

Terto, M., Gomes, J., Araújo, D., Silva, T., Ferreira, J.,Souza, J., Silva ,M., and Tavares, J.(2020). Photoprotective activity of *Plectranthus amboinicus* extracts and HPLC quantification of rosmarinic acid. Revista Brasileira de Farmacognosia 30: 183-188.

Tungmunnithum, D. , Garros, L. , Drouet, S. , Renouard,S., Lainé, E. , and Hano, C.(2019) Green ultrasound assisted extraction of trans rosmarinic acid from *Plectranthus scutellarioides* (L.) R.Br. Leaves. Plants 8:50.

Türkez, H. , Celik , K. ,and Toğar, B. (2014). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology 66: 597-603.

Vichai , V. , and Kirtikara , K.(2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols 1: 1112-1116.

Viswanathaswamy, A., Koti, B., Gore, A.Thippeswamy, A., and Kulkarni, R. (2011). Antihyperglycemic and antihyperlipidemic activity of *Plectranthus Amboinicus* on normal and alloxan-induced diabetic rats. Indian Journal of Pharmaceutical Sciences 73:139-145.

Yanishlieva, N., Marinova, E., Gordon, M., and Raneva, V.(1999).Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry 64:59-66.

Zhao, L. , Chen, J. , Su, J. , Li, L. , Hu , S. ,and Li , B.(2013) In vitro antioxidant and antiproliferative activities of 5-Hydroxymethylfurfural. Journal of Agricultural and Food Chemistry 61:10604-10611.

Zhang, J., Ma, S. , Du, S. , Chen, S. , and Sun, H. (2019) . Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. Journal of Food Science and Technology 56: 2611-2620.

التركيب الكيميائى والنشاطات المضادة للاكسدة والتورم والفطريات للمستخلصات الميثانولية لأوراق الكاليوس والزعتر الجبلى والسلفيا

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تهدف الدراسة الحالية الى دراسة التوصيف الكيميائى للمستخلصات الميثانولية لاوراق الكاليوس والزعتر الجبلى والسلفيا وكذلك تقييم نشاطاتها المضادة للاكسدة وللتورم وللفطريات. وقد أظهر مستخلص الزعتر الجبلى اعلى محتوى للفينولات الكلية وللفلافونيدات الكلية بتركيز 122.2 جم مكافئات حامض الجاليك/كجم ، 41.1 جم مكافئات كيورستين /كجم مستخلص على التوالى . تبعا للتحليل الكروماتوجرافى السائل فان مستخلص الزعتر الجبلى احتوى على حامض الروزمارنيك كمكون فينولى اكثر سيادة بتركيز 1.021 جم/كجم . كما اظهر التحليل الكروماتوجرافى الغازى المتصل بمطياف الكتلة ان الكارفاكرول هو المكون الاساسى لنفس المستخلص بنسبة 7.59% . اوضح كذلك مستخلص الزعتر الجبلى أقل قيم IC_{50} و EC_{50} ضد الشارد الحر DPPH وللقدرة الاختزالية بقيم 0.042 و 0.327 جرام/لتر على التوالى بما يؤكد قدرته المرتفعة كمضاد للاكسدة . بالمثل فان مستخلص الزعتر الجبلى اعطى اعلى قدرة مضادة للخلايا السرطانية ضد خلايا A549 و MCF7 و HEPG2 بقيم IC_{50} 0.041 ، 0.047 ، 0.043. جرام/لتر على التوالى . بينما تفوق مستخلص السلفيا انتخابيا ضد خلايا MCF7 بقيمة IC_{50} 0.040 جرام/لتر . وفى النهاية أظهر مستخلص الزعتر الجبلى تفوقه فى تثبيط نمو ميسليا فطر *Sclerotinia sclerotiorum* بنسبة 83.0% وفطر *Cochliobolus cynodontis* بنسبة 82.1 % وفطر *Botrytis cinerea* بنسبة 77.8% وفطر *Bipolaris hawaiensis* بنسبة 74.1%، وفطر *B. spicifera* بنسبة 73.9%، وفطر *Alternaria alternata* بنسبة 73.7% وذلك عند استخدامه بتركيز 32 جرام/لتر.