Egypt. J. Bot. **Vol. 59,** No. 1, pp. 81 - 94 (2019)

In vitro Evaluation of *Syzygium aromaticum* L. Ethanol Extract as Biocontrol Agent against Postharvest Tomato and Potato Diseases

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ETHANOLIC extract of dried flower bud of *Syzygium aromaticum* L. **(**clove) was investigated to evaluate its antifungal activity against local fungal isolates causing post-harvest infections form potato and tomato. Four fungal isolates; *Geotrichum candidum, Alternaria alternata, Fusarium oxysporum* and *Mucor hiemalis* were identified. All fungal isolates were found to be inhibited by the extract; whereas the smallest inhibition zone) of *S. aromaticum* extract was 2.5% *for M. hiemalis, A. alternata, G. candidum* and 12.5% for *F. oxysporum.* Quantitative assessment of phytochemical compounds in clove plant revealed the presence of phenolic acids (20.80%); flavonoids (26.81%); tannins (4.90%), saponins (2.60%), alkaloids (1.60%), total protein (17.83%), total carbohydrate (2.23%) and total oil (0.90%). Biochemical profile was revealed by GC/MS which detect 18 different chemical compounds. The major compounds were eugenol (17.27%), trans-caryophyllene (0.50%), humulene (3.33%), anthracenedione (3.35%), cedran-diol (0.61%), citroflex A (5.45%) and lucenin 2 (8.25%). TLC technique and different solvent systems were employed to select the proper one based on its capacity to isolate the maximum number of fluorescent spots in crude extract. Chloroform-acetone-butanol (CAB) 85:15:20 could efficiently separate three bands (A, B and C). Each detected band was individually screened for its antifungal activity against the isolated fungi. Only band C showed a significant antifungal activity. Mass spectroscopy (MS) was used to investigate characteristics, purity as well as expectation of both chemical formula and molecular weight of the isolated compounds; which finally proved that glucoside β-sitosterol was the main compound represented in fraction -C.

Keywords: *Syzygium aromaticum*, Antifungal, Post-harvest, Secondary metabolites, TLC, GC/MS.

Introduction

Fungi are common pathogens and the main cause of crop diseases. They affect most of fruits and vegetables during storage and transport. Mold growth depends on many factors such as pH, water activity, temperature, atmosphere and time. Fruit infection by fungi may appear during the growth, or due to the improper harvesting, handling, packaging, transportation, post-harvest stocks and marketing conditions, or after purchase by the consumer (Abdullah et al., 2016). Much of these losses are due to the attack of several fungal pathogens because, after harvest, fruits have lost most of the intrinsic resistance that protect them while they are attached to the plant. In addition, most of the post-harvest pathogens require a wound for their penetration (Droby et al., 1992). Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted because fungicides represent a danger to the health of humans, animals, and the environment (Harris et al., 2001). In recent years, effort to reduce the use of chemical pesticides in agriculture has increased. Concerns have been raised about their environmental impact and the potential health risk related to the use of these compounds. Most of the synthetic pesticides are non-biodegradable and thus have the potential to disturb the ecological balance. Development of pesticide resistance in pests is also a major issue in today's agriculture which subsequently leads to an application of greater dose of the pesticide to counteract the resistance. This in turn causes a worsening of the ambient pollution problem. Because of growing

#Corresponding author email: elbousmona@gmail.com
DOI: 10.21608/ejbo.2018.3838.1180
Edited by: Prof. Dr. Salama A. Ouf, Faculty of Science, Cairo University, Cairo, Egypt.

concerns about health and environmental safety, the use of toxic, carcinogenic and environmentally damaging chemicals is currently being discouraged. Therefore, there is a need to develop alternative agents for the control of pathogenic fungal diseases in plants (Bhuyan & Das, 2012).

Clove *(Syzygium aromaticum* L.) is an aromatic dried flower bud of a tree that grows in hot tropical climates, it used as spicy allover the world because the aroma of the clove is pleasant (Bhowmik et al., 2012). Moreover, it has many useful and medical purposes. It is used to control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain (Elujoba et al., 2006; Sulieman et al., 2007 and Tanko et al., 2008). In addition, the *S. aromaticum* are highly antimutagenic (Miyazawa & Hisama, 2003) anti-inflammatory (Kim et al., 1998), antioxidant (Chaieb et al., 2007), antithrombotic (Srivastava & Malhotra, 1991). Recently, antibacterial and antifungal activities of *S. aromaticum* was reported (Kumar et al., 2014 and Hamini-Kada et al., 2014).

The objective of the present research was to evaluate the antifungal activity of ethanolic extract of *S. aromaticum* dried flower buds against some fungi isolated from infected post-harvested potato tubers and tomato fruits collected from the Egyptian markets in addition to preliminary identification of the most dominant secondary metabolites in the crude extract.

Materials and Methods

Collection of specimens

Infected potato tubers and tomato fruits showing symptoms of rotting were purchased from local markets at Damietta Governorate and then transferred under aseptic conditions to isolate infectious fungi.

Isolation of fungi

Infected pieces were cut into $1\times1cm^2$ segments and then dipped in 70% ethanol momentary and soaked in solution of 5% sodium hypochlorite for 1-3min. The sterilized pieces then washed once with sterilize distilled water, then transfer to petri dishes containing Czapek's yeast extract agar medium CYA as described by Gams et al. (1998) which was supplemented with chloramphenicol as an antibacterial agent ($0.5gL^{-1}$). Two to three pieces were distributed onto surface of the medium. All petri-dishes were then incubated at 25-28°C then checked regularly every day, up to 6 days. A portion of growing mycelia was transferred onto a new sterile petri-dishes containing CYA medium for further purification purposes. All purified fungal isolates were re inoculated into new healthy individuals of potato tubers and tomato fruits to investigate the symptoms of rotting and subsequently, performing the pathogenicity test that ensure the ability of the investigated fungi for infection. Finally, all fungal isolates were identified according to their macroscopic and microscopic characteristics (Domsch et al., 1980).

Collection and preparation of plant material

Dried flower buds of *S. aromaticum* were purchased from local market in Damietta, Egypt. Then they were washed by tap water 3 times followed by sterilized distilled water. They were pounded to powder and then subsequently sieved to remove coarse particles.

Plant material was dissolved in 95% ethanol (1:10 w/v); each 1g sample had been dissolved in 10ml of solvent and extracted on shaker at 150rpm for 24h at room temperature. Mixtures were then filtered through sterile layer of gauze to remove any solid plant materials and then through Whatman No. 1 filter paper. The filtrate was concentrated *via* evaporation process which was carried out by using rotary evaporator at 35-40°C.

Evaluation of antifungal activity of S. aromaticum ethanolic extract

Antifungal activity test

Antifungal activity was determined by well diffusion method which was described by Rojas et al. (2006). Petri dish containing 20ml of potato dextrose agar (PDA) media according to Beever and Bollard (1970) was inoculated with 250µL spore suspension (1×10^5cfu/ml) of seven days-old fungal culture. Six mm wells were made using a sterile cork borer; in each well, 50µL from plant crude extract, antibiotic (ciclopirox-olamine) (positive control) and ethanol (blank) were applied using sterilized dropping micropipettes and then incubated at 27 C) for 6 days.

Different concentrations (50%, 25%, 12.5%, 5%, 2.5% and 1%) of clove ethanolic extract were prepared in 95% ethanol. Well diffusion method described by Al-Kuraishi et al. (2013) was followed to detect inhibitory activity of *S.*

aromaticum. A Petri dish containing 20ml of PDA media seeded with 250µL spore suspension (1×10^5cfu/ml) of seven days-old fungal cultures was prepared .Wells were made by using sterilized cork borer (6 mm). In each well 50µL volume of each dilution was applied (triplicate). The plates were incubated at 27°C for 6 days, after incubation period, the diameter of inhibition zones around wells were recorded in millimeters. Tests were performed in triplicate.

Preliminary phytochemical screening of S. aromaticum

The ethanolic plant extract was subjected to preliminary qualitative tests for testing the presence of alkaloids, saponins, tannins, steroids and flavonoids according to Harborne (1998).

Quantitative phytochemical screening of S. aromaticum.

Estimation of total flavonoid content

Flavonoid content of extract was determined by colorimetric method described by Chang et al. (2002). The plant extract was separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1 M potassium acetate, and 2.8ml of distilled water, and left at room temperature for 30min. The absorbance of the reaction mixture was measured at 415nm using spectrophotometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of rutin/ gm of extract).

Estimation of total tannins

The total tannin content in the plant extract was determined by Folin-Deins reagent method adopted by Polshettiwar et al. (2007). The absorbance was measured at 755nm.

Estimation of total saponins

Twenty grams of plant powder was dispersed in 200ml of 20% ethanol. The suspension was heated over a water bath for 4h with stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. Twenty ml of diethyl ether was added to the concentrate and shaken vigorous. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n-butanol was added. The combined n-butanol extract were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was evaporated. Then, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage according to Okwu & Ukanwa (2007) determination.

Estimation of total alkaloids (gravimetric method)

Ten grams of the plant powders was extracted with 90% ethanol till exhaustion tested with Mayer's reagent using the standard procedure described by Woo & Püls (1977).

Estimation of total phenolic content

Determination of total phenolics in plant extract was determined by using modified Folin-Ciocalteu method (Maurya & Singh, 2010). Gallic acid solution (sigma chemical) was used as a standard and prepared in various concentration 2-10µg/ml to be a standard curve. Concentration of 1mg/ml of plant extract was also prepared and 0.5ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml 0f 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30min at room temperature before the absorbance was read at 760nm spectrophotometrically. All determination was performed in triplicate. Determination of total phenol content in the extracts were calculated using the linear regression equation of the calibration curve and expressed as gallic acid equivalent per gram of extract.

Carbohydrates content

Total, soluble and insoluble carbohydrates were estimated by using the method described by Chaplin & Kennedy (1994).

Total carbohydrates were extracted by dissolving 1g of powder in 2-5ml of 2M HCl in a sealed tube. The sealed tube was heated at 100ºC for a period of 2-5h. The extracted sugars were estimated using the general phenol- sulfuric acid assay. The absorbency was measured at 490nm after 30min.

For soluble carbohydrates half gram plant

powders were extracted with ethanol/water (80% v/v) by reflux for 2h. The alcohol was removed from the alcoholic extract by evaporation under reduced pressure. The aqueous extract was clarified using Carrez reagent, and then its volume was completed to 100ml with dist. water.

Then calculate insoluble carbohydrates= Total carbohydrates - soluble carbohydrates.

Total nitrogen and protein content

The total nitrogen and protein content of *S. aromaticum* was determined using Kjeldahl method (James, 1995).

Steam distillation of volatile oils

Fifty grams of fresh plant were subjected to steam distillation to extract volatile oils (Balbaa et al., 1981).

Chromatographic characterization of the crude extract of clove

Gas chromatography/mass spectrophotometer GC/MS

The sample was injected by injector (250°C) into GC/MS equipment (Thermo Scientific TRACE 1310 Gas Chromatograph) which was attached with ISQ LT single quadrupole mass spectrometer (Thermo fisher scientific). The run was done by some help of helium gas with a flow rate of 1.5Psi/min. Analysis was done by GC/MS provided with a column (DB5-MS, 30m: 0.25mm ID (J&W Scientific), with EI ionization model and ionization voltage of 70e. v. The temperature program was; 50°C (1min), 150°C (1min) at 7°C/min, 250°C (5min) at 5°C/min, 290°C (2min) at 10°C/min. The chromatogram was presented by Xcalibur software which was connected with built-in library (WILEY & NIST MASS SPECTRAL DATA), referring to detector involved with a temperature of 300°C.

Thin layer chromatography (TLC)

A sample of ethanolic crude extract was spotted on silica gel plate (60 SF 254e. Merch 20x20) using a clean capillary tube.

Different types of mobile phases were investigated under the technique of try and error concept (T/E). Since the ethanol as an extracting solvent gives a polar nature of the crude extract so, multiple running systems related to polar systems were selected such as butanol – acetic acid – water 3:1:1 (BAW), as well as those related to slightly nonpolar ones such as chloroform – ethanol 9:1 (CE) and chloroform – acetone – butanol 85:15:20 (CAB).

Development chamber containing developing solvent (BAW) was prepared, then a TLC plate was placed inside the closed chamber. When the solvent phase reached the top, the plate was air dried and visualization process was accomplished by both naked eye and long UV (365nm). By the same way, both of CE and CAB solvent system were settled in development chamber individually.

Assessment of antifungal activity for partially purified fractions

All detected bands on TLC plate were screened for antifungal activity against all investigated fungi by the same way described for screening of antifungal activity of the crude extract.

This experiment was designated to determine the most potent fraction as well as comparing the results with those of the original crude extract.

Chromatographic characterization of the partially purified fraction with antifungal activity

Total soluble protein concentration

Total soluble proteins were estimated in both crude extract and fraction -C, by the method of Lowry et al. (1951) using bovine serum albumin as a standard protein; the color was read at 750nm using spectrophotometer Speko II. The concentration of protein was calculated from the standard curve.

Total soluble carbohydrates

The total soluble carbohydrate content was estimated by the method of Hedge & Hofreiter (1962). 0.2-1ml of working standard solution of five different test tube was taken then 4ml of anthrone reagent was added and the contents were mixed. The covered test tubes were put in water bath for 10min then cooling to room temperature. Blank was prepared with 1ml of distilled water and 4ml of anthrone reagent. The optical density was measured in a photoelectric colorimeter at 620nm. Compute the concentration of the carbohydrates in the sample from the calibration curve.

Mass spectroscopy (MS)

Mass spectrum was carried out on Direct Probe Controller Inlet part to Single Quadropole mass analyzer (Thermo Scientific; GC/MS model ISQ LT) using Thermo X-Calibur software at the

Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo. The Mass spectroscopy system was used to confirm the purity of the compounds as well as to explore the characteristics of the isolated fraction and the expected molecular weight.

Statistical analysis

Statistical analysis of the obtained data was carried using one-way analysis of variance (ANOVA) according to Sendecor & Cochran (1980)

Results

Preliminary identification of fungal pathogens

A total of 15 fungal isolates related to four genera were recovered. The isolates identification was depending on microscopic and macroscopic examination according to Domsch et al. (1980). Only 4 fungal species were isolated; namely *Geotrichum candidum* (40%), *Alternaria alternata* (33.3%), *Fusarium oxysporum* (20%) and *Mucor hiemalis* (6.7%) (Fig. 1).

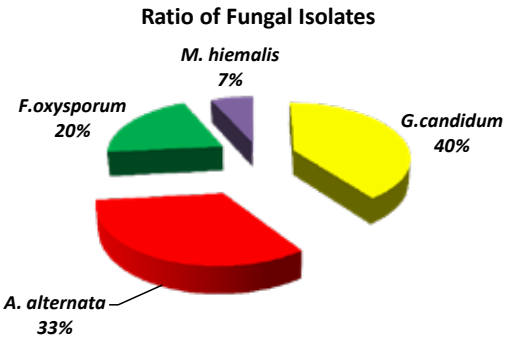

Fig. 1. Percentage of fungal isolates.

Evaluation of antifungal activity of ethanolic extracts of S. aromaticum

S. aromaticum extract recorded high values of inhibition zone diameter in corresponding to positive control (Table 1).

Effect of S. aromaticum in radial growth of the investigated fungi (test fungi)

Different concentrations of ethanolic extract of *S. aromaticum*; 1%, 2.5%, 5%, 12.5%, 25% and 50% were used for revealing the inhibitory effect of different concentrations of plant extract against each fungal species. Table 2 displays the effect of different concentrations of ethanolic *S. aromaticum* extract against test fungi which were affected by different degrees.

Phytochemical screening of S. aromaticum

The analysis of the preliminary phytochemical tests of the plant powder showed the presence of chemical constituents such like alkaloids, flavonoids, tannins, saponins, protein and carbohydrates; results were quantified to detect the concentrations of each constituent.

Clove extract appeared to be rich in phenolic acids as well as flavonoids in addition to alkaloids, tannins and saponins. Also, its content of proteins and nitrogenous compounds was much higher than the content of carbohydrates and oil (Table 3).

Chromatographic characterization of the crude extract of S. aromaticum

Chromatographic analysis of ethanolic crude extract by GC/MS

Chemical profile for ethanolic crude extract of clove indicated that it contains 18 different chemical compounds (Table 4 and Fig. 2). Four of these compounds (ethanol, 1,2-propanediol, dimethyl sulfoxide and dimethyl sulfone) belonged to the used solvents and their derivatives, other compounds such as hexadecenoic and octadecanoic esters were considered as precursors for other structural components such as fatty acids and lipid components. On the other hand, there were some compounds such as eugenol, trans-caryophyllene, humulene, anthracenedione, cedran-diol, citroflex A and lucenin 2 were detected.

TABLE 1. Antifungal activity of crude ethanolic plant extracts against the tested fungi measured as diameter of inhibition zone in (mm).

Fungal species	*A. alternate*	*F. oxysporum*	*G. candidum*	*M. hiemalis*
Diameter of positive control (mm)	40.33±1.98	40.33±1.98	39.10±1.50	41.56±1.50
Diameter of extract inhibition zone (mm)	68.33±1.50	44.66±1.50	51.66±1.50	61.66±1.98

Data given are mean of triplicates ± standard error (SE).

TABLE 2. Effect of different concentrations of ethanolic *S. aromaticum* extract against test fungi expressed as diameters of inhibition zone in (mm).

Concentration of ethanolic extract (%)	Diameters of inhibition zone in mm.			
	A. alternata	*F. oxysporum*	*G. candidum*	*M. heimalis*
1	-	-	-	-
2.5	11.66±2.20	-	12.00±1.50	11.33±1.50
5	13.00±1.90	-	18.66±2.22	18.00±1.10
12.5	37.33±1.50	20.00±2.88	24.00±1.50	33.66±1.10
25	44.33±1.50	34.00±1.98	40.33±1.98	45.00±1.98
50	55.00±1.20	36.66±1.11	43.00±1.10	46.66±1.22

TABLE 3. Quantitative assessment of constituents in extracted materials of *S. aromaticum*.

Constituents	Conc.%
Total phenolic acid	20.80
Total flavonoids	26.8 1
Total carbohydrate	2.23
Total protein	17.83
Total alkaloids	1.60
Total tannins	4.90
Total saponins	2.60
Total oil	0.90

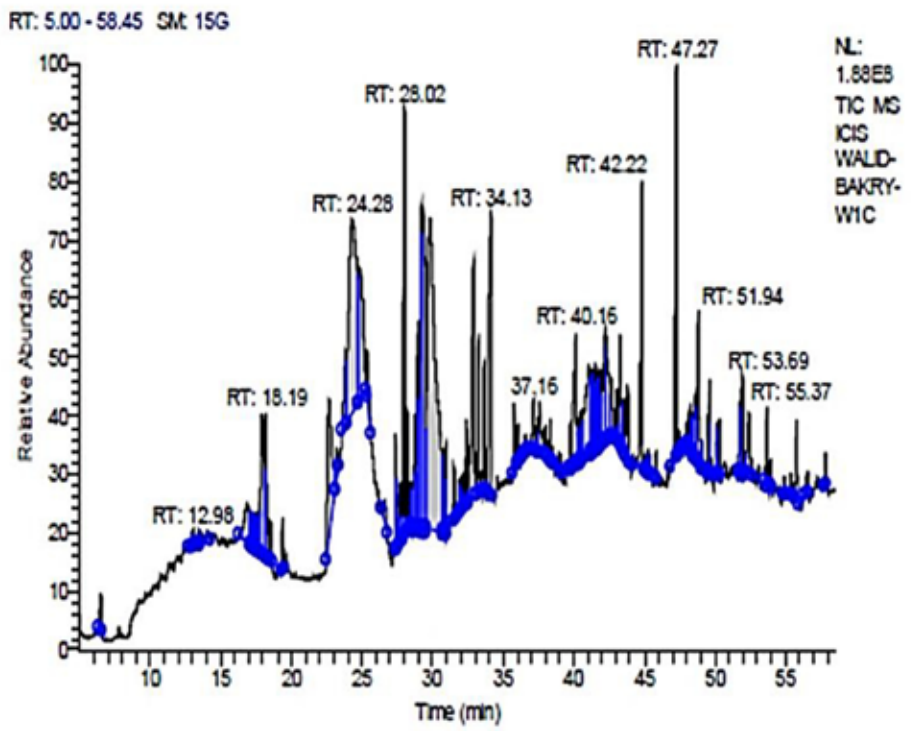

Fig. 2. GC/MS chromatogram of ethanolic crude extract of clove.

TABLE 4. Chemical profile of ethanolic crude extract of *S. aromaticum* revealed by GC/MS

No	RT (min)	Compound name	Area %	Molecular formula	M. weight
1	6.53	Ethanol	0.28	C_2H_6O	46
2	13.52	1,2-Propanediol	0.77	$C_3H_8O_2$	76
3	17.95	Dimethyl Sulfoxide	6.39	C_2H_6OS	78
4	18.53	Dimethyl sulfone	0.29	$C_2H_6O_2S$	94
5	19.39	3-Nonenoic acid, methyl ester	0.34	$C_{10}H_{18}O_2$	170
6	24.28	Eugenol	17.27	$C_{10}H_{12}O_2$	164
7	27.44	Nothosmyrnol	0.65	$C_{11}H_{14}O_2$	187
8	27.75	trans-Caryophyllene	0.50	$C_{15}H_{24}$	204
9	28.02	Humulene	3.33	$C_{15}H_{24}$	204
10	29.82	Phenol,2-methoxy-4-(2 propenyl) -acetate	25.39	$C_{12}H_{14}O_3$	206
11	31.52	Cedran-diol	0.61	$C_{15}H_{26}O_2$	238
12	34.13	11,13-Dihydroxy-tetradec-5-ynoic acid, methyl ester	10.70	$C_{15}H_{26}O_4$	270
13	36.07	Octadecanoic acid,17-hydroxy-, methyl ester	0.47	$C_{19}H_{38}O_3$	314
14	41.07	Hexadecanoic acid,2,3-dihydroxypropyl ester	7.17	$C_{19}H_{38}O_4$	330
15	41.85	10-Hydroxy-5,7-dimethoxy-2,3-dimethyl-1,4-anthracenedione	3.35	$C_{18}H_{16}O_5$	312
16	44.83	Citroflex A	5.45	$C_{20}H_{34}O_8$	402
17	47.24	Oleic acid, eicosyl ester	3.55	$C_{38}H_{74}O_2$	562
18	48.81	Lucenin 2	8.25	$C_{27}H_{30}O_{16}$	610

Thin layer chromatography (TLC)

Chromatographic separation by BAW completely failed with no evidence demonstrating the migration of loaded sample from application position, thus, BAW was neglected and subsequently the pure polar nature of mobile phase in BAW was modified to another slightly nonpolar mobile phase which was CE. Chromatographic separation by CE did not succeed completely because only one band was detected showing the ability of solvent system to upload the specimen but it was not able to fractionate this mixture to its subcomponents, hence, CE was also ignored and subsequently, CAB as running system was used.

CAB as a mobile phase was proposed to separate three bands (A, B and C) with Rf values 0.52, 0.76 and 0.87 ascendingly.

Assessment of antifungal activity for partially purified fractions

Three fractions were investigated by agar well diffusion technique of which only one induced antifungal activity. This fraction showed Rf value 0.87. This compound exerted inhibitory effect against *G. candidum*, *A. alternata* and *M. heimalis* with diameter of inhibition zone 18.7, 11.4 and 9.8mm, respectively. Whereas, it hadn't any fungal activity against *F. oxysporum*.

It should be noted that the values of inhibition zone belong to the purified fraction were much little than those belong to the original crude extract.

Total soluble protein concentration and carbohydrate contents in both crude extract and fraction - C

Total soluble proteins were estimated in both crude extract and fraction -C; the measurements clarified that the total protein concentration in the crude extract is 85mg ml^{-1} which was extremely reduced to 35mg ml^{-1} in case of fraction -C.

Total soluble carbohydrates were estimated in both crude extract and fraction -C, the measurements clarified that the total soluble carbohydrates content in the crude extract is 505mg g^{-1} in corresponding to 162.5mg g^{-1} with extreme loss of carbohydrates content. Eventually, it could be deduced that the compound in charge had a core of chemical structure linked actually with a portion of glycoprotein, the core of this compound would be predicted by GC/MS.

Mass spectrum characterization of the partially purified fraction

GC/MS equipment was used to present the mass spectrum for fraction -C to test the purity and predict the structure of the compounds. GC/MS exhibited the presence of only one peak (Fig. 3) as an indication for high purity of the fraction. Thus, it was easy to identify the compound via the prediction introduced by the searching library connected to GC/MS. The compound quietly may be glucoside β-sitosterol (Figs. 4 and 5).

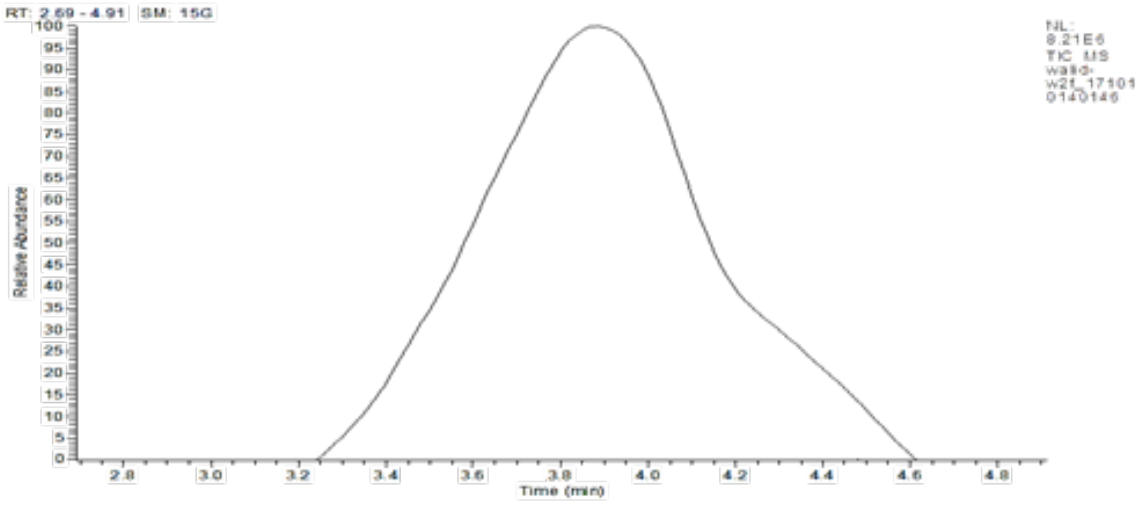

Fig. 3. GC/MS exhibited the presence of only one peak of the partially purified fraction -C.

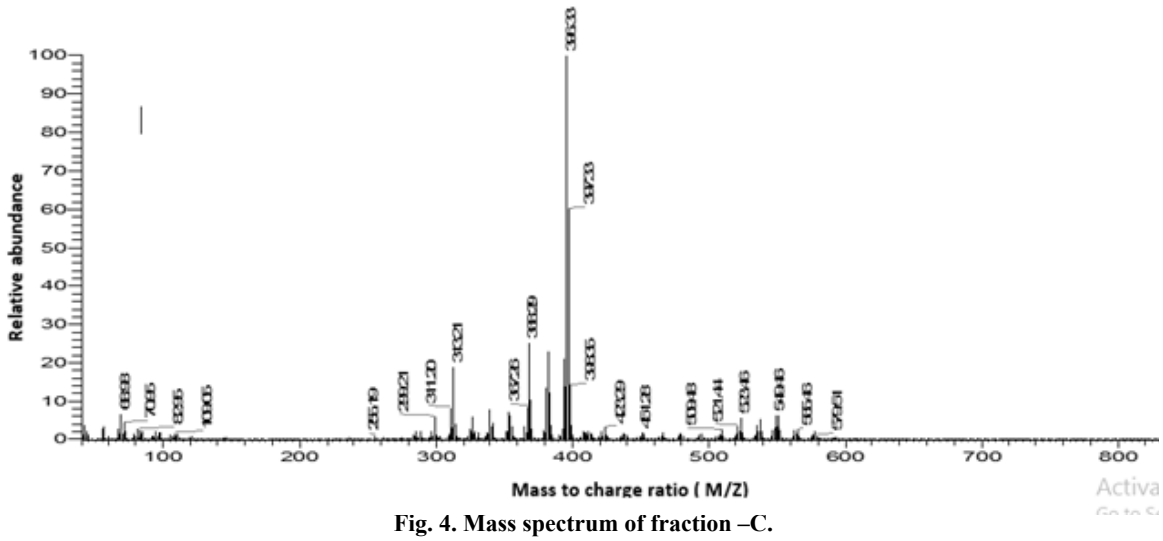

Fig. 4. Mass spectrum of fraction –C.

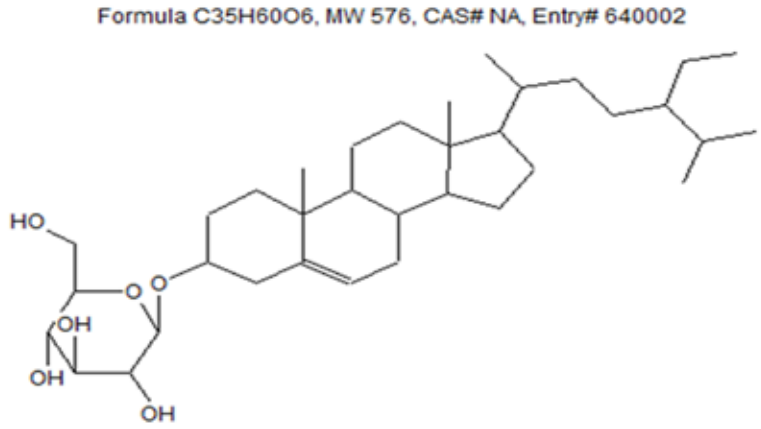

Fig. 5. The chemical structure of identified compound" Glucoside β-sitosterol".

Furthermore, The spectrum of component was compared with the spectrum of the component stored in the NIST library version (NIST Chemistry Web Book) (Joulain & König, 1998) and a database of chemical molecules https:// pubchem.ncbi.nlm.nih.gov/search/search.cgi

Discussion

The results of this study showed that the ethanolic extract of *S. aromaticum* L. showed strong antifungal effect against all tested fungi. Different concentration of *Syzygium aromaticum* extract showed variation among fungal species. The smallest inhibition zone was observed at concentration of 2.5% against *A. alternata*, *G. candidum* and *M. heimalis*; with diameter of 11.66, 12.0 and 11.33mm, respectively. While *F. oxysporum* gives minimum inhibition zone of 20.0mm in diameter at relatively higher concentration (12.5%).

These results agree with the results recorded by Bander (2011) who reported that the alcoholic extract of *S. aromaticum* plant and its essential oil exhibited strong effect against all tested Trichophyton species.

Antifungal activity exhibited by *S. aromaticum* may attribute to the presence of secondary metabolites. These compounds can interfere with pathogens by different mode of action. Freiesleben & Jäger (2014) had previously investigated the correlation between the biosynthetic group of secondary metabolites in plants and their antifungal mechanisms of action. From looking at the composition of the fungal cell, at least 6 different antifungal mechanisms can be suggested.

These mechanisms include inhibition of cell wall formation, cell membrane disruption, dysfunction of the fungal mitochondria, inhibition of cell division, inhibition of RNA/DNA synthesis or protein synthesis and inhibition of efflux pumps.

Different types of secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, resins, fatty acids, gums have been identified as antifungal and antibacterial agent in plants (Dorman & Deans, 2000; Wallace, 2004; Han & Paik, 2010; Gokhale & Wadhwani, 2015 and Ryu et al., 2016).

In the present investigation, the chemical nature of active components in *S. aromaticum* was identified by qualitative and quantitative preliminary phytochemical tests, it appeared to be rich in phenolic acids, flavonoids, alkaloids, sterol, tannins and saponins. In addition to proteins, nitrogen, carbohydrates and oil. The phytochemical results obtained in this study agree with Tanko et al. (2008) who concluded the presence of flavonoids, resins, glycosides, tannins, saponins and alkaloids in the ethanolic extract of *S. aromaticum*. In another study done by Upadhyaya et al. (2018), phytochemical screening was carried out on ethanol, chloroform and distilled water extracts of clove for its chemical composition. Qualitative phytochemical analysis of these extracts confirms the presence of alkaloids, terpenoids, flavonoids, saponins, steroids and tannins.

Investigation chemical nature of active antifungal component in *S. aromaticum* was carried out by using GC/MS to predict the chemical profile of the crude ethanolic extract of clove. The results revealed that the crude extract contained 18 different chemical compounds with different concentrations such like eugenol, trans-caryophyllene, humulene, anthracenedione, cedran-diol, citroflex A and lucenin 2. This result is consistent with that of Wagn et al. (2017) who found that the major compounds in clove alcoholic extract analysis by GC/MS were eugenol, acetyl eugenol , caryophyllene , and humulene followed by a-farnesene and caryophylleneoxide. These notable differences in composition may be due to extraction methods as well as genetic diversity and agronomic treatments (Mahanta et al., 2007).

Separation the components of the crude extract and purification the effective compound in charge using TLC technique revealed that only one fraction with Rf value 0.87 showed antifungal activity against all tested fungi except *F. oxysporum*. It was also observed that the values of inhibition zone belong to the purified fraction were too much little than those belong to the original crude extract and this may be due to the nature of the compound in charge which might have either a portion of protein or a portion of carbohydrate via glucosidal bond. This hypothesis is consistent with Khan & Nasreen (2010), in the fact that the antifungal activity exhibited by plants might be attributed to the presence of either single or synergetic effect of more than one compound. Synergism is observed when the effect of the combined substances is greater than the sum of the individual effects (Burt, 2004). It has been also demonstrated that the synergistic effect of some secondary metabolites is probable due to the interaction of these components with different proteins or enzymes (Bassolé & Juliani, 2012). So that the purified fraction was estimated for detection of proteins as well as carbohydrates and also would be compared with the content on proteins and carbohydrates in the crude extract.

Protein and carbohydrate contents in the crude extract were reduced in case of purified fraction (C). It was previously suggested that carbohydrates influence on production of secondary metabolites (Sørensen & Giese, 2013). Eventually, it could be deduced that the compound in charge had a core of chemical structure linked actually with a portion of glycoprotein, the core of this compound would be predicted by GC/MS. Mass spectrum characterization of the partially purified fraction showed only one peak as an evidence for high purity of the fraction. It was clear that the compound may be glucoside β-sitosterol which approved the expectation of glucosidal bond especially when the results of total carbohydrates were taken in consideration.

Glucoside β-sitosterol was previously isolated from different plant species and exhibited antimicrobial activity. It was isolated from ethanolic extract of the root heart wood of *Acacia senegal*, as white granules (Jain et al., 2012). Further researches have indicated that such phytosterol compounds serve to stabilize phospholipid bilayers in plant cell membranes and display antifungal properties against human and plant origin (Moreau et al., 2002; Khan et al., 2007; Kuigoua, 2010, Singh et al., 2011 and Arora & Kalia, 2013).

Moreover, other studies emphasized the isolation of β-sitosterol, β-sitosterol 3-O-β-D-glucopyranoside and other five compounds namely oleanolic acid lactone, nigricin, flavaellagic acid, 2α- hydroxyoleanolic acid, 3β-hydroxy-11-oxo-olean-12-en-28-oic acid from *S. aromaticum* (Begum et al., 2014).

The focus should be shifted to exploit *S. aromaticum* as a suitable, ecofriendly and safe alternative for chemical pesticides and its potential use as biological fungicide for the control of postharvest infections which affecting two of the most important economic plants in Egypt.

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(*Received* 15/5/2018;
accepted 11/10/2018)

في المختبر: تقييم تأثير المستخلص الكحولي للقرنفل كعامل مكافحة بيولوجية ضد أمراض ما بعد الحصاد للطماطم والبطاطس

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تقوم الدراسة على عزل وتعريف الفطريات الممرضة لنباتى الطماطم والبطاطس فى مرحلة ما بعد الحصاد وهى *Fusarium oxysporum*، *Geotrichum candidum*، *Mucor hiemalis*، *Alternaria alternata* لاختبار مدى تأثير المستخلص الكحولى للبراعم الجافة للقرنفل على هذه الفطريات. وأظهرت النتائج أن المستخلص له فاعلية كبيرة فى تثبيط الفطريات المختبرة، ومن ثم تم اختبار تركيزات مختلفة للمستخلص. وأوضحت النتائج أن أقل منطقة تثبيط كانت عند تركيز 2.5% لكل من فطر *Alternaria alternata*، *Mucor hiemalis*، *Geotrichum candidum*، بينما كان عند تركيز 12.5% لفطر *Fuarium oxysporum*. أظهر التحليل الكيميائى الكمى والكيفى الأولى لنبات القرنفل أنه يحتوى على العديد من المركبات الكيميائية ونواتج الأيض الثانوية المتمثلة في المركبات الفينولية، قلويدات، الصابونين، الفلافونويد، التانين بالإضافة إلى محتواه من البروتين والكربوهيدرات والزيوت.

كما تم التعرف على مكونات المستخلص الأيثانولى للقرنفل بجهاز الكروموتوجرافى الغازى المزود بمطياف GC/MS للتنبؤ بالمظهر الكيميائي للمستخلص والذي أظهر أن المستخلص الكحولى للقرنفل يتألف من 18 مركب كيميائي مختلف بتركيزات مختلفة مثل: استرات أحماض هيكساديكانويك و أوكتاديكانويك و الأوليك وأحماض نوتسميرنول و النونوينيك بالإضافة إلى ايوجينول، ترانس كاريوفيلين، هومولين، أنثراسينيديون، سيدران-ديول، سيتروفلكس أ و لوسينين 2.

كما تمت عملية فصل مكونات المستخلص الكحولى للقرنفل باستخدام (TLC) كروماتوغرافيا الطبقة الرقيقة والذى نجح فى فصل ثلاثة أجزاء، وأظهر جزء واحد فقط (Rf= 0.87) نشاط مضاد للفطريات المختبرة. كذلك تم تحديد نسبة كلا من البروتين و الكربوهيدرات فى كلا من المستخلص والجزء المفصول. كما تم التعرف على مكونات الجزء المفصول باستخدام جهاز الكروماتوجرافى الغازى المزود بمطياف الكتلة (GC/MS) والذى رجح أن المادة الفعالة هى β-sitosterol glucoside.