Egypt. J. Chem. **Vol. 64,** No. 6 pp. 2833 - 2843 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antimicrobial activity of bioactive compounds extract from *Saussurea costus* against food spoilage microorganisms

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Abstract

Saussurea costus had a wide range of antimicrobial activities which used as alternative for synthetic preservatives that threaten human health. This study aimed to identify the bioactive compounds in *S. costus* extract (SCE) and to evaluate its antimicrobial activity against some pathogenic microorganisms. HPLC and GC-MS were used to quantify the bioactive compounds in SCE. The results indicated that ethanol and ethyl acetate extracts had the highest levels of polyphenols followed by n-butanol, and then n-hexane extracts. The main phenolic compounds are Naringenin, Chlorogenic acid, Ferulic acid, Ellagic acid, Gallic acid and coffeic acid followed by taxifolin, catechin, syringic acid, methyl gallate, vanillin, kaempferol, cinnamic acid and rutin. GC-MS results showed 14 compounds in S. costus extract. The antibacterial activity of *S. costus* ethanol extract increased by increasing the concentration of extract from 10 µl to 50 µl for each wells .The inhibition zones were 13 mm and 23mm for *S. typhi* and *Staphylococccus aureus*, respectively. Gram (+ve) bacteria found to be more sensitive to SCE than Gram (-ve) bacteria. Similarly; the antifungal activity was increased by increasing the concentration of SCE the inhibition zones were 15.5 mm and 22.5 mm for *P. verecossum* and *A. ochraceous*, respectively. *A. ochraceous* appeared to be more sensitive towards all concentration of the extract. The minimum inhibitory concentration (MIC) of SCE for both bacteria and fungi strains ranged from 0.08 - 0.3 mg/ml and 0.25 -1.17 mg/ml, respectively. The results revealed that the SCE can play an important role against the human multi-drug resistant pathogens and can alternate the antibiotics as well as chemical preservatives to control infection and food spoilage contaminants.

***Keywords**: Saussurea costus*, Phenolic compounds, Antibacterial, antifungal, food spoilage contaminants, HPLC, GC-MS.

1. Introduction

Most common genera of fungi in foods are *Aspergillus, Penicillium and Fusarium*, which have the ability to produce mycotoxins. *Aspergillus flavus* and *Aspergillus parasiticus* are important pathogens of cotton, corn, peanuts and other oil-seed crops, producing aflatoxins in the field and during storage. *Fusarium verticillioides* and *F. proliferatum* proliferatum are capable to produce carcinogenic fumonisins, which pose serious hazards to human and animal health **[1-4]**. Among fungi strains, *Aspergillus ochraceus, Aspergillus carbonarius* and *Aspergillus niger* produce ochratoxin in foods e.g., cereals, beans, spices, dried fruits, nuts and oilseeds that has been reported around the world **[5,6]**.

Food borne pathogens are responsible for a wide range of illnesses, many of which have serious consequences for human health and economy. The traits of the most common pathogenic bacteria (Bacillus cereus, *Staphylococccus aureus, (Clostridium botulinum, Clostridium perfringens, Esherichia coli, Listeria monocytogenes, Salmonella spp., Shigella spp., Vibrio spp. Bacillus cereus* and *Yersinia enterocolitica*), are investigated, as well as certain significant outbreaks. Nowadays, leading experts and organizations are recommending that

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Receive Date: 27 March 2021, Revise Date: 07 April 2021, Accept Date: 11 April 2021
DOI: 10.21608/EJCHEM.202169572.3528

certain risks be managed using a risk-based food safety strategy **[7,8].**

Medicinal plants possess active compounds can alternate the traditional chemical preservatives, which may used for preservation of food products that have negative effects on consumer health. Several biological activities have been reported for plant extracts, which show antiviral, antibacterial and antifungal properties **[9-12].** Medicinal plants, which contain a wide range of phytochemicals are one of the most readily available sources of such compounds **[13-17].**

Sausurea costus (*S. costus*) is well known in Islamic medicine, and is recorded in the holy hadiths that Prophet Muhammad (peace and blessings be upon him) said **[18]**. It is known in the Arab countries as " "Al-Kost Al-Hindi" and has been used by traditional healers since the era of Islamic civilization. *S. costus* is traditionally used as a stimulant, antiseptic, repellent, sedative, and bronchodilator **[19]**. The biological activities of *S. costus* roots (synonymous with *S. lappa*) are extensively investigated and revealed that it contains anti-trypanosomal activity **[20].** It has "complement inhibitor" substances useful in treating some diseases associated with excessive activation of the complementary system, such as rheumatoid arthritis, respiratory distress and systemic lupus erythematosus **[21].** *S. costus* has been reported to have good anticancer activity on tested cell lines **[22].** Ethanol extract from *S. lappa* (synonym of *S. costus*) recorded a broad antimicrobial activity against some human pathogens **[23].** Presence of bioactive properties of *S. costus* roots such as anti-ulcer, anti-inflammatory, anti-immune, and antiviral activity have been reported in several studies **[24, 25]**. Based on these practical standpoints, the main goal of the current study is to quantify the bioactive compounds by HPLC and GC-MS in the natural SCE as an antibacterial and antifungal agent.

2. Materials and Methods

2.1. Preparation of SCE

50 grams of *S. costus* powder that obtained from Egyptian herbal market, Dokki, Giza, was extracted with 95% Ethyl alcohol and ultra-sonicated for 40 min using ultrasonic processor (DAIGGER ULTRASONIC Model GEX 750, USA), then filtered through filter paper Whatman No. 1, this step was repeated three times and the combined ethanol extract was evaporated using rotary evaporator at 45ºC till dryness **[26]**.

The obtained ethanol extract was used for antibacterial and antifungal assay. Also, the ethanol extract was sequentially fractionated with n-hexane, ethyl acetate and n-butanol (3x50 ml each) by adding distilled water. The n-hexane, ethyl acetate and n-butanol extracts were collected and evaporated separately by rotary evaporator till dryness and filtered for HPLC and GC-MS analysis.

2.2. Polyphenols analysis of S. costus extracts using HPLC

The *S. costus* extracts were analyzed using HPLC (Agilent 1260 series). The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase was water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a gradient as follows: 0 min (82% A); 0-5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The column temperature was maintained at 35 °C.

2.3. GC-Ms analysis of ethyl acetate extract

Trimethylsilyl (TMS) derivatization was conducted on based on the protocol described previously by **Moldoveanu and David [27]**. Briefly, the dried extract were re-suspended in 10 μL and 50 μL of *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) then, incubated at 70 °C for 60 min in a dry block heater. The GC-MS system (Agilent Technologies) equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre. The GC equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analysis were carried out using helium as a carrier gas at a flow rate of 1.0 ml/min at a split ratio of 50:1, injection volume of 0.5 μl and the following temperature program: 50 °C for 1min; rising at 8 °C /min to 300 °C and held for 20 min. The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 30-700 and solvent delay 8 min. The mass temperature was 230°C and Quad 150 °C. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

2.4. Antimicrobial activity of S. costus ethanol extract

2.4. 1. Culture media and Microorganisms

The antibacterial assay was conducted against three Gram (+ ve) pathogenic bacteria; *Bacillus cereus* EMCC 1080, *Staphylococcus aureus* ATCC 13565 and *Staphylococcus sciuri* and four Gram (-ve) bacteria; *Escherichia coli* O157-H7 ATCC 51659, *Salmonella typhi* ATCC 15566 *Pseudomonas aeruginosa* NRRL B-272 and *Salmonella entrica*. The strains were grown on nutrient agar slants at 37ºC for 24 hr. and kept in refrigerator at 4ºC until subsequent use. The antifungal assay was carried out against seven fungal species including *Aspergillus flavus* NRRL 3357, *A. parasiticus* SSWT 2999, *A. carbonarius* ITAL 204, *A. ochraceus* ITAL 14, *A. niger*, *Fusarium verticillioides* ITEM 10027 and *F. proliferatum* MPVP 328 grown on Potato Dextrose Agar (PDA) media. The fungal species were obtained from applied mycology department Cranfeield , UK.

2.4.2 Antibacterial assay

Assay was conducted using well diffusion method on nutrient agar. The tested microorganisms were inoculated to Trypticase soya broth tubes and incubated at 37ºC for 4 hrs. The turbidity of these cultures was adjusted by using 0.5 Mc-Farland. A uniform bacterial lawn was developed by sterile cotton swabs on the surface of solid nutrient agar plates. Cork borer was used for made a well in diameter (8 mm). Each well was filled with different volumes of SCE (10, 20 and 50 µl). Ceftriaxone (1.0 mg/ ml) used as a positive control. The plates were incubated at 37ºC for 24 hours. Antibacterial activities of the extracts were determined by measuring the diameter of zone of inhibition (mm) **[28,29].**

2.4.3 Antifungal assay

The extracts were tested for antifungal activity using well diffusion method. From tested fungi spore suspension 100µl was spread onto the surface of solidified potato dextrose agar (PDA) plates using a glass rod and the plates were left to dry before applying the extracts. Well filled with tested extracts. Once all wells are saturated, the plates were incubated at 28ºC for 24- 48hr. Miconazole (Sigma-Aldrich) with concentration of 1.0 mg/ml was used as a control positive. The inhibition zones (observed with naked eye) were measured including the diameter **[29]**.

2.4.4Estimation of minimum inhibitory concentration (MIC)

The tube dilution method according to **Wiegand *et al.*, [30]** was used for MIC determination. Cultures of tested bacterial pathogens ware diluted in 10 ml of tryptic soya broth (TSB) medium to get 0.5 McFarland standard turbidity (10^8cfu ml^{-1}). Ten concentrations of SCE (2.0, 1.5, 1.0, 0.75, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.05 mg ml^{-1} in DMSO) were prepared and each tube was inoculated with 100µl of bacterial suspension and incubated at 37°C for 24h. The bacterial growth in broth is designated by turbidity of the broth and the lowest concentration which inhibited the growth of the test organism was taken as the minimum inhibitory concentration (MIC).

The technique of **Perrucci *et al.*, [31]** was used for MIC determination against fungi**.** The *S. costus* extract at tested concentrations were separately dissolved in 0.5 ml of 0.1% Tween 80 and mixed with 9.5 ml of melting PDA and poured into Petri dish (6 cm). The solidified plates were centrally inoculated with 3µl of fungal spore suspension (10^8 CFU ml^{-1}; 0.5 McFarland standards). The plates were incubated at 25°C for 48h. At the end of the incubation period, mycelial growth was monitored and MIC was determined

2.5Statistical Analysis

The obtained results were subjected to one-way analysis of variance (ANOVA) of the general liner model (GLM) using **SAS [32]** statistical package. The results were the average of three replicates ($p \leq 0.05$).

3. **Results and Discussion**

Quantitative analysis of phenolic compound of SCE

The results indicated that phenolic compounds are detected in all extracts of *S. costus* with different concentration levels. Ethanol and ethyl acetate extracts had the highest contents of polyphenols followed by n-butanol, and then n-hexane extracts. Polyphenol profile of *S. costus* extracts are depicted in Table 1 and Fig 1 a, b.

The results indicated that the highest phenolic compounds are found in order; Naringenin, Chlorogenic acid, Ferulic acid, Ellagic acid, Gallic acid and coffeic acid followed by other compounds. Rutin and pyro catechol were found in ethanol and ethyl acetate extracts, respectively however, coumaric acid was not detected in all extracts. Presence of polyphonlic compounds in the extracts may be increasing the biological activity of the extract due to the synergistic effect.

The polyphenol compounds are bioactive compounds commonly disseminated in plants which are consumed by human. Polyphenols possess antibacterial and antifungal activities. Catechin is a polyphenol with antibacterial activity against different bacterial strains such as *S. aureus, Streptococcus mutans* and *E.coli* **[33].** Similarly, **Karthikeyan *et al.* [34]** reported that ethanol extract of costus possess high amount of phenolic compounds than the acetone and aqueous extract. The

main phenolic compounds found in the ethanol extract were ferulic acid (23.46 ppm), coumarin (25.63 ppm) as well as phloroglucinol (1.151 ppm).

Arima *et al.*, [35]reported the synergistic effect of rutin to enhance the antibacterial activity of flavonoids. The activities of galangin, Kaempherol, myricetin, and fisetin were enhanced in the presence of rutin when *S. enteritidis* was used as a test organism. Similarly, catechol and pyrogallol were examined for their activity against three bacterial and two fungal species and catechol showed activity against the tested organisms; *P. putida, P. pyocyanea* and *Corynebacterium* as well as antifungal activity against *Fusarium* and *Penicillium* however; pyrogallol was effective against bacterial species only **[36].** An aqueous extract of costus (*Saussurea lappa*) was found to be rich in phenolic compounds and had antioxidant properties and antimicrobial activities **[37]**. Moreover, **Sagar *et al*.[38]** demonstrated that the methanol extract of *S. lappa* showed antibacterial activity against *E. coli, S. aureus* and *Y. pestis* greater than that obtained by acetone and ethanol extracts.

GC-MS analysis of S. costus ethyl acetate extract

GC-MS analysis of *S. costus* ethyl acetate extract presented in table (2) and Fig (2). The obtained data indicated the presence of 14 compounds. Five of them represent over 80% of the constituents. Butanedioic acid, 2TMS derivative recorded the highest percentage with 20.4% followed by D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1), Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-, Caffeic acid, 3TMS derivative and L-(-)-Sorbofuranose, pentakis(trimethylsilyl) ether with area percentage 18.4, 16.5, 14.4 and 11.4%, respectively.

Srinivasan *et al* [39] identified the chemical compounds in the essential oil from costus and found that n- hexadecanoic acid to be the major constituent in all examined essential oil accompanied with other fatty acids, hydrocarbons and mono-, di- and sesquiterpenes.

Sesquiterpene lactones are the most distinctive secondary metabolites of the members of the Compositae (Asteraceae). Several plant families, such as Acanthaceae, Amaranthaceae, Apiaceae, Magnoliaceae, and Costaceae have a diversity of chemical structures and a wide range of biological activities, including antitumourogenic, insect antifeedant, plant growth regulating, antibacterial, antifungal and cytotoxic properties **[40, 17]**.

Antimicrobial activity of SCE

Different volumes (10, 20 and 50 µl) of SCE at concentrations 50 mg/ml were assayed for their antimicrobial activity against pathogenic bacteria and fungi using well diffusion method. The antibacterial activity of SCE presented in Table (3) and Fig (3). The results indicated that the antibacterial activity of *S. costus* is increased by increasing the volume of SCE at concentration of 50 mg/ml against all investigated bacteria with inhibition zones ranged from 13 mm to 23 mm for *S. typhi* and *Staph. Aureus*, respectively. Gram (+ve) bacteria found to be more sensitive to SCE than Gram (-ve) bacteria. Among the examined bacterial strains, *Staph. aureus* was the most sensitive with inhibition zone reached 23mm. in the same respect, *S.typhi* was found to be the most resistance to *SCE*. The *S. costus* extract showed antibacterial effect on the tested Gram (+ ve) strains higher than that observed by the antibacterial agent 10µl ceftriaxone (1mg/ml). The same concentration level of ceftriaxone showed the highest activity against all gram (-ve) bacteria (24.8 mm to 29.3 mm). These results are in agreement with those results reported by **Arihan *et al,* [41]and Duraipandiyan *et al.*[42]** who found that gram **(+**ve**)** bacteria tend to be more sensitive than gram (- ve). **Malabadia [43]** reported the antibacterial activity of different extracts of *Costus speciosus* against pathogenic strains of bacteria including *Staphylococcus aureus, E. coli, Sheigilla, K. pnumonia, Psudomonas aeruginosa, Bacillus subtilis* and *Salmonella*. Additionally they reported that the aqueous extract had no antibacterial effects against these strains. In the same regards, **Salim *et al.* [44]** tested the antibacterial activity of hot and cold ethanol extract of *Costus speciosus* rhizome with different concentrations against Gram (+ve) and Gram (-ve) bacteria and revealed that *Psudomonas aeruginosa* found to be more susceptible to all hot ethanol extracts with inhibition zones ranged from 12 mm to 24 mm at concentration of 1:16 and 1:1, respectively. The same results obtained by **Fakhra and Shilpa [45]** who concluded that cold and hot aqueous extract of *Costus speciosus* has antibacterial activity against five pathogens with *Psudomonas aeruginosa* most susceptible organism. The antibacterial activity of different extracts of Costus was studied by **Ariharan *et al.* [41]** against Gram (+ve) (*Staph. aureus* and *Staph epodermidis*) and Gram (-ve) bacteria *E. coli, Salmonella typhimurium* and *Psudomonas aeruginos.*

Table (4) represents the antifungal activity of SCE. The obtained data revealed that increasing the inhibition zone by increasing the volume of extract in the well. The 50µl concentration showed the highest inhibition zones ranged from 15.5mm to 22.5 mm for *P. verecossum and A. ochraceous,* respectively.

A. ochraceous appeared to be more sensitive towards all concentration of extract than other fungi with inhibition zones ranged from 12.5mm to 22.5mm followed by *A. parasiticus* (11.0 mm to 16.5 mm) by increasing the volume from 10µl to 50µl.

TABLE 1. Phenolic compounds of *Saussurea costus* extracts analysed using HPLC.

Phenolic compounds	Phenolic compounds Concentrations (µg/g)			
	Ethanol extract	Ethyl acetate	n-Hexane	n-Butanol
Gallic acid	24.8	0.00	75.2	3,830
Chlorogenic acid	27.4	18,269	14.5	10,436
Catechin	0.00	1,708	0.00	256
Methyl gallate	0.56	638.5	0.00	121
Coffeic acid	1.60	3,050	0.00	285
Syringic acid	9.79	1,109	13.6	92.3
Pyro catechol	0.00	1,776	0.00	0.00
Rutin	5.39	0.00	0.00	0.00
Ellagic acid	78.2	6710	43.8	572
Coumaric acid	0.00	0.00	0.00	0.00
Vanillin	17.0	802.31	22.6	73.1
Ferulic acid	19.9	8016.17	18.3	96.3
Naringenin	74.3	133,880	74.3	3,343
Taxifolin	60.9	3,514	53.2	209
Cinnamic acid	47.6	143	55.0	0.00
Kaempferol	16.8	316	14.9	0.00

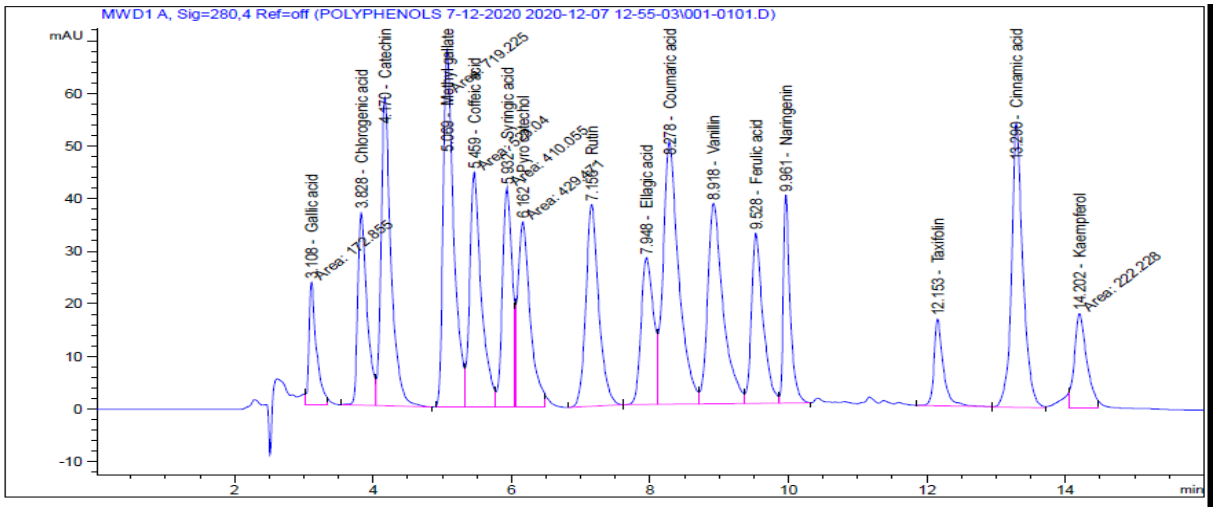

Fig. 1a. HPLC Chromatogram of phenolic compounds profile of reference material.

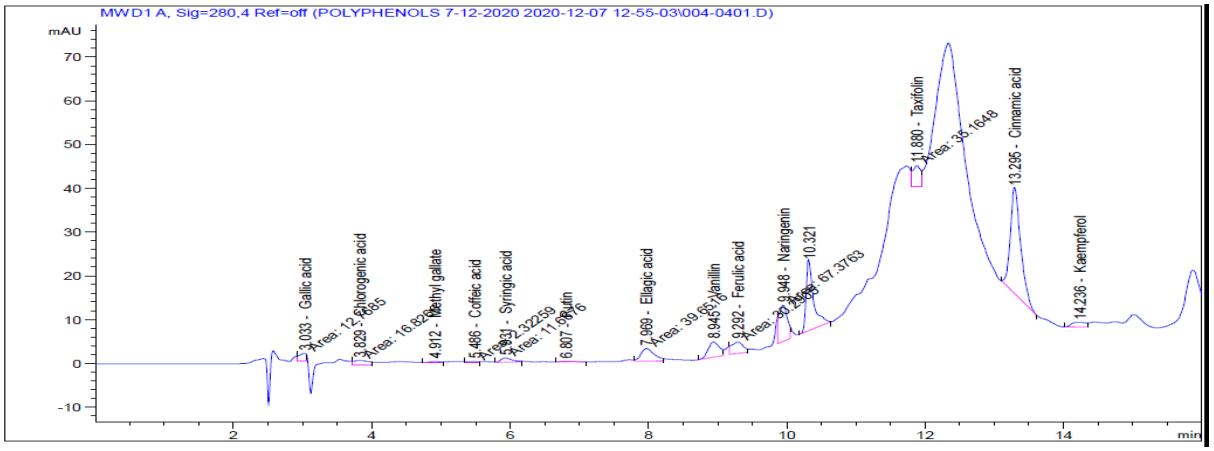

Fig. 1b. HPLC Chromatogram of phenolic compounds profile of *S. costus* extract.

TABLE 2. Chemical compounds identified in *S.costus* ethyl acetate extract by GC-MS analysis.

Peak	RT	Name	Formula	Area Sum %
1	10.888	Glycerol, 3TMS derivative	C12H32O3Si3	2.80
2	11.370	Butanedioic acid, 2TMS derivative	C10H22O4Si2	20.4
3	11.808	2-Butenedioic acid (E)-, bis(trimethylsilyl) ester	C10H20O4Si2	1.14
4	13.186	2-Hydroxycyclohexane-1-carboxylic acid, di-TMS	C13H28O3Si2	0.96
5	13.646	Malic acid, 3TMS derivative	C13H30O5Si3	3.16
6	16.871	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]m	C27H52O4Si2	2.11
7	17.271	D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether (isomer 1)	C21H52O6Si5	18.4
8	17.346	L-(-)-Sorbofuranose, pentakis(trimethylsilyl) ether	C21H52O6Si5	11.4
9	17.662	D-Erythro-Pentofuranose, 2-deoxy-1,3,5-tris-O-(trimethylsilyl)-	C14H34O4Si3	1.08
10	18.928	Doconexent	C22H32O2	1.34
11	19.381	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	C21H34O2	16.5
12	20.255	Caffeic acid, 3TMS derivative	C18H32O4Si3	14.4
13	28.016	1,4-Bis(trimethylsilyl)benzene	C12H22Si2	2.29
14	29.576	BENZENE, 1,4-BIS(TRIMETHYLSILYL)-	C12H22Si2	3.99

RT: Retention Time

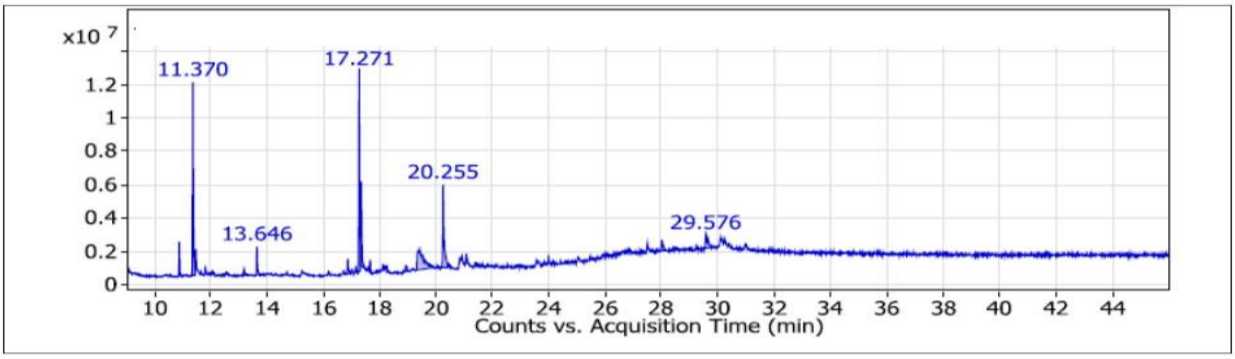

Fig. 2. The chemical compounds in *S. costus* by ethyl acetate extract identified by GC-MS.

TABLE 3. Antibacterial activities of SCE against pathogenic bacteria (Mean± SD)

Tested volume	Inhibition zones in mm (Mean ± SD)						
	B. cereus	***Staph. aureus***	***Staph. Sciuri***	***S. typhi***	***S. entrica***	***E. coli***	***P. areugnosa***
10 µl	18.5±0.71^{b}	16.5±2.12^{b}	18.5±2.12^{a}	10±0.71^{b}	13.3±1.77^{b}	11.25±1.06^{c}	14.3±1.77^{b}
20 µl	20.0±0.71^{ab}	19.5±0.71^{ab}	20.8±0.35^{a}	14.0±2.8^{b}	16.3±1.06^{b}	13.3±1.06^{bc}	14.0±2.8^{b}
50 µl	22.8±1.06^{a}	23.0±2.83^{a}	21.3±1.77^{a}	13±2.12^{b}	17.3±1.06^{b}	15.5±0.71^{b}	16.3±1.06^{b}
Ceftriaxone 1mg/ml (10 µl)	10.5±1.4^{c}	15.0±1.4^{b}	17.8±3.2^{a}	25.3±1.1^{a}	24.8±2.5^{a}	29.3±1.1^{a}	25.5±2.1^{a}

Means with the same letter in the same column are not significantly different, (n=3)
Ceftriaxone (1.0 mg/ ml) :Positive control.

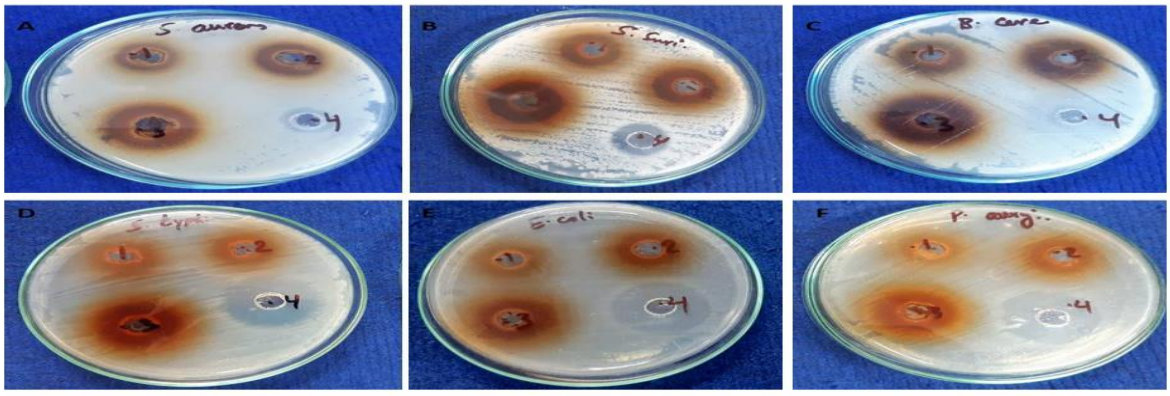

Fig. 3. Antibacterial activity of ethanol extract (50 mg/ml) using well diffusion method filled with different volume 1 (10 µl), 2 (20 µl), 3 (50 µl) and 4 (positive control 10 µl.

TABLE 4. Antifungal activities of SCE against tested fungal spp. (Mean± SD)

Tested volume	Inhibition zones in mm (mean± SD)						
	A. flavus	***A. carbonareous***	***A. niger***	***A. parasiticus***	***A. ochracious***	***P. verecossum***	***F. proliferitum***
10 µl	9.5±0.71^{d}	10.5±0.71^{d}	9.5±0.71^{c}	11.0±1.41^{c}	12.5±0.71^{c}	10.5±0.71^{c}	9.5±0.71^{d}
20 µl	11.5±0.71^{c}	13±0.00^{c}	12.0±0.00^{c}	13.5±0.71^{bc}	19.0±1.41^{b}	12.0±0.00^{c}	12.0±0.00^{c}
50 µl	16.5±0.71^{b}	17±1.4^{b}	17.0±1.41^{b}	16.5±0.71^{b}	22.5±0.71^{ab}	15.5±0.71^{b}	16.5±0.71^{b}
Miconazol 1 mg/ml (10 µl)	28±0.00^{a}	30.5±0.71^{a}	29.0±1.4^{a}	31.5±2.1^{a}	26.0±2.8^{a}	25.5±0.7^{a}	19.0±1.4^{a}

Means with the same letter in the same column are not significantly different, (n=3)

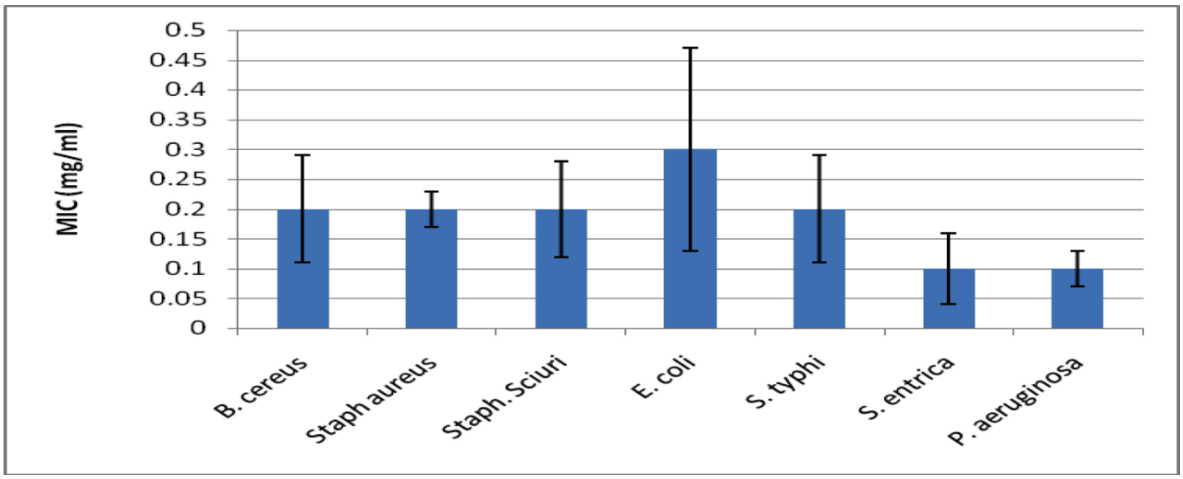

Fig .4. Minimum Inhibitory Concentrations of *SCE* for tested bacteria.

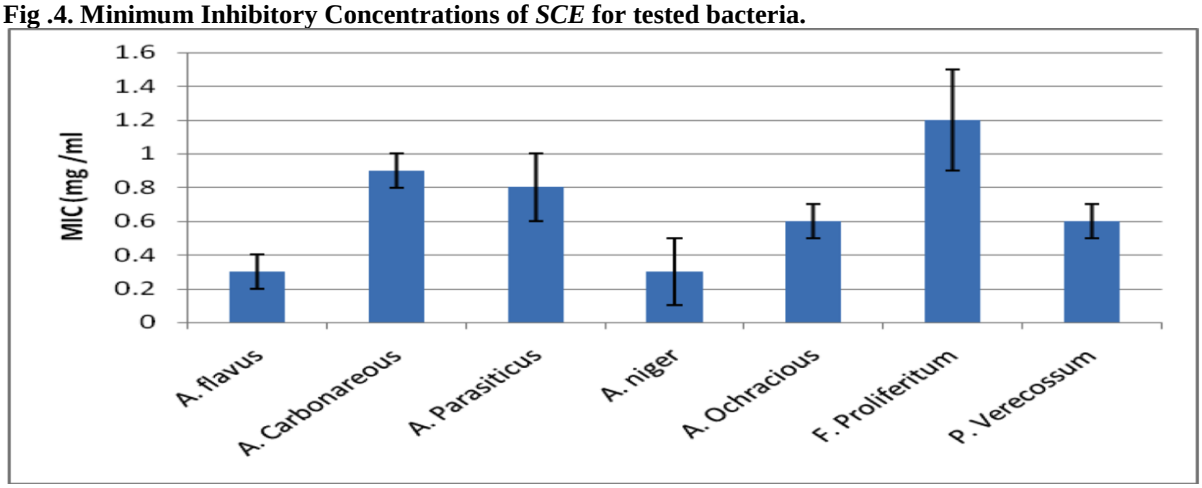

Fig. 5. Minimum Inhibitory Concentrations of SCE for tested fungi.

Pencillium verecossum found to be the most resistance to extract among the tested fungi with inhibition zones from 10.5 mm to 15.5 mm with increasing the concentration from 10µl to 50 µl. The inhibition zones by 50 µl of extract were closed to that developed by the 10 µl of antifungal agent Miconazol 1mg/ml with inhibition zones ranged from 19.0 mm to 31.5 mm for *F. proliferitum* and *A. parasiticus*, respectively. SCE had an inhibition activity against different fungi **[42, 46]** Herbal medicine is oldest and experienced of various types of disease and many research are currently looking for good alternatives to reduce the health hazardous associated with regular usage of antibiotics **[47]. Salim *et al.* [44]** announced that the ethanol extract on cold and hot of costus rhizome showed antifungal activity against three tested fungal species (*Penicillium* species, *Fusarium* species, and *Aspergillus fumigates*) at concentration ranging from (1:1- 1:4) and (1:1 – 1:8) in case of hot and cold extract, respectively. The antifungal action of the ethyl acetate extract of *Costus speciosus* rhizome

against fungal species like *A. niger, Botrytis cinerea Trichophyton rubrum, T. simii* and *Candida albicans***[50].** The same antifungal activity of Costus hexan extract was reported by **Duraipandiyan *et al.*[42].** Moreover; the inhibitory activity of Costus methanolic extract against *Penicillium* spp. and *Mucor spp* **[51].** Plant-based remedies have been widely used for the management of variable diseases due to their safety and less side effects **[52]**. The screened the activity of Costus root and leaf essential oil against pathogenic fungi which showed moderate antifungal activity against *A. tamari* and *Rhizopus stolonifer* **[39]**.

Minimum inhibitory concentration (MIC) values of Costus extracts

MIC is defined as the lowest concentration of an extract that completely inhibits the growth of microorganisms within 24 hr. As shown in Fig (4) the minimum inhibitory concentration (MIC) of *S. costus* extract against different bacteria showed in Fig. 4. The highest activity of *S. costus* extract was recorded against *E. coli* with MIC value of 0.3 mg/ml, however, the lowest MIC was observed against *P. areugnosa* (0.08 mg/ml). These results are agreement with those reported by **Sulakshana, *et al.*[49]** who used different extracts of courts against *E. coli* (0.5 mg/ml) followed by *P. aerugnosa* (0.25 mg/ml). Moreover; the determined of MIC by ethanol extract of *S. costus* which ranged between 2 and 8 µg/ml *Saussurea lappa* extracted by ethanol as antibacterial effect on *Helicobacter pylori* **[23]**. **Lammari et al.[52]** identified compounds by GC/MS in *Saussurea lappa* (known as costus) that a significant effect of biological interests include(β-eudesmol, a sesquiterpene). Moreover, recently this compound was shown as an effective inhibitor of SARS-CoV-2 main protease **[53]**.

Whereas, Fig (5) showed the MIC values of SCE against different tested fungi. The highest activity was recorded against *F. prolifertum* (1.17 mg/ml) followed by *A. carbonaceous* (0.92 mg/ml). The lowest MIC was recorded against *A. flavus* 0.25 mg/ml and *A. niger* 0.32 mg/ml. The impact of polyphenol compounds that can act on the cytoplasmic membrane and change both structure and function on microorganisms. As a result of bacteria lose the structural integrity of the cell membrane, especially in Gram (+ve) bacteria **[54].** On the other hand, *Saussurea lappa* is considered as a rich source of triterpenoids, flavonoids, steroids and sesquiterpene lactones, and is reported to have several biological potentials including antimicrobial, antibacterial **[55-57**].

4. Conclusion

The SCE contains a potent active compounds could be used successfully as antibacterial and antifungal agents against broad range of microorganisms. These results might be open the field to be used as food preservatives alternative to traditional chemical preservatives that threaten the human health. This work revealed that the *S. costus* extracts can play a great role in the defense action against the human multi-resistant pathogens and can alternate the antibiotics in the treatments of some infections.

5. Conflict of Interests

There are no conflicts of interest declared by the Authors.

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