Chemical Composition and Antimicrobial Activity of Oleoresin of *Capsicum annuum* Fruits

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Abstract

Capsicum annuum is consumed as a part of human diet since prehistoric period. The present study was designed to analyze the composition of oleoresin of C. annuum fruits and evaluate its antimicrobial potential. Study involved extraction of oleoresin by percolation of the pulverized dried ripe fruits, which were further analyzed using GC/GC-MS and evaluated for antimicrobial activity using standard disc diffusion method. The predominant constituents in capsicum oleoresin included linoleic acid (33.81%), 2,3-dihydroxypropyl oleate (16.80%), palmitic acid (15.57%), capsaicin (10.28%), and dihydrocapsaicin (6.09%). The capsicum oleoresin exhibited significant antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Candida albicans and Aspergillus niger. Antimicrobial potential of C. annuum oleoresin was based on determination of zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) through disk diffusion method. Oleoresin exhibited maximum ZOI and MIC against B. subtilis (12.0±0.5 mm and 25 µL/mL) and A. niger (30.0±0.5 mm and 40 µL/mL). This study concluded capsicum oleoresin to contain large number of fatty acids and their esters, amides, monoterpenes, diterpenes, triterpenes, sesquiterpenes, and phytosterols. It also exhibited significant antimicrobial activity against pathogenic microorganisms.

Keywords*:* capsicum, oleoresin, GC-MS, zone of inhibition, minimum inhibitory concentration

1. Introduction

Capsicum annuum (CA), commonly called as chilli pepper that belongs to Solanaceae family, is traditionally used in most of cuisines and food products attributed to its distinctive flavor, color, pungency and aroma (Dzoyem *et al.*, 2017). The CA fruits are reportedly applied to allay gout, arthritis, anorexia, sciatica, dyspepsia, flatulence, cardiac debility, cough, malaria, intermittent fevers, cholera, muscular spasms (of arm, shoulder, and spines), neuralgia, lumbago, chilblains, cancer, bronchitis, headache and arrhythmias (Vijayalakshmi *et al.*, 2010; Al-Snafi, 2015; Saleh *et al.*, 2018). CA is an annual herbaceous plant having glabrous, lanceolate leaves, white flowers and fruits (Bargavi and Elumalai, 2010).

Mexico is considered as the place of origin of CA while other species are known to originate in South America. C*apsicum* species are widely cultivated throughout the entire region of Southeast Asia (Smith and Heiser, 1957). Different varieties of pepper have been developed around the world and broadly classified into five major categories, namely CA var. *cerasiforme* (cherry pepper), CA var. *conoides* (cone pepper), CA var. *fasciculatum* (red cone pepper), CA var. *grossum* (bell or sweet pepper), and CA var. *longum* (cayenne or chilli pepper).

Pepper fruits are consumed in food preparations as spices due to pungent taste attributed to presence of capsaicin present in fruits, seeds, and placental tissue. Pepper fruits create a burning sensation that can last for several hours (Saleh *et al.*, 2018). CA fruits contain capsaicinoids that are group of compounds responsible for its characteristic pungent taste. Capsaicinoids are also known for their therapeutic properties in rheumatoid arthritis and gastric ulcers (Basith *et al.*, 2016; Batiha *et al.*, 2020). Capsaicinoids found in the capsicum are 9-11 carbon chain, branched fatty acid vanillylamides, of which capsaicin and di-hydrocapsaicin are most abundant (Nadi *et al.*, 2020). They are accountable for 90% of total pungency of pepper fruits (Othman *et al.*, 2011). The major capsaicinoids reported in capsicum oleoresin are capsaicin (48.6%) followed by 6,7-dihydro capsaicin (36%), nordihydrocapsaicin (7.4%), homodihydrocapsaicin (2%), homocapsaicin (2%), capsanthin and capsorubicin.

The CA fruits are consumed globally as fresh, processed natural color as paste, oleoresin and paprika (Perva-Uzunalić *et al.*, 2004; Giuffrida *et al.*, 2013). Capsicum oleoresin serves as source for antimicrobials, antioxidants, food

colors and flavors (Zhu *et al.,* 2015). The CA fruits extract called oleoresin comprises around 100 different volatile constituents that function in different ways from pure capsaicin. Capsaicinoids in capsicum collectively contribute around 0.1-0.2%, and their quantity varies according to soil, climate, variety, and harvest time. The variation in composition of oleoresin is based on various factors like geographical origin, extraction procedures, post-harvest treatment, processing, drying condition, and temperature.

Capsaicin is crystalline, colorless and pungent principle, and its chemical structure is elucidated as 8-Methyl-6-nonenoyl vanillyl amide (Govindarajan and Sathyanarayana, 1991; Johnson, 2007; Vani, 2017). Other phyto-constituents found in CA are carotenoids like capsorubin, capsanthin, lutein and carotene among others. CA also contains minute quantity of a liquid alkaloid, a saponin capsicidin. CA is also rich in fats (9-17%) and protein (12-15%) (Mohd Hassan *et al.,* 2019). Other parts of CA plant contain steroidal alkaloid glycosides (solanine, solanidine and solasodine), steroidal glycosides (capsicoside A-D), and Scopoletin (coumarin) (Yahara *et al.,* 1994; McKenna *et al.,* 2002). Also, it has been reported to have high quantity of vitamin C (ascorbic acid) and Zinc – the two nutrients which are vital for a strong and healthy immune system. CA has vitamins (A and C), rutin (bioflavonoid), β-carotene, iron, calcium, and potassium in high abundance. Capsicum also contains magnesium, phosphorus, sulphur, B-complex vitamins, sodium, and selenium (Khan and Abourashed, 2011).

Reports suggest humans to possess 1:1 bacteria and cells. Little disruption in human microflora may lead to various infections. Use of various conventional antibiotics to treat several infections leads to multiple drug resistance, immune suppression, allergic reactions, and high mortality risk. Hence, this creates utmost need for natural alternatives (Hang *et al.,* 2020). Pathogenic microorganism that has developed resistance to conventional antibiotics and other medications used for treatment of infections are known as superbugs (Chopra, 2000). Recognizing the problem of resistance against conventional antibiotics, investigators attention is slowly shifted towards biologically active compounds that are isolated from plants, which are commonly used as herbal medicines, as they may provide a new source of natural antibacterial, antifungal and antiviral agents (Maiyo *et al.,* 2010).

Medicinal plants biosynthesize various secondary metabolites which possess potential antimicrobial properties that could be used in the treatment of infectious diseases (Adams, 2001; Matasyoh *et al.,* 2009). Hence, this study

intended to explore the chemical composition of oleoresin of dried ripe fruits of CA from North India using gas chromatography-mass spectrometry (GC/GC-MS) and investigated its antimicrobial potential.

2. Methodology

2.1 Plant Material

Dried ripe fruits of CA were procured from local market in Ghaziabad, North India. The plant material was identified and authenticated by Dr. K.C. Bhatt, Senior Scientist, National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi, India. Voucher specimen with reference number NHCP/NBPGR/2009/2 was deposited in the herbarium of R. V. Northland Institute.

2.2 Isolation of Capsicum Oleoresin

Capsicum oleoresin was prepared by extracting the crushed/pulverized dried ripe fruits of CA (1 kg) with volatile solvent (ethanol) using percolation method (Tandon *et al.*, 1964; Koleva *et al.*, 2013; Lu, *et al.*, 2017). The dark extract comprising not less than 10% of total soluble solids were drawn off and distilled under reduced pressure for the removal of excess solvent to offer dark red viscous liquid. The CA oleoresin was collected, measured, and stored at 4 °C in the dark. This oleoresin was analyzed by GC-MS and further evaluated for its antimicrobial activity.

2.3 GC Analysis

The GC analysis of CA oleoresin was executed on GC-2010 (Shimadzu, Japan) equipped with flame ionization detector (FID) and Rxi-5SilMS column (30 m x 0.25 mm x 0.25 μm). Injector and detector (FID) temperatures were maintained at 260 and 270 °C, respectively. Carrier gas used was nitrogen at flow rate of 1.21 mL/min with column pressure of 115.6 kPa. The sample (1 μL) was injected into column with split ratio of 1:100. The biochemical components were separated following linear temperature programming from 100 to 280 °C at a rate of 4 °C/min and then held at 280 °C for 29 min, with total run time of 65 min. Constituents percentage was based over FID peaks area electronic integration.

2.4 GC-MS Analysis

The GC-MS analysis was carried out on GC-MS-QP 2010 Ultra (Shimadzu, Japan) fitted with a Column Rxi-5SilMS (60 m x 0.25 mm i.d., film thickness 0.25 µm) using carrier gas of helium maintained with 1.21 mL/min flow rate. Initially, temperature of column oven was fixed at 100 °C for 10 min and increased up to 250 °C at the rate of 6 °C/min. Next, temperature was tuned to 250 °C for 10 min followed by increment up to 280 °C at a rate of 1 °C/min and then held at 280 °C for 10 min. The split flow was maintained at 28.4 mL/min with a split ratio of 1:100, injector temperature of 260 °C, at detector temperature of 270 °C, and with an injection volume of 1.0 µL. The ionization energy (voltage) supplied was 70 eV and mass scan range (m/z) was 40-650 amu. The percent composition of CA oleoresin was estimated from FID peak area.

2.5 Antimicrobial Activity

2.5.1 Test Microorganisms

The microorganisms selected were *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), and fungi species, namely *Candida albicans* (MTCC 227), and *Aspergillus niger* (MTCC 404). All experimental strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. These strains were maintained at 4 °C on nutrient agar slants throughout the experiment and used as stock cultures.

2.5.2 Media and Preparation

Nutrient agar media was composed of beef extract (1 g), yeast extract (2 g), peptone (5 g), NaCl (5 g), agar (15 g) and distilled H_2O (1 L). Sabouraud dextrose agar (SDA) media comprised dextrose (40 g), peptone (10 g), agar (15 g) and distilled H_2O (1 L).

The nutrient agar medium (28 g) was accurately weighed and suspended in 1000 mL of distilled H_2O. The mixture was heated to boiling to dissolve the medium completely in a conical flask. The flask was then plugged using non-absorbent cotton plug and covered with aluminum foil. It was sterilized by autoclaving at 15-lbs/in^2 pressure and 121 °C for 15 min. The SDA medium was suspended in 1000 mL of distilled H_2O and boiled to dissolve the medium completely in a conical flask. The conical flask containing SDA medium was plugged with non-absorbent cotton plug and covered properly with aluminum foil. It was sterilized by autoclaving at 15-lbs/in^2 pressure and 121 °C for 15 min.

2.5.3 Microorganisms (Inoculum) Preparation

Inoculation of test microorganisms was done over freshly prepared medium slants. Slants incubation was done at 37 °C for minimum 24 h. Test microorganisms from slants after washing with saline solution (3 mL) were re-incubated at 37±2 °C for a minimum 24 h. The developed organisms from the nutrient media were washed using distilled H_2O (50 mL). Dilution factor when determined at 530 nm offered 25% of light transmission. The quantity of suspension required for nutrient broth (100 mL) was estimated based on test plate or broth. Apart from that, the test organisms were stored in refrigerator.

2.5.4 McFarland Turbidity Standard

A McFarland standard number 0.5 was used for the purpose of quality control. It was prepared by adding 0.5 mL of a 1.175 % (w/v) barium chloride dihydrate ($BaCl_2.2H_2O$) solution to 99.5 mL of 1 % (v/v) sulphuric acid.

2.5.5 Antimicrobial Standard

A stock solution for each tetracycline (antibacterial) and fluconazole (antifungal) standard drug was prepared in a concentration of 100 µg/mL by adding 10 mg drug (standard) in 100 mL of 1% dimethyl sulfoxide (DMSO). The prepared stocks of tetracycline and fluconazole were further converted into 10 µg/mL concentration through dilution of 1 mL stock solution up to 10 mL of 1% DMSO.

2.5.6 Susceptibility Testing (Zone of Inhibition)

Antimicrobial susceptibility of oleoresin was investigated by standard disc diffusion method in triplicate using 24-48 h grown strains reseeded on nutrient media (Bayer *et al.*, 1966). The cultures were adjusted with saline H_2O to obtain a suspension at concentration of $1x10^6$ CFU/mL with a McFarland standard number 0.5, and then the 100 µL of suspension was spread on nutrient agar media plates to obtain uniform microbial growth. Sterile filter paper discs (Whatman's number 5, 6 mm in diameter) were impregnated with 10 µL of the oleoresin and placed on the surface of the agar test plates. Control discs were saturated with tetracycline (10 µg/disc). Plates were subsequently incubated for 24 h at 37 °C. The zones of inhibitions (ZOI) were calculated by measuring the diameter in mm. In the case of fungi, the test was performed in sterile Petri dishes containing SDA. The CA oleoresin was adsorbed over sterile paper discs and placed on the surface of medium previously inoculated

with a suspension of fungus. Control discs were saturated with fluconazole (10 µg/disc). All Petri dishes were sealed with a sterile laboratory film to avoid evaporation of the test samples and incubated at 27 °C for 48 h. The ZOI was determined by measuring the diameter in mm of clear zone around each disc.

2.5.7 Determination of Minimum Inhibitory Concentration (MIC)

Oleoresin of CA was tested for determination of MIC using disc diffusion method (Daw *et al.*, 1994). MIC can be stated as minimum test samples concentration which shows no microbial growth after 24 or 48 h incubation at 37 °C. The Petri dishes contained the nutrient agar (15 mL) for bacteria and SDA for fungi, supplemented by the test strains at a density of $1x10^6$ CFU/mL. Four discs of 6 mm diameter were placed in each of the agar plates. The CA oleoresin was dissolved in 1% DMSO and two-fold serial dilutions were made in concentration range from 10 to 1 µL/mL. DMSO blank was used as negative control. Bacterial strains inoculated petriplates were subjected to incubation for 24 h at 37 °C, whereas the petriplates inoculated with fungal strains were subjected to incubation for 48 h at 27 °C. Minimum concentration of oleoresin showing a clear of inhibition was considered as MIC of oleoresin. The experiment was performed in triplicate and results were calculated as standard means (±SD). Difference in statistical data were considered as significant at $p < 0.05$.

3. Results and Discussion

Constituents of CA oleoresin were identified using GC and GC-MS analyzer. Individual compounds were identified based on retention time (RT) of peaks obtained using Innowax fused silica capillary column supported with literary data, matching against the standard library spectra (Chen *et al.*, 2017;) and built up using pure substances and components of known oleoresins. Further identification was carried out by comparing spectra obtained by GC-MS analysis with those stored in spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature (McLafferty and Stauffer, 1994; Adams, *et al.*, 2001). Relative amounts of identical components were based on peak area obtained without correction of FID response. The yield of oleoresin obtained from the dried ripe fruits of CA was found to be 9.5%. The composition of CA oleoresin is mentioned in Table 1 with retention time and respective percentage.

Table 1. The GC based chemical composition of oleoresin from dried fruits of CA

S. No.	Components	Retention Time	% Area
1	(R)-(+)-Citronellic acid	9.02	0.11
2	*n*-Hexadecane	11.68	0.10
3	5,6,7,7a-Tetrahydro-4,2(4H)-benzofuranone	13.56	0.05
4	Dodecanoic acid	14.06	0.26
5	*n*-Tricosane	15.78	0.08
6	*n*-Heptadecane	16.47	0.13
7	*n*-Butyl-9-octadecenamide	17.08	0.05
8	5,6,7,7a-Tetrahydro-6,2(4H)-benzofuranone	17.61	0.04
9	Myristic acid	17.08	1.15
10	*n*-Pentadecanoic acid	18.88	0.40
11	6,10,14-Trimethyl-2-pentadecanone	19.11	0.04
12	Methyl palmitate	20.46	0.25
13	1-Tetradecanoyl-piperazine-3,5-dione	20.71	0.06
14	*n*-14-Pentadecenoic acid	20.91	0.20
15	Demycarosylturimycin H	21.03	0.06
16	Palmitic acid	21.47	15.60
17	3,7,11,15-Tetramethylhexadec-1-en-3-ol	23.52	0.03
18	Linoleic acid	24.41	33.81
19	Stearic acid	24.60	0.79
20	11-Acetamidooctadecanoic acid	25.54	0.49
21	Dodecyl-guanidine monoacetate	25.80	0.33
22	(Z)-13- Docosenamide	27.23	0.22
23	1-N-Pentadecyl-decahydronaphthalene	28.11	0.08
24	N-{4-[2-(1,1-dimethylethyl)-5-oxo-1,3-dioxol formamide	28.91	0.41
25	N-(4-Hydroxy-3-methoxybenzyl) nonanamide	29.17	1.04
26	2-Hydroxy-1-(hydroxymethyl) ethyl palmitate	29.59	0.55
27	Nonivamide	29.83	0.26
28	Acetyl oleylate	30.03	0.41
29	Capsaicin	30.65	10.28
30	Dihydrocapsaicin	30.94	6.09
31	(+)-2-exo-Hydroxycineole	31.16	0.06
32	tert-Butyldimethylsilyl-5,5-dimethyl-1,3-dioxane-2-ethanol	31.53	0.78
33	3,5-Dimethyl-1-butylpyrazole	31.71	0.10
34	1-Methoxy-4-[1-(methyl-D) propyl-1-D] benzene	31.80	0.06
35	2,3-Dihydroxypropyl oleate	32.25	16.80
36	Squalene	33.65	0.26
37	3,4,4a,5,6,8a-Hexahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran	33.89	0.18
38	α-Epoxycedrene	34.49	0.13
39	2-Methyl-3,4,4-trimethoxy-2-cyclobutene-1-one	34.88	0.08
40	γ-Tocopherol	37.58	0.23
41	4,4-Dimethyl-5α-androstan-3β-ol	38.34	0.32
42	Stigmast-5-en-3-ol	38.58	0.10
43	2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol	39.38	1.80
44	24-Methyl-5-cholestene-3-ol	41.92	0.81
45	Stigmasterol	42.81	0.26
46	*n*-Hexatriacontane	43.51	0.19
47	β-Sitosterol	44.65	1.44
48	β-Amyrin	45.77	0.14
49	Methyl commate D	47.40	0.37
50	Stigmast-4-en-3-one	49.59	0.08
51	Phytol acetate	54.70	0.26

The GC-MS analysis of the oleoresin of CA exhibited presence of eight fatty acids (52.39%), their three esters (52.60%), two vanillyl amides (16.37%), four aliphatic alkanes (0.50%), monoterpenes, sesquiterpenes, diterpenes, triterpenes, phytosterols, and aromatic constituents. The predominant constituents were linoleic acid (33.81%), 2,3-dihydroxypropyl oleate (16.80%), palmitic acid (15.57%), capsaicin (10.28%), dihydrocapsaicin (6.09%). The phytoconstituents occurring in minor amounts included myristic acid (1.15%), N-(4-hydroxy-3-methoxybenzyl) nonanamide (1.04%), 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (1.80%) and β-sitosterol (1.44%). The literary evidence suggests that capsaicin and dihydrocapsaicin usually predominates among capsaicinoids of chilli extracts (Maokam *et al.*, 2014). However, the present GC and GC-MS study over extract of CA fruits determined relatively lesser percentage of capsaicin and dihydrocapsaicin. This could be attributed to the geographical conditions of this plant.

The fatty acids and their esters detected in the oleoresin were lauric acid, myristic acid, pentadecanoic acid, methyl palmitate, 14-pentadecenoic acid, palmitic acid, stearic acid, linoleic acid, 11-acetamido-stearic acid, 2-Hydroxy-1-(hydroxymethyl) ethyl palmitate and 2,3-dihydroxypropyl oleate. The aliphatic constituents, namely *n*-hexadecane, *n*-tricosane, *n*-heptadecane and hexatriacontane were present in trace amounts. The phytosterols included β-sitosterol, stigmasterol, stigmast-4-en-3-one, 24-methyl-5-cholestene-3-ol and β-sitosterol oleate. (R)-(+)-Citronellic acid, (+)-2-exo-hydroxycineole and α-epoxycedrene were the monoterpenes and sesquiterpene present in the oleoresin. The diterpenes determined in the oleoresin were phytol acetate and 3,7,11,15-tetramethyl hexadec-1-en-3-ol. The triterpenes detected were squalene and β-amyrin. The aromatic compounds present in the trace amount in CA oleoresin included 5,6,7,7a-tetrahydro-4,2(4H)-benzofuranone, 5,6,7,7a-Tetrahydro-6,2(4H)-benzofuranone, 1-N-pentadecyl decahydro-naphthalene, N-(4-hydroxy-3-methoxybenzyl)-nonanamide, capsaicin, dihydrocapsaicin, 1-methoxy-4-[1-(methyl-D)propyl-1-D]-benzene and 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-2H-1-benzopyran.

Antimicrobial potential of CA oleoresin was tested against *A. niger*, *B. subtilis*, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. aureus*. The CA oleoresin exhibited significant antimicrobial activities when compared with standard drugs (tetracycline and fluconazole) (Table 2).

Table 2. ZOI (mm) and MIC (µl/ml)

Microorganisms	ZOI (mm) ±SD (n = 3)			MIC (µL/mL) ±SD (n = 3)	
	Oleoresin	DMSO	Standard	Oleoresin	Standard
Gram Positive Bacteria					
B. subtilis	12.0±0.5	No Zone	12.0±0.6	25.0±0.4	8
S. aureus	10.0 ± 0.3	No Zone	20.0±0.4	12.0±0.5	8
Gram Negative Bacteria					
E. coli	4.0±0.4	No Zone	25.0±0.3	45±0.1	8
P. aeruginosa	8.0±0.5	No Zone	10.0±0.2	10.0±0.2	8
Fungi					
C. albicans	15.0±0.3	No Zone	10.0±0.1	25.0±0.5	0.5
A. niger	30.0±0.5	No Zone	38.0±0.2	40.0±0.4	6

ZOI – zone of inhibition, oleoresin concentration = 10 µL/disc, negative control = dimethyl sulfoxide (DMSO), standard (positive control) = tetracycline (10 µg/disc for bacteria) and fluconazole (10 µg/disc for fungi), MIC – minimum inhibitory concentration (oleoresin concentration ranged from 1-100 µL/mL)

The ZOI ranged from 4.0-12.0 mm and 15.0-30.0 mm for microbial strains – bacteria and fungi, respectively. Tetracycline exhibited a ZOI of 10-25 mm against bacterial strains while fluconazole had a ZOI of 10-38 mm against fungal strains. Significant antibacterial activity was observed in CA oleoresin against *B. subtilis* and *P. aeruginosa* followed by *S. aureus* which was in parity with standard (positive control) while weaker than standard against *E. coli*. The ZOI was less than 10 in gram negative bacteria which indicated CA oleoresin in moderate activity. For *E. coli*, it was less than 5 showing its weak activity. However, antifungal activities seemed promising. CA oleoresin exhibited strong antifungal potential against *C. albicans* which was stronger than fluconazole (positive control) followed by *A. niger*. Antimicrobial activity of CA oleoresin was assessed by determination of MIC.

CA oleoresin exhibited significant inhibitory effect against all test micro-organisms with MIC values ranged from 10-45 and 25-40 µL/mL for microbial strains – bacteria and fungi, respectively. The CA oleoresin offered greater inhibitory action against *P. aeruginosa* followed by *S. aureus* (Table 2). The MIC value of CA was higher against *E. coli* but lesser against *P. aeruginosa.* The standard drug tetracyclin exhibited 8 µL/mL of MIC against *B. subtilis, E. coli, S. aureus,* and *P. aeruginosa* (Hussin and El-Sayed, 2011; Adimpong, *et al.*, 2012). CA oleoresin also showed greater activity towards *C. albicans* followed by *A. niger*. The drug fluconazole was chosen as positive control against *C. albicans* and *A. niger* based on the literary evidence (Ahmad *et al.*, 2019). The standard drug fluconazole exhibited MIC of 0.5 µg/mL and

6 µg/mL against *C. albicans* and *A. niger*, respectively (Borman *et al.*, 2017; Nagy *et al.*, 2018).

The antimicrobial potential of oleoresin of CA fruits could be attributed to presence of its bio-constituents. Soetarno *et al.* (1997) highlighted that capsaicinoids are the major constituents of ethanolic extract of CA fruits and responsible for their antimicrobial potency. The antimicrobial results of the present study were also in agreement with other standard research studies of Soetarno *et al.* (1997) and Nurjanah *et al*. (2014). These findings support the traditional application of CA fruits in treatment of oral thrush due to *C. albicans*.

4. Conclusion

The present study established that predominant constituents of the oleoresin of CA fruits are linoleic acid (33.81%), 2,3-dihydroxypropyl oleate (16.80%), palmitic acid (15.57%), capsaicin (10.28%) and dihydrocapsaicin (6.09%). The phytoconstituents occurring in minor amounts included myristic acid (1.15%), N-(4-hydroxy-3-methoxybenzyl) nonanamide (1.04%), 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (1.80%) and β-sitosterol (1.44%). The oleoresin mainly contained fatty acids (52.39%), esters (52.60%), and amides (16.37%). Hence, it was concluded that oleoresin of CA fruits possessed significant antimicrobial activity against pathogenic microorganisms. The presence of capsaicin, dihydrocapsaicin and β-sitosterol in oleoresin made CA fruits of high medicinal value.

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