ORIGINAL ARTICLE

Preparation and *in vitro* antimicrobial activity of supercritical fluid extracts of selected Indian plants against oral pathogens and their phytochemicals and statistical analysis

Rosy Kumari, Ratish Chandra Mishra, Jaya Parkash Yadav

Department of Genetics, Maharshi Dayanand University, Rohtak - 124001, Haryana, India

Abstract

Introduction: In this study, extracts of *Acacia nilotica, Elettaria cardamomum, Psidium guajava,* and *Glycyrrhiza glabra* were prepared using supercritical fluid extraction (SFE) at different pressures and 50°C constant temperature. The antimicrobial activities of extracts were evaluated against oral pathogens, namely, *Enterococcus faecalis*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. **Materials and Methods:** The antimicrobial activities of extracts were evaluated against oral pathogens using agar well diffusion method. Phytochemical analysis of *A. nilotica* twig was done using gas chromatography–mass spectrometry (GCMS). Statistical analyses of data were performed by one-way ANOVA using MS-Excel and principal component analysis was performed using statistical software XLSTAT 2018. **Results:** All plants extracts exhibited significant activity at $P < 0.05$ with inhibitory zones ranging from 8 to 42 mm and minimum inhibitory concentration (MIC) values from 0.19 to 3.12 mg/ml. *A. nilotica* twig extract obtained at 400 bar pressure showed the highest zone of inhibition (42.07 mm) and lowest MIC (190 µg/mL). *E. cardamomum* and *G. glabra* extracts showed moderate activity while *P. guajava* extracts showed least activity against the oral pathogens. GC-MS analysis of *A. nilotica* twig confirm the presence of functional moieties of stigmasterol, clionasterol, betulinaldehyde, eugenol, α-terpinyl acetate, and 22,23-dihydrobrassicasterol in the extract which could be responsible for its antimicrobial efficacy and may prove beneficial in oral care products. **Conclusion:** The extraction of antimicrobial agents from plant materials using SFE at low temperatures avoids the thermal degradation and use of toxic solvents. *A. nilotica* twig at 50°C and 400 bar showed the significant antimicrobial potential, hence it can be processed to obtain effective and cheaper drug due to higher biomass availability. Chemical profiling of SFE extract by GC-MS analysis proved helpful in the identification of compounds. Furthermore, bioactive compounds should be explicated for their exact mechanism of action with the target pathogens.

Key words: *Acacia nilotica,* antimicrobial activity, *Elettaria cardamomum,* gas chromatography–mass spectrometry, *Glycyrrhiza glabra, Psidium guajava*, supercritical fluid extraction

INTRODUCTION

Oral hygiene is an important part of human health. The oral microbiota is maintained in a balanced way in healthy individuals. Disruptions in the homoeostasis lead to a wide variety of oral diseases. *Enterococcus faecalis*, *Streptococcus mutans*, *Staphylococcus aureus, Porphyromonas gingivalis, Candida albicans,* etc., are the major pathogens responsible for various oral infections.[1] Many antimicrobial chemical agents including chlorhexidine, fluorides, and sodium lauryl sulfate are used in oral care products. These chemicals cause many side effects such as hypersensitivity reactions, tooth staining, desquamation of oral mucosa, alteration in taste perception, and calculus formation.[2,3] Hence, their adverse effects outrage the benefits and compelled the researchers to

Address for correspondence:
Jaya Parkash Yadav, Department of Genetics, Maharshi Dayanand University, Rohtak - 124 001, Haryana, India.
E-mail: yadav1964@rediffmail.com

Received: 24-04-2019
Revised: 19-03-2020
Accepted: 08-05-2020

search for an alternative. Medicinal plants are the source of novel bioactive compounds and these days consumers are embracing the herbal products a lot.

Acacia nilotica commonly known as Desi Kikar or Babul; is a tannin rich medicinal plant of Fabaceae family. The plant is native to Arabian Peninsula and now widely distributed throughout the tropical and subtropical regions of the world. Conventionally, it is used to treat leukoderma, gonorrhea, diarrhea, paralysis, eye pain, and tooth and gum diseases.[4]

Psidium guajava or Guava popularly known as "poor man's apple of the tropics"; belongs to the Myrtaceae family. The plant is considered to be originated in tropical South America and is widely grown in tropical areas such as India, Bangladesh, and West Indies. All parts of the plant are used in traditional medicine such as in malaria, dengue, gastroenteritis, vomiting, diarrhea, wounds, acne, rheumatism, toothache, and inflamed gums.[5,6]

Elettaria cardamomum or green cardamom or chhoti elaichi belongs to family Zingiberaceae. This spice is native of Western Ghats of India and Sri Lanka from where it has spread to other tropical countries. Cardamom fruit is used in cardiac disorders, renal and vesicular calculi, gastrointestinal disorders, dyspepsia, debility, anorexia, asthma, bronchitis, bad breath, and cancer.[7,8] *Glycyrrhiza glabra* is commonly known as licorice or Mulheti belongs to family Fabaceae. It is native to the Mediterranean region, central to Southern Russia and Asia and now is widely cultivated throughout the world.[9] In traditional Siddha system of medicine, liquorice is used as a demulcent, expectorant, sweetener, antitussive, laxative and to treat anemia, gout, corneal inflammation, sore throat, and tonsillitis.[10]

Medicinal plants are rich in a wide variety of secondary metabolites, such as phenols, flavonoids, alkaloids, glycosides, saponins, tannins, and terpenoids which makes them effective against microbial infections.[11] There are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated.[12] There is no single method available as standard; for extracting bioactive compounds from plants. The efficiencies of conventional and non-conventional extraction methods mostly depend on the critical input parameters; understanding the nature of plant matrix; and chemistry of bioactive compounds and scientific expertise.[13]

Recent advances in separation of natural products have made supercritical fluid extraction (SFE) as a preferred choice superceding Soxhlet or sonication, which require longer extraction times and conditions. Supercritical fluids can easily effuse through solids like a gas and dissolves materials like a liquid. Hence, these fluids substitute a wide range of organic solvents. The low temperature separation process prevents the degradation of the bioactive compounds.[14] At supercritical state, small changes in temperature or pressure result in large changes in density, allowing many properties of a supercritical fluid to be controlled. CO_2 is generally the most desirable solvent in SFE because it has a readily accessible critical point, i.e., 31°C and 1071 psi.[15] In addition CO_2 is cheap, inert, ecofriendly and gives a clean solvent free extract.[16] Many bioactive compounds such as eugenol and germacrene from tulsi, curcuminoids from turmeric,[17] vanillic and ferulic acid from pomegranate,[18] and L-carnitine from oyster mushroom[19] have been extracted using SFE-CO_2.

These days gas chromatography–mass spectrometry (GC-MS) is widely used for the identification of compounds in analytical research and development, quality control of active pharmaceutical ingredients, bulk drugs, and formulations.[20] GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analytes to be identified on the basis of their mass. GC requires the analytes to have significant vapor pressure between 30 and 300°C.[21]

In the present study, the extracts of *A. nilotica*, *E. cardamomum*, *Psidium guajava,* and *G. glabra* were prepared using SFE and antimicrobial activities of the extracts were evaluated against selected oral pathogens using agar well diffusion method. Phytochemical investigation of *A. nilotica* twig extracts was also carried out using GC-MS due to their highest antimicrobial activity against all pathogens.

METERIALS AND METHODS

Collection of Plant Samples

Locally available medicinal plant samples, namely; *A. nilotica* (twig), *P. guajava* (leaves), *G. glabra* (root), and *E. cardamomum* (seed pod) were collected. The samples were air-dried and powdered using a mixer grinder.

Extract Preparation using SFE

Plant extracts were prepared by SFE at different pressures including 150, 250, 300, 350, and 400 bar with constant temperature at 50°C and extraction time of 40 min. The solvent used was 99.9% CO_2 and 1 ml methanol with a flow rate of 1 min/ml through the extraction vessel.

Antimicrobial Activity and Minimum Inhibitory Concentration (MIC) Determination

The antimicrobial activity of SFE extracts against the pathogens, namely, *C. albicans* (ATCC 3018), *E. faecalis* (ATCC 29212), *S. mutans* (MTCC 497), and *S. aureus*

(ATCC 259323) was analyzed by agar well diffusion method.[22-24] The MIC values were determined by micro-broth dilution method using 96-well plates.

GC-MS Conditions

The extracted compounds of *A. nilotica* twig which showed significant activity were identified using GC-MS at AIRF center, JNU, New Delhi. The analysis was performed using a gas chromatography unit Shimadzu GCMS-QP2010 Plus comprising AOC-20i+s autosampler and equipped with the RTX-5 capillary column; with column flow rate of 1.21 mL/min; injection temperature 250°C; column oven temperature 60°C; ion source temperature 230°C; interface temperature 270°C; and pressure at column inlet 73.3 kPa. The method of electron-impact ionization was applied. All data were obtained by collecting the full scan mass spectra with scan speed of 3333 within the scan range 40–650 m/z. The compounds were identified comparing the data with the software libraries including WILEY8.LIB, NIST11.lib, and NIST11s.lib.

Statistical Analysis

Each experiment was done in triplicate. The results are expressed in terms of mean ± standard deviation. Statistical analyses of data were performed by one-way ANOVA using MS-Excel software and a significant difference was defined as $P < 0.05$. Principal component analysis (PCA) was performed using statistical software XLSTAT 2018.[24]

RESULTS

Antimicrobial Activity

The selected plant extracts derived using SFE pressure from 150 bar to 400 bar showed the antimicrobial activities against the tested oral pathogens [Table 1]. All plants extracts exhibited significant activity at $P < 0.05$ with inhibitory zones ranging from 8 to 42 mm and MIC values from 0.19 to 3.12 mg/ml. *A. nilotica* twig extract at 400 bar pressure showed highest zone of inhibition (42.07 mm). *E. cardamomum* and *G. glabra* extracts showed moderate activity while *P. guajava* extracts showed least activity against the oral pathogens. One-way ANOVA showed (F_{cal} = 32.99 >F_{crit} = 1.88) the major difference between antimicrobial activities at $P < 0.05$ and reject the null hypothesis. In PCA, F1 represents horizontal axis which is positively correlated with antimicrobial activity against *S. mutans,* followed by *E. faecalis*, *C. albicans,* and *S. aureus*. F2 represents vertical axis that is positively linked with activity against *S. aureus* only as depicted in Figure 1. Twig of *A. nilotica* extract at 50°C temperature and 400 bar pressure (S1) is most influential extract with highest F1 score [Table 2].

MIC

Quantitative evaluation of the antimicrobial activity of selected SFE extracts was carried out against tested oral

Table 1: Antimicrobial activity of supercritical fluid extraction extracts against oral pathogens

Plant name	Temp. in °C/ pressure in bar	Code	Zone of inhibition (mm) ± SD			
			Enterococcus faecalis	*Staphylococcus aureus*	*Streptococcus mutans*	*Candida albicans*
Acacia nilotica	50/400	S1	38.04 ±0.11	28.04±0.65	40.12±0.21	42.07±0.43
	50/300	S2	14.05±1.33	15.01±0.29	12.09±1.32	20.08±1.04
	50/250	S3	13.04±1.43	15.03±0.43	12.07±1.21	21.07±1.05
	50/150	S4	-	10.05±0.23	11.08±1.42	10.06±1.54
Elettaria cardamomum	50/400	S5	14.06±0.87	15.04±0.44	12.06±0.09	17.03±.07
	50/300	S6	14.07±0.86	15.05±0.54	12.05±0.08	17.05±0.06
	50/250	S7	13.09±0.77	14.01±0.43	11.06±0.03	16.03±0.03
	50/150	S8	-	-	-	10.01±0.07
Psidium guajava	50/400	S9	11.05±0.34	8.07±0.12	8.05±0.34	10.07±0.41
	50/300	S10	11.07±0.22	8.04±0.09	8.03±0.24	10.08±0.36
	50/250	S11	11.06±0.32	8.07±0.12	8.05±0.34	10.09±0.42
	50/150	S12	-	-	-	-
Glycyrrhiza glabra	50/400	S13	14.01±0.21	16.01±0.33	13.03±0.45	14.02±0.22
	50/300	S14	14.02±0.13	16.02±0.44	13.02±0.54	14.03±0.41
	50/250	S15	14.04±0.21	16.03 ± 0.53	13.05±0.65	14.04±0.32
	50/150	S16	-	-	-	-

pathogens by microbroth dilution method using 96-well plates. MIC value ranged from 0.19 to 3.12 mg/mL [Table 3 and Figure 2]. Interestingly, SFE extract of *A. nilotica* obtained at 400 bar having highest antimicrobial activity showed the lowest MIC against all the tested pathogens, i.e., 190–220 µg/ML against the tested oral pathogens. *A. nilotica extract* at 400 bar pressure showed lowest MIC (190 µg/mL).

Table 2: Correlations between sample's activity and factors

Code	F1	F2
S1	5.813	−0.613
S2	0.784	0.007
S3	0.781	−0.022
S4	−0.944	0.288
S5	0.620	0.174
S6	0.622	0.174
S7	0.384	0.145
S8	−2.272	−0.706
S9	−0.634	−0.136
S10	−0.635	−0.140
S11	−0.632	−0.137
S12	−2.812	−0.169
S13	0.577	0.434
S14	0.578	0.435
S15	0.582	0.435
S16	−2.812	−0.169

F1: Horizontal axis, F2: Vertical axis

GC-MS Analysis

GC-MS analysis of *A. nilotica* extract obtained at 400 bar pressure was carried and various compounds were identified. Phytochemicals with different retention times and peak areas are depicted in Figure 3 and Table 4. Bioactive such as betulinaldehyde, stigmasterol, eugenol, α-terpinyl acetate, 22,23-dihydrobrassicasterol, 1,1'-sulfonylbis[2-(methylthio) ethane], glycolaldehyde dimethyl acetal, 1,4-dimethoxy-2,3-butandiol, fluoroacetic acid, and 3-ethoxy-1,2-propanediol presents in the extract.

DISCUSSION

Based on long history of traditional use, fewer side effects and being less expensive; medicinal plants are a popular developmental route of many natural drugs.[25] In this study, extracts of *A. nilotica, E. cardamomum, P. guajava,* and *G. glabra* were prepared using SFE at different pressures and 50°C constant temperature. The antimicrobial activities of extracts were evaluated against oral pathogens, namely, *E. faecalis*, *S. mutans*, *S. aureus,* and *C. albicans*. All plants extracts exhibited significant activity at $P < 0.05$ with inhibitory zones ranging from 8 to 42 mm and MIC values from 0.19 to 3.12 mg/ml. *A. nilotica* twig extract at 400 bar pressure showed the highest zone of inhibition (42.07 mm) and lowest MIC (190 µg/mL) hence, proceeded for GC-MS analysis. Stigmasterol, clionasterol, betulinaldehyde, eugenol, α-terpinyl acetate, and 22, 23-dihydrobrassicasterol were functional moieties in the extract which could be responsible for its antimicrobial efficacy and may prove beneficial in oral care products.

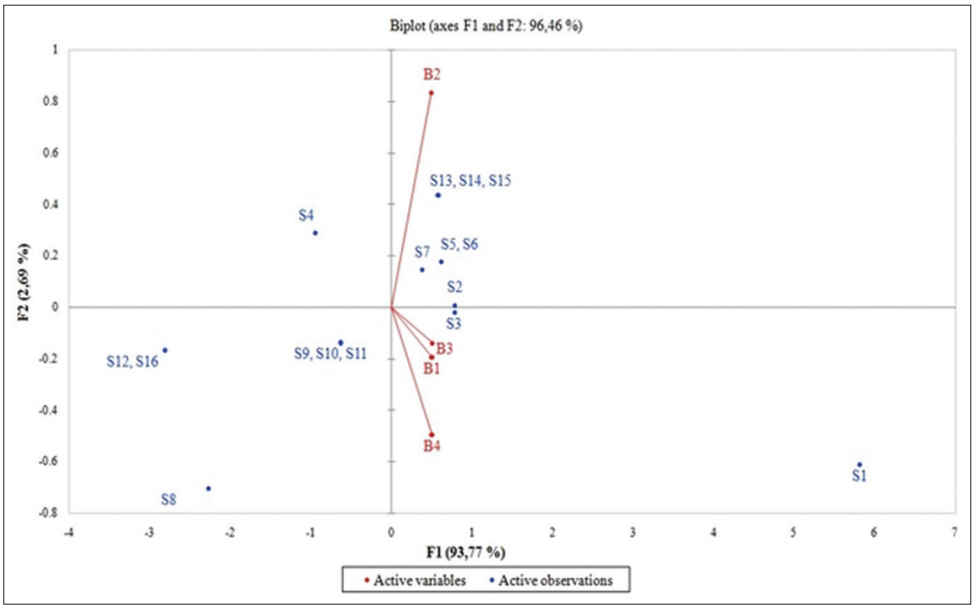

Figure 1: Principal Component Analysis of antimicrobial activity of selected plant extracts.

Table 3: Minimum inhibitory concentration of supercritical fluid extraction extracts against oral pathogens

Plant name	Temperature/pressure in °C/bar for supercritical fluid extraction	Minimum inhibitory concentration in mg/mL			
		Enterococcus faecalis	***Staphylococcus aureus***	***Streptococcus mutans***	***Candida albicans***
Acacia nilotica	50/400	0.22	0.19	0.19	0.19
	50/300	0.38	0.54	0.39	1.45
	50/250	0.39	0.78	0.39	1.56
	50/150	-	0.88	0.76	1.87
Elettaria cardamomum	50/400	0.67	0.78	0.57	0.68
	50/300	0.39	0.39	0.39	3.12
	50/250	1.21	0.67	0.76	1.34
	50/150	-	0.69	0.58	0.98
Psidium guajava	50/400	0.78	0.67	0.85	0.69
	50/300	1.05	1.24	0.76	1.56
	50/250	0.39	0.78	0.39	3.12
	50/150	1.25	0.95	0.84	1.45
Glycyrrhiza glabra	50/400	0.75	0.71	0.66	0.59
	50/300	0.96	0.84	1.24	0.87
	50/250	0.39	0.32	0.43	3.12
	50/150	1.57	0.98	0.78	1.59

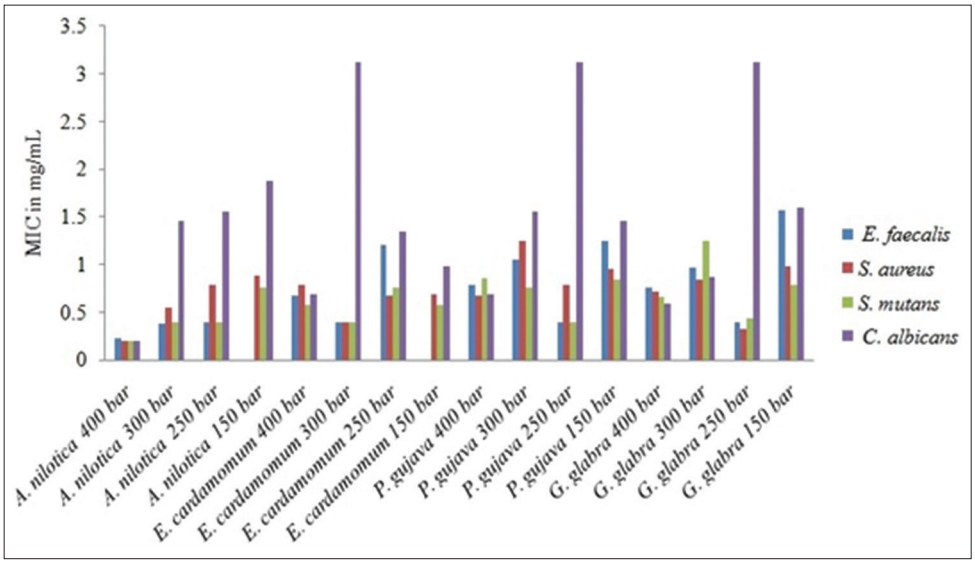

Figure 2: Minimum inhibitory concentration of supercritical fluid extraction extracts against oral pathogens

There was an increase in antimicrobial activity of *A. nilotica* extracts obtained with SFE pressure from 150 bar to 400 bar. The possible reason could be the presence of highly polar bioactives in *A. nilotica* that may require high pressure for extraction. A lot of work has been done on antimicrobial activity of bark, leaf, and pod of *A. nilotica;*[26] however, literature is scarce in respect of the phytochemical analysis and efficacy of *A. nilotica* twig extract as an antimicrobial agent. In the previous studies conducted in our lab, bactericidal effect of different solvent extract of *A. nilotica* leaves was reported against 9 bacterial strains, namely, *Shigella flexneri*, *Enterococcus faecalis*, *S. aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Serratia marcescens*, *Klebsiella pneumonia*, *Escherichia coli,* and *Pseudomonas aeruginosa.*[27] Similarly, in another study, the pod, bark, and leaves extract showed significant antibacterial potential against five oral pathogens, namely, *Lactobacillus acidophilus*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Aggregatibacter actinomycetemcomitans*.[28]

Table 4: Chemical profile of supercritical fluid extraction extract of Babul twig using gas chromatography–mass spectrometry

Peak	Retention time	Area %	Phytochemicals	Molecular formula	Molecular weight (g/mol)
1	4.808	2.75	Diethylene Glycol	$C_4H_{10}O_3$	106.121
2	5.348	1.22	1,3,5,7-Tetroxane	$C_4H_8O_4$	120.10
3	5.587	3.95	Fluoroacetate	$C_2H_3FO_2$	78.04
4	7.607	3.52	3-Ethoxy-1,2-Propanediol	$C_5H_{12}O_3$	120.14
5	9.972	25.01	1,1'-Sulfonylbis[2-(Methylthio)Ethane]	$C_6H_{14}O_2S_3$	214.36
6	10.617	0.61	1-Deoxy-D-Ribitol,	$C_5H_{12}O_4$	136.14
7	12.802	0.33	3-Ethoxy-1,2-Propanediol	$C_{10}H_{18}O$	154.25
9	15.023	0.62	Nerol	$C_4H_{10}O_3$	106.12
10	15.308	24.10	Glycolaldehyde Dimethyl Acetal	$C_4H_{10}O_3$	106.12
11	15.630	0.33	Cinnamaldehyde	C_9H_8O	132.16
12	17.622	0.66	α-Terpinyl acetate	$C_{12}H_{20}O_2$	196.29
13	17.755	0.74	Eugenol	$C_{10}H_{12}O_2$	164
15	19.915	0.14	Coumarin	$C_9H_6O_2$	146.15
16	20.114	10.50	1,4-Dimethoxy-2,3-Butandiol	$C_6H_{14}O_4$	150.17
17	20.938	0.15	1-Dodecanol	$C_{12}H_{26}O$	186.34
18	21.716	0.27	2,4-Bis(1,1-Dimethylethyl)- Phenol	$C_{14}H_{22}O$	206.33
19	23.034	0.54	Nerolidol	$C_{15}H_{26}O$	222.37
20	24.459	2.98	1,4-Dimethoxy-2,3-Butandiol	$C_6H_{14}O_4$	150
21	25.402	0.21	Ar-Turmerone	$C_{15}H_{20}O$	216.32
22	26.063	0.18	2-Propenoic Acid	$C_{17}H_{32}O_2$	268.43
23	26.938	0.51	Mome Inositol	$C_7H_{14}O_6$	174
24	27.542	0.17	Myristic Acid	$C_{14}H_{28}O_2$	228.38
25	27.933	0.11	Rosifoliol	$C_{15}H_{26}O$	222.37
26	28.430	0.93	1,4-Dimethoxy-2,3-Butanediol	$C_{24}H_{26}O_6$	410.47
27	29.151	0.36	Neophytadiene	$C_{20}H_{38}$	278.52
28	29.623	0.24	Pentadecanoic Acid	$C_{15}H_{30}O_2$	242.40
29	30.026	0.15	Phytol	$C_{20}H_{40}O$	296.54
30	30.937	0.18	Methyl Palmitate	$C_{17}H_{34}O_2$	270.46
31	31.473	0.16	Butyl Isodecyl Phthalate	$C_{22}H_{34}O_4$	362.51
32	31.768	2.30	Palmitic Acid	$C_{16}H_{32}O_2$	256.43
33	32.077	0.29	Ethoxy(Methoxy)Methylsilane	$C_4H_{11}O_2Si$	119.22
34	33.998	0.12	1-Octadecanol	$C_{18}H_{38}O$	270.50
35	34.115	0.21	Methyl Linoleate	$C_{19}H_{34}O_2$	294.48
36	34.248	0.09	Methyl Elaidate	$C_{19}H_{36}O_2$	296.50
38	34.940	0.38	Linoleic Acid	$C_{18}H_{32}O_2$	280.45
39	35.048	0.30	Cis-9-Hexadecenal	$C_{16}H_{30}O$	238.42
40	35.341	0.10	Linoleic Acid	$C_{18}H_{32}O_2$	280.45
41	35.446	0.28	Cysteamine S-Sulfate	$C_2H_7NO_3S_2$	157.20
43	40.929	0.63	Docosanol	$C_{22}H_{46}O$	326.61
44	41.501	0.75	Phthalic Acid	$C_6H_4(COOH)_2$	166.14

(Contd...)

Table 4: (*Continued*)

Peak	Retention time	Area %	Phytochemicals	Molecular formula	Molecular weight (g/mol)
45	47.300	0.28	Squalene	$C_{30}H_{50}$	410.72
46	48.591	0.25	Tetratriacontyl Heptafluorobutyrate	$C_{38}H_{69}F_7O_2$	690
47	49.127	0.21	3-Methoxy-Estra-1,3,5(10),8-Tetraen-17-One	$C_{19}H_{22}O_2$	282
49	51.872	0.80	1-Heptacosanol	$C_{27}H_{56}O$	396.73
50	54.044	0.91	22,23-Dihydrobrassicasterol	$C_{28}H_{48}O$	400.69
51	54.657	1.55	Stigmasterol	$C_{28}H_{48}O$	412.69
53	56.243	5.08	Clionasterol	$C_{29}H_{50}O$	414.72
54	58.478	0.61	Betulinaldehyde	$C_{30}H_{48}O_2$	440.71
55	60.130	0.54	Dihydrodigoxigenin	$C_{23}H_{36}O_5$	392.54

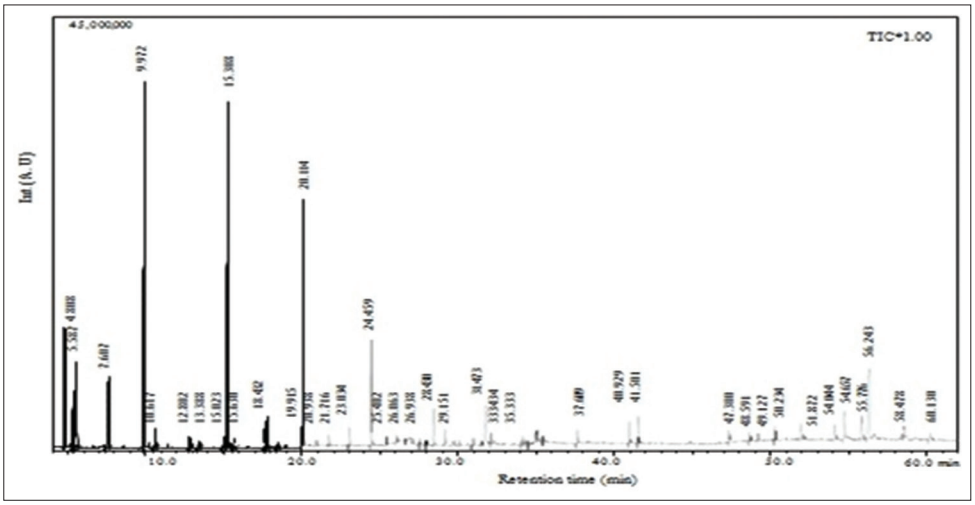

Figure 3: Gas chromatography–mass spectrometry chromatogram for the supercritical CO_2 extract of *Acacia nilotica* twig

SFE extracts of *E. cardamomum* seed pod obtained at 300 bar were the second most active extract with zone of inhibition in the range of 12.05–17.05 mm. The earlier study done on antimicrobial activity of cardamom has also been reported activity against *C. albicans*, *S. mutans,* and *S. aureus*.[29] GC–MS studies of green cardamom seed resulted in the identification of bioactive compounds with α-terpinyl acetate (38.4%), 1,8-cineole (28.71%), linalool acetate (8.42%), sabinene (5.21%), and linalool (3.97%).

P. guajava and *G. glabra* extracts obtained at 250 bar showed the highest growth inhibition, beyond that there was no effect of pressure on extract's activity. Therefore, in each case, best extraction was achieved when the polarities of the fluid and bioactive in the plant samples were coincident. *G. glabra* root showed a moderate activity (13.03–16.01 mm inhibition zone). *G. glabra* root possess (5–24%) glycyrrhizin, (3–16%) sugar, (30%) starch, and (6%) ash.[30] Earlier study showed that aqueous extract of *G. glabra* root can be effective for decreasing the severity of oral mucositis in head-and-neck cancer patients undergoing radiotherapy.[31] *P. guajava* leaves extract showed zone of inhibition in the range of 8.65–11.06 mm. The previous studies reported that methanol extract of *P. guajava* leaves exhibited significant activity against *S. mutans*.[32,33]

The methanol was used as solvent modifier in the SFE process which may increase the solvation power of SC-CO_2 and the recovery of bioactive compounds.[34] Supercritical carbon dioxide is intrinsically non-polar and addition of cosolvent in SFE makes it effective in the extraction of polar compounds embedded in the cell wall of plant samples.[35] An optimum temperature of 50°C was chosen for all extracts, as a very high temperature may lead to degradation of thermo-labile compounds.

GC-MS analysis phytochemicals with different retention times and peak areas are depicted in Figure 3 and Table 4. Bioactive such as betulinaldehyde,[36] stigmasterol,[37,38] eugenol,[39] α-terpinyl acetate,[40] and 22, 23-dihydrobrassicasterol[41] found in the extract, has been reported to exhibit antimicrobial activities against oral pathogens. Although few compounds such as 1,1'-sulfonylbis[2-(methylthio) ethane], glycolaldehyde dimethyl acetal, 1,4-dimethoxy-2,3-butandiol, fluoroacetic acid, and 3-ethoxy-1,2-propanediol present in the extract have been evidenced toxic, for human

consumption.[42,43] Therefore, further research is needed to isolate active compounds of the extract and to be assessed for their cytotoxic effects.

CONCLUSION

It can be concluded from the results that pressure plays an important role in SFE. Preparation of plant extracts using SFE will be beneficial if incorporated in oral care products as it is a clean and eco-friendly process. *A. nilotica* twig at 50°C and 400 bar showed significant antimicrobial potential and hence it can be processed to obtain effective and cheaper drug due to higher biomass availability. Chemical profiling of SFE extract by GC-MS analysis proved helpful in identification of compounds. Furthermore, bioactive compounds should be explicated for their exact mechanism of action with the target pathogens.

ACKNOWLEDGMENT

The research was financially supported by UGC under UGC-SAP program (F.3-20/2012, SAP-II). Rosy Kumari and Ratish Chandra Mishra gratefully acknowledged UGC New Delhi for the award of UGC BSR fellowship (F.25-1/2013-14).

REFERENCES

1. Esteban-Fernández A, Zorraquín-Peña I, Ferrer MD, Mira A, Bartolomé B, González De Llano D, *et al.* Inhibition of oral pathogens adhesion to human gingival fibroblasts by wine polyphenols alone and in combination with an oral probiotic. J Agric Food Chem 2018;66:2071-82.
2. Jain P, Ranjan M. Role of herbs in root canal irrigation-a review. IOSR J Pharm Biol Sci 2014;9:6-10.
3. Sakaue Y, Takenaka S, Ohsumi T, Domon H, Terao Y, Noiri Y. The effect of chlorhexidine on dental calculus formation: An *in vitro* study. BMC Oral Health 2018;18:52.
4. Raheel R, Aslam MS, Sghar S, Ashraf M. Phytochemical, ethnopharmacological review of *Acacia nilotica* (Desi Kikar) and taxo-pharmacology of genus *Acacia*. Indian Res J Pharm Sci 2014;1:65-72.
5. Joseph B, Priya M. Review on nutritional, medicinal and pharmacological properties of guava (*Psidium guajava* Linn.). Int J Pharm Bio Sci 2011;2:53-69.
6. Laily N, Kusumaningtyas RW, Sukarti I, Rini MR. The potency of guava *Psidium guajava* (L.) leaves as a functional immunostimulatory ingredient. Procedia Chem 2015;14:301-7.
7. Sultana S, Ali M. Analysis of volatile oil of the fruits of *Elettaria cardamomum* (L.) maton and its antimicrobial activity. World J Pharm Pharm Sci 2014;3:1798-808.
8. Vutakuri N, Somara S. Natural and herbal medicine for breast cancer using *Elettaria cardamomum* (L.) Maton. Int J Herb Med 2018;6:91-6.
9. Shah SL, Wahid F, Khan N, Farooq U, Shah AJ, Tareen S, *et al*. Inhibitory effects of *Glycyrrhiza glabra* and its major constituent glycyrrhizin on inflammation-associated corneal neovascularization. Evid Based Complement Altern Med 2018;2018:8438101.
10. Damle M. *Glycyrrhiza glabra* (Liquorice)-a potent medicinal herb. Int J Herb Med 2014;2:132-6.
11. Kapoor A, Kaur G, Kaur R. Antimicrobial activity of different herbal plants extracts: A review. World J Pharm Pharm Sci 2015;4:422-59.
12. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Altern Med 2009;2011:680354.
13. Azmir J, Zaidul IS, Rahman MM, Sharif KM, Mohamed A, Sahena F, *et al*. Techniques for extraction of bioactive compounds from plant materials: A review. J Food Eng 2013;117:426-36.
14. Vieitez I, Maceiras L, Jachmanian I, Albores S. Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology. J Supercrit Fluids 2018;133:58-64.
15. Gupta AM, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. Int J Appl Nat Sci 2012;1:8-26.
16. Handa SS, Khanuja SP, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. Trieste: The International Centre for Science and Technology, United Nations Industrial Development Organization; 2008.
17. Capuzzo A, Maffei ME, Occhipinti A. Supercritical fluid extraction of plant flavors and fragrances. Molecules 2013;18:7194-238.
18. Mushtaq M, Sultana B, Anwar F, Adnan A, Rizvi SS. Enzyme-assisted supercritical fluid extraction of phenolic antioxidants from pomegranate peel. J Supercrit Fluids 2015;104:122-31.
19. Radfar S, Ghoreishi SM. Experimental extraction of L-carnitine from oyster mushroom with supercritical carbon dioxide and methanol as co-solvent: Modeling and optimization. J Supercrit Fluids 2018;140:207-17.
20. Rasheed NM, Nagaiah K, Waheed MA. Recent analytical techniques in quality control of indigenous system of medicine. Ann Phytomed 2013;2:44-58.
21. Chauhan A, Goyal MK, Chauhan P. GC-MS technique and its analytical applications in science and technology. J Anal Bioanal Tech 2014;5:1-5.
22. Perez C, Pauli M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Bio Med Exp 1990;15:113-5.
23. Mishra RC, Kumari R, Yadav JP. Comparative study of antidandruff efficacy of *Punica granatum* peel and its biosynthesized silver nanoparticles. J Bionanosci 2018;12:508-14.

24. Kumari R, Mishra RC, Yadav A, Yadav JP. Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of *Acacia nilotica* extract. Indian J Tradit Knowl 2019;18:162-8.
25. Chatatikun M, Yamauchi T, Yamasaki K, Chiabchalard A, Aiba S. *Phyllanthus acidus* (L.) skeels and *Rhinacanthus nasutus* (L.) kurz leaf extracts suppress melanogenesis in normal human epidermal melanocytes and reconstitutive skin culture. Asian Pac J Trop Med 2019;12:98-105.
26. Sadiq MB, Tharaphan P, Chotivanich K, Tarning J, Anal AK. *In vitro* antioxidant and antimalarial activities of leaves, pods and bark extracts of *Acacia nilotica* (L.) del. BMC Complement Altern Med 2017;17:372.
27. Yadav A, Yadav M, Kumar S, Yadav JP. Bactericidal effect of *Acacia nilotica*: *In vitro* antibacterial and time kill kinetic studies. Int J Curr Res 2015;7:22289-94.
28. Pote M, Hirapure P. Antimicrobial potential of *Acacia nilotica* extracts on few dental pathogens. Int J Pharm Sci Res 2014;5:4756-9.
29. Asghar A, Butt MS, Shahid M, Huang Q. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*. J Food Sci Technol 2017;54:2306-15.
30. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, *et al*. Antimicrobial potential of *Glycyrrhiza glabra* roots. J Ethnopharmacol 2008;116:377-80.
31. Najafi S, Koujan SE, Manifar S, Kharazifard MJ, Kidi S, Hajheidary S. Preventive effect of *Glycyrrhiza glabra* extract on oral mucositis in patients under head and neck radiotherapy: A randomized clinical trial. J Dent (Tehran) 2017;14:267-74.
32. Shekar BC, Nagarajappa R, Jain R, Singh R, Thakur R, Shekar S. Antimicrobial efficacy of *Acacia nilotica*, *Murraya koenigii* (L.) sprengel, *Eucalyptus* hybrid, *Psidium guajava* extracts and their combination on *Streptococcus mutans* and *Lactobacillus acidophilus*. Dent Res J 2016;13:168-73.
33. Besra M, Kumar V. *In vitro* investigation of antimicrobial activities of ethnomedicinal plants against dental caries pathogens. 3 Biotech 2018;8:257.
34. Hrnčič MK, Cör D, Verboten MT, Knez Ž. Application of supercritical and subcritical fluids in food processing. Food Qual Saf 2018;2:59-67.
35. Khaw KY, Parat MO, Shaw, PN, Falconer JR. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. Molecules 2017;22:1186-208.
36. Chung PY, Chung LY, Navaratnam P. Potential targets by pentacyclic triterpenoids from *Callicarpa farinosa* against methicillin-resistant and sensitive *Staphylococcus aureus*. Fitoterapia 2014;94:48-54.
37. Yinusa I, George NI, Shuaibu UO, Ayo RG. Bioactivity of stigmasterol isolated from the aerial part of *Spillanthes acmella* (Murr) on selected microorganism. Int J Curr Microbiol Appl Sci 2014;3:475-9.
38. Correa G, Abreu VG, Martins D, Takahashi JA, Fontoura HS, Cara DC, *et al*. Anti-inflammatory and antimicrobial activities of steroids and triterpenes isolated from aerial parts of *Justicia acuminatissima* (*Acanthaceae*). Int J Pharm Pharm Sci 2014;6:75-81.
39. Marchese A, Barbieri R, Coppo E, Orhan IE, Daglia M, Nabavi SF, *et al*. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit Rev Microbiol 2017;43:668-89.
40. Cutillas AB, Carrasco A, Martinez-Gutierrez R, Tomas V, Tudela J. Composition and antioxidant, antienzymatic and antimicrobial activities of volatile molecules from Spanish *Salvia lavandulifolia* (Vahl) essential oils. Molecules 2017;22:1382.
41. Tanaka A, Shimizu K, Kondo R. Antibacterial compounds from shoot skins of moso bamboo (*Phyllostachys pubescens*). J Wood Sci 2013;59:155-9.
42. Xu H, Nie Z, Zhang Y, Li C, Yue L, Yang W, *et al*. Four sulfur mustard exposure cases: Overall analysis of four types of biomarkers in clinical samples provides positive implication for early diagnosis and treatment monitoring. Toxicol Rep 2014;1:533-43.
43. Leong LE, Khan S, Davis CK, Denman SE, McSweeney CS. Fluoroacetate in plants-a review of its distribution, toxicity to livestock and microbial detoxification. J Animal Sci Biotechnol 2017;8:55.

Source of Support: Nil. **Conflicts of Interest:** None declared.