IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)
An International Open Access, Peer-reviewed, Refereed Journal

EXTRACTION OF ESSENTIAL OIL FROM *FOENICULUM VULGARE* SEED AND *CHRYSOPOGON ZIZANIOIDES* ROOT AND ANALYSIS OF ITS PHYTOCHEMICAL PROPERTIES

Dr. Krishnaveni M[1],Srinivasan G[2], Tamilselvan.T[3]

[1]Assistant professor, Department of Biochemistry, Periyar University, Salem-636 011.

[2,3]M.Sc Student, Department of Biochemistry, Periyar University, Salem-636 011.

Abstract

Plant oils were used pharmacologically for many years. Recently, it has been generated widespread interest as a source of natural antimicrobials, anticancer agent. Considering the importance of essential oil natural resources *Foeniculum vulgare* seed and *Chrysopogon zizanioides* root were selected for the study.The yield obtained was found to be 1% for*Foeniculumvulgare* seeds and 0.75% for *Chrysopogon zizanioides* roots. The functional groups were assessed by FTIR. While, GC-MS showed highest peak area percent of 44.54 for Anethole from*Foeniculum vulgare* seed and Cinnamaldehyde with 8.18, tau-cadinol with 7.28 from *Chrysopogon zizanioides* root essential oil. The levels of secondary metabolites, phytonutrients, antioxidant activity was found to high in essential oil extracted from *Chrysopogon zizanioides* root when compared to *Foeniculum vulgare* seeds. No significant antibacterial activity was observed with both samples when tested against *Escherichia coli* MTCC 1692, *Klebsiella pneumonia* MTCC 7403,*Pseudomonas aeruginosa* MTCC 2581,*Staphylococcus aureus* MTCC 7443,*Salmonella enteric* MTCC 8587.Results of anticancer activity studied by MTT assay showed IC50 value of 169.3µg/ml for essential oil from *Foeniculum vulgare* seed. While, it was 178.2µg/ml for *Chrysopogon zizanioides* root.

Key words: Antibacterial, Anticancer, Antioxidant, Essential oil, FT IR, GC-MS.

1Introduction

Plants served as a limitless resource of food and feed for domesticated animals, humans, fibers for clothes, as well as medicines. Mankind were greatly gained by plants and its secondary metabolites. Among the several different plant products, essential oils deserve main focus. Essential oil is a complex mixtures of hydrocarbons, oxygenated hydrocarbons, consisting of monoterpenes, sesquiterpenesand find application in pharmaceutical, nutritional, cosmetic field, cancer or bacterial infectionstreatment (Freires IA and Denny C, 2015), (Russo R and S Corasaniti MT, 2015) and also find application in hand was preparationsto cope up with superficial skin infections(Mansi C and Rakesh KS, 2020), in dental caries prevention, in aromatherapy to treatment alzheimer, cardiovascular disorders. Considering the benefits of essential oil, in the present study we decided to extract essential oil from *Foeniculum vulgare* Mill seeds, *Chrysopogan zizanioides* (L.) Roberty roots, and analysed the functional groups present by FTIR, phyto-constituents present via GCMS, biochemical parameters like phytonutrient, secondary metabolites, antioxidant analysis as well as antibacterial activity against *Escherichia coli* MTCC 1692*, Klebsiella pneumonia* MTCC 7403,

Pseudomonas aeruginosa MTCC 2581, *Staphylococcus aureus* MTCC 7443, *Salmonella enteric* MTCC 8587 and anticancer activity against HSC-3 human oral squamous carcinoma cell line.

2 Materials and methods

2.1 Sample Collection

The dry *Foeniculum vulgare* seeds and *Chrysopogon zizanioides* roots were purchased at Salem, Tamil Nadu, Indiaand cleaned thoroughly. The samples used for the study was identified by Dr. A. Balasubramanian, Executive Director, ABS Herbal Garden, Salem and Former Siddha Research Consultant (AYUSH), Ministry of Health and Family Welfare. The authentication number for the seed of *Foeniculumvulgare* Mill was AUT/PU/166C and for *Chrysopogan zizanioides* (L.) Roberty AUT/PU/166D.

2.2 Essential Oil Extraction

Essential oil was extracted from 100gm of dried *Foeniculum vulgare* seeds and *Chrysopogon zizanioides* rootsvia hydro-distillation by using Clevenger's apparatus at 100°C for 2hrs (*Foeniculum vulgare* seeds) and 4hrs for (*Chrysopogon zizanioides* roots). The essential oil extracted was dried over anhydrous sodium sulphate, stored in a vial away from light at 4°C (FadilM and Farah Ihssane A, 2015) . The extracted essential oil was used for the biological analysis by dissolving in 90% ethanol (1:1 ratio). The essential oil yield obtained was 1% for *Foeniculum vulgare* seeds and 0.5% for *Chrysopogon zizanioides* roots. The yield of essential oil was calculated by formulae : Yield (%) = Amount of essential oil extracted / Amount of sample used (g) x 100

2.3 Fourier transform infrared analysis

Potassium bromide pellet method was used for the FTIR analysis (Frank AS, 1997). The spectrum were recorded using Bruker Tensor 27 spectrometer.

2.4 Gas Chromatography-Mass Spectrophotometry

Chromatogram of GC-MS was obtained using Scion 436-GC Bruker-Triple quadruple mass spectrophotometer (IIFPT,Thanjavur) with fused silica capillary column BR-5MS (5% Diphenyl95% Dimethyl poly siloxane),30m x 0.25mmID x 0.25m df(Franelyne PC and Agnes LC, 2016) .

2.5 Determination of Total phenol

Folin-ciocalteau method was used(Kaur C and Kapoor HC, 2002) .To 0.1ml essential oil, added Folinciocalteau reagent (5 ml, 1:10 dilution), incubated for 5min.,added $NaCo_3$ (4ml, 1M) again incubated at room temperature for 15min., read at 765nm and phenol served as a standard, expressed in terms of Gallic acid equivalent (mg/g).

2.6 Estimation of flavonoids

Total flavonoid was analysed by Aluminium chloride method(Chang CC and Yang MH, 2002) . To 0.1ml essential oil, added $AlCl_3$ (0.1ml, 10%) mixed well. After 30 minutes, the absorbance was measured at 415nm. Quercetin served as a standard, expressed the results as mg quercetin equivalent/g.

2.7 Nitric oxide scavenging activity

To 0.1ml essential oil, added sodium nitroprusside (10mM) in phosphate buffered saline. After incubating at room temperature for 150min added 0.5ml Griess reagent, measured the absorbance at 546nm. Positive control used was Quercetin(Lee HS, 1992) (Chakraborthy GS, 2009) .

2.8 Reducing power assay

To 0.1ml essential oil, added phosphate buffer (2.5ml, 0.2M, P^H 6.6), potassium ferricyanide (2.5ml, 1%), kept at 50°c for 20min.,addition of 1.0 ml trichloro acetic acid (10%) stops the reaction, centrifuged at 3000rpm (10min.).To the supernatant added equal volume of distilled water, $FeCl_3$ (0.1ml, 0.1%), mixed well, after 10min. measured the absorbance at 700nm. Vitamin C acted as a reference(Oyaizu M, 1986).

2.9 Total antioxidant activity

Total antioxidant activity was assessed by Phospho-molybdenum method(Prieto P and Pineda M, 1999).

3.0 Metal chelating activity

To 0.1ml essential oil, added 0.05ml 2mM $FeCl_2$. The reaction was initiated with 160µl(5mM) Ferrozine.Measured the absorbance at 562nm after 10min incubation(Dinis, TCP and Madeira VMC, 1994).

3.1 Estimation of carbohydrate

The carbohydrate was estimated by Anthrone method(Hedge JE and Hofrelter BT, 1962) . To 0.1ml essential oil, added 4ml anthrone reagent, kept in the water bath for 8min., cooled, read at 630nm. The standards were developedwithglucose.

3.2 Estimation of protein

The total protein was estimated by Lowry's method(Lowry OH and Rosebrough NJ, 1951). To 0.1ml essential oil, added 2ml alkaline copper reagent, mixed well, after 10min. incubatiuon added 0.2ml Folinciocalteau reagent (1: 2), kept in dark for 30min., read at 660nm. Bovine serum albumin served as a standard.

3.3 Estimation of aminoacid

The amino acid was assessed via Ninhydrin method(Yemm EW and Cocking EC, 1955). The readings were taken using UV spectrophotometer Schimadzu Model 1800. Each experiments were done six times. The Mean/Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$.

3.4 Antibacterial activity - Disc Diffusion Assay

Foeniculum vulgare seed and *Chrysopogon zizanioides* root essential oil was tested against *Escherichia coli* MTCC 1692, *Klebsiella pneumonia* MTCC 7403, *Pseudomonas aeruginosa* MTCC 2581, *Staphylococcus aureus* MTCC 7443, *Salmonella enteric* MTCC 8587 bydisc diffusion method.Agar plates were inoculated with 0.1% inoculum suspension, solidified. In the Kirby-Bauer test, small filter disks soaked with essential oil 5µl,10µl,15µl and chloramphenicol 30µg/disc (standard antibiotic) was kept on the surface of the agar allowed to diffuse,incubated at 37°C for zone of inhibition formation, which was measured in millimeter(Bauer AW and Kirby WMM, 1966).

3.5 Anticancer activity

The extracted essential oil from *Foeniculum vulgare* seed and *Chrysopogon zizanioides* root was tested for in vitro cytotoxicity against HSC-3 cells by MTT assay(Mosmann T, 1983).The cultured HSC-3 cells were harvested by trypsinization, pooled in a 15ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml/well into the 96 well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48h at 37°C. The wells were washed with sterile PBS, treated with various concentrations of the extracted essential oil, and incubated at 37°C in a humidified 5% CO_2 incubator for 24h. After the incubation period, MTT (20 µL, 5mg/ml) was added into each well and the cells were incubated for another 2-4h until purple precipitates were clearly visible under an inverted microscope. Each well were aspirated, washed with 1X PBS. Later, the formazan crystals formed were dissolved in DMSO and the absorbance was measured at 570nm using a microplate reader (Thermo Fisher Scientific, USA) and the cell viability (%) using Graph Pad Prism 6.0 software (USA).

4 Results and Discussion

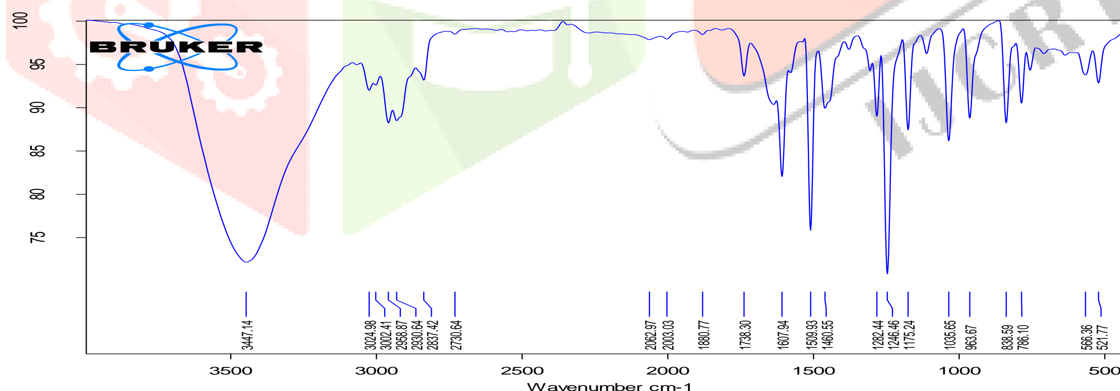

Fig.1 FTIR spectrum of *Foeniculum vulgare* seed

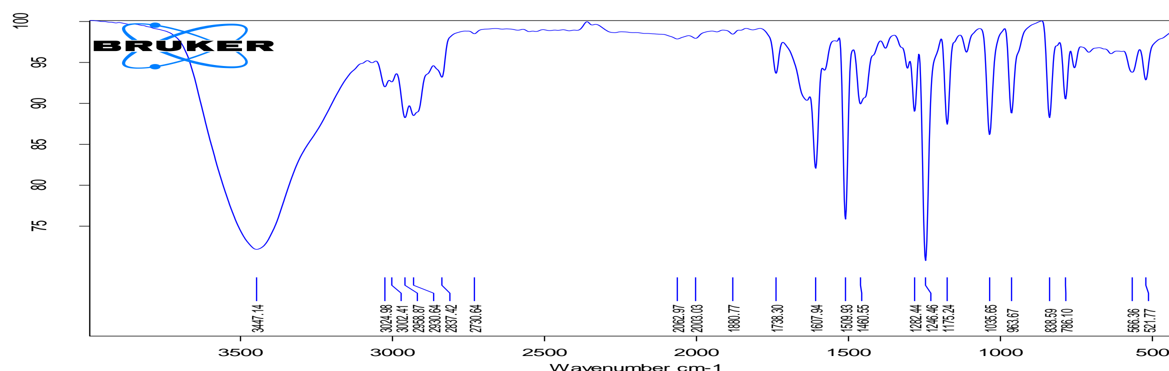

Fig.2 FTIR spectrum of *Chrysopogon zizanioides* root

Figure.1,2 showed the spectrum obtained for *Foeniculum vulgare* seed, *Chrysopogon zizanioides* root. Infrared absorption below 1000cm^{-1}are due to C-H bending vibrations, the absorptions at 997 to 1130cm^{-1} range shows stretching vibrations of C-O (monosaccharides, oligosaccharides), while the absorption at 1150- 1270cm^{-1}represents carbonyl C-O, or O-H bending. Likewise, absorptions at region 1300 – 1450cm^{-1}demonstrates stretching vibrations of C-O amide, C-C stretching from phenyl groups.Absorption within 1500-1600cm-1 region represent aromatic domain, N-H bending vibration. The absorptions from 1600-1760cm^{-1}symbolize the bending vibrations of N-H amino acids, C=Ostretching induced by aldehyde, ketone, esters. The absorptions within 2800-2900 cm^{-1} are mainly due to C-H stretching specific to CH_3, CH_2 from lipids.methoxy derivatives, C–H aldehyde, cis double bonds. Absorption at 3350 signifies vibrations of the OH group from water, alcohols, phenols, amides, aldehyde, ketone, esters.

Table. 1GC-MS of *Foeniculum vulgare* seed essential oil

No	RT (min)	Name of the compound	Molecular formula	Molecular Weight	Peak Area %
1	3.03	Limonene	C10H16	136	0.70
2	3.29	ç-Terpinene	C10H16	136	0.55
3	3.80	Fenchone	C10H16O	152	12.30
4	4.69	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	C10H16O	152	0.61
5	5.50	Estragole	C10H12O	148	35.74
6	6.15	Fenchyl acetate	C12H20O2	196	0.41
7	6.48	Anisaldehyde	C8H8O2	136	4.01
8	7.07	Anethole	C10H12O	148	44.54
9	8.54	2-Propanone,(p-methoxyphenyl)-	C10H12O2	164	0.36
10	9.52	1-Propanone, 1-(4-methoxyphenyl)-	C10H12O2	164	0.21
11	11.12	3-Methoxycinnamaldehyde	C10H10O2	162	0.11
12	11.83	Apiol	C12H14O4	222	0.46

Table.1 shows the results of GC-MS of *Foeniculum vulgare* seed essential oil. The peak area observed was high for Anethole , Estragole, Fenchone. All other compounds were found to be very less.

Table. 2 GC-MS of *Chrysopogon zizanioides* root essential oil

	RT(min)	Nameofthecompound	Molecular Formula	Molecular Weight	PeakArea%
1	3.74	Fenchone	C10H16O	152	0.06
2	5.45	Estragole	C10H12O	148	0.28
3	6.83	Cinnamaldehyde,(E)-	C9H8O	132	8.18
4	6.97	Anethole	C10H12O	148	0.72
5	8.41	Biphenylene,1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	C14H2O	188	0.21
6	9.40	Aceticacid, cinnamylester	C11H12O2	176	0.60
7	10.08	á-Guaiene	C15H24	204	0.22
8	10.46	Ylangene	C15H24	204	0.35
9	10.64	(Z)-2-Methoxycinnamaldehyde	C10H10O2	162	0.46
10	11.15	2Naphthalenemet hanol,decahydroà, à,4atrimethyl-8-methylene-,[2R	C15H26O	222	3.43

		(2à,4aà,8aá)]-			
11	11.54	á-Acorenol	C15H26O	222	0.64
12	11.70	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3methylenetricyclo[4.4.0.02,7]decanere-	C15H24	204	2.16
13	11.83	4a(2H)-Naphthalenol,1,3,4,5,6,8a hexahydro-4,7-diMethyl-1-(1-methylethyl)-,(1S,4R,4aS,8aR)-	C15H26O	222	0.60
14	11.91	Selin-6-en-4à-ol	C15H26O	222	2.77
15	12.04	ç-Himachalene	C15H24	204	1.37
16	12.32	tau.-Cadinol	C15H26O	222	7.28
17	12.46	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)prop-2-en-1-ol	C15H24O	220	1.21
18	12.60	(2R,3R,3aR,6R,8aS)-3,7,7-Trimethyl-methyleneoctahydro-1H-3a,6-methanoazulen-2-ol	C15H24O	220	1.46
19	12.76	ç-Gurjunenepoxide-(2)	C15H24O	220	1.00

Table.2 shows the results of GC-MS analysis of *Chrysopogon zizanioides* root essential oil. The highest peak observed was with Cinnamaldehyde,(E)- , tau.-cadinol all the other peaks were found to be very low.

Table. 3 Biochemical parameters studied in essential oil extracted from *Foeniculum vulgare* seed and *Chrysopogon zizanioides* root

Parameters	*Foeniculum vulgare* seed (mg/g)	*Chrysopogon zizanioides* root (mg/g)
Secondary metabolites		
Total phenolics	0.10±0.01	0.27±0.17
Total flavonoid	0.27±0.17	0.38±0.09
Antioxidant activity		
Phosphomolybdenum	0.19±0.06	0.36±0.12
Nitric oxide scavenging	0.18±0.02	0.89±0.58
Reducing power	0.18±0.02	1.21±0.22
Metal chelating	0.14±0.02	0.31±0.04
Phytonutrient assay		
Carbohydrate	0.04±0.00	0.23±0.02
Protein	0.11±0.02	1.07±0.09
Aminoacids	0.04±0.00	0.11±0.00

The results of secondary metabolites, antioxidant activity, nutrient content was found to be high with *Chrysopogon zizanioides* root essential oil when compared to essential oil from *Foeniculum vulgare* seed. (Table.3) Presence of terpene, phenol, other volatile compounds impart natural antioxidant property(Fellah S and Diouf PN, 2006) that could neutralize free radical induced stress preventing diseases(Halliwell B, 1996). Antioxidant activity was low with *Foeniculum vulgare* seed essential oil but when tested against HSC 3 cancer cells , it was found to be effective.

4.1 Antibacterial activity of essential oil from*Foeniculumvulgare* seed and *Chrysopogonzizanioides*root

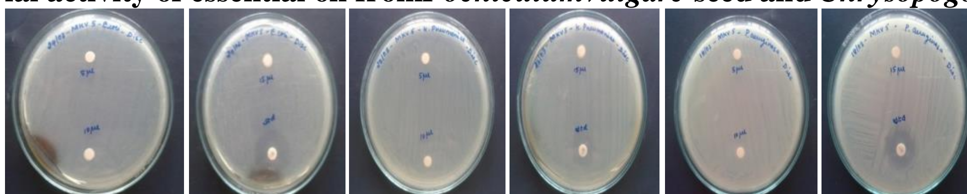

Fig.3A

Fig. 3B

Fig.3A and 3B Antibacterial activity of *Foeniculumvulgare* seed essential oil

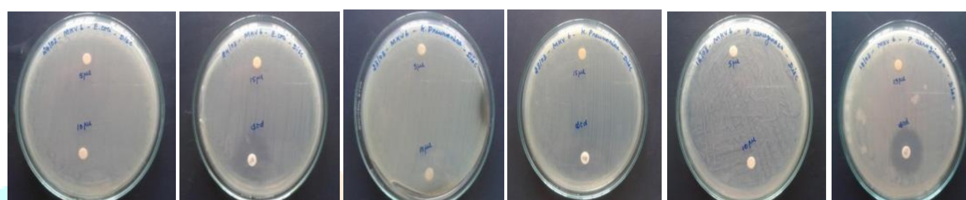

Fig.3C

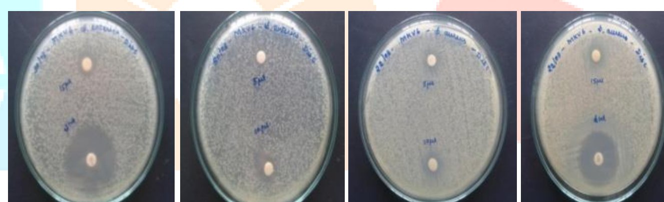

Fig.3D

Fig.3C and 3D Antibacterial activity of *Chrysopogon zizanioides* root essential oil

The antibacterial activity was tested against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enteric*for the *Foeniculum vulgare* seed essential oil. No antibacterial activity was observed with *Salmonella enteric, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,Klebsiella pneumonia* at studied concentration 5, 10, 15µl when compared with standard.(Figure.3A and B) Likewise, the antibacterial activity of *Chrysopogon zizanioides* root essential oil was very low with *S. enteric*, *S.aureus* but no antibacterial activity was observed with *E.coli, K.pneumonia, P.aeruginosa* at selected concentration when compared to standard chloramphenicol. (Figure.3C and D) Theobserved lowantimicrobial activity of essential oil might be mainly due to the disruption of bacterial cell wall leading to the membrane lysis, leakage of intracellular contents causing cell lysis. The results of our study were found to be contrary when compared with available literature for essential oil extracted from other natural sources.

4.2 Methyl ThiazolTetrazolium (MTT) assay using HSC-3 cellswith essential oil extracted from *Foeniculum vulgare* seeds and *Chrysopogon zizanioides* root

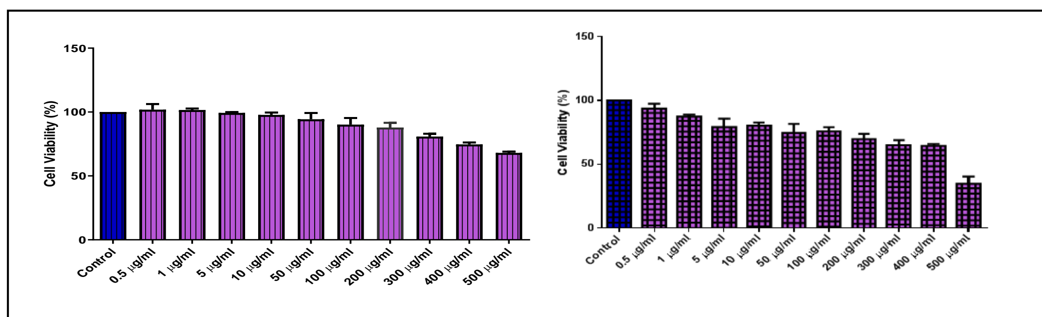

Fig.4A *Foeniculum vulgare* seed **Fig.4B *Chrysopogon zizanioides* root**

MTT assay was performed to study the dehydrogenase activity in mitochondria to assess the cell viability of human oral squamous carcinoma cells(Fig.4A &4B). The observed IC 50 value was found to be 169.3µg/ml for essential oil extracted from *Foeniculum vulgare* seeds. While, for *Chrysopogon zizanioides* root essential oil it was 178.2 µg/ml. Essential oil is important in most of the pharmaceutical applications.As per literature Wintergreen and Rosemary essential oil were found to be positive when tested against *Streptococcus mutans* which causes dental caries, hence we tried testing our essential oil samples against human oral squamous carcinoma cells - HSC-3 and the results were found to be positive. The secondary metabolites and active constituents of essential oil help in reducing the cancer.

5 Conclusion

From the obtained results, we would like to conclude that among the extracted essential oil from *Foeniculum vulgare* seed and *Chrysopogon zizanioides* root, essential oil from *Chrysopogon zizanioides* root possess significant antioxidant, anticancer activity.

6 Acknowledgment

The author wishes to acknowledg former Vice Chancellor, Periyar University, Prof. Dr. T. Balakrishnan and also thank Indian Institute of Food Processing Technology (IIFPT), Thanjavur and also family.

References

1.Freires, IA, Denny C, Benso B, de Alencar, SM andRosalen, PL. 2015.Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: A systematic review. Molecules.20(4):7329–58.

2. Russo, R, Corasaniti,MT, Bagetta, G andMorrone, LA. 2015.Exploitation of cytotoxicity of some essential oils for translation in cancer therapy. Evid.Based Complement.Alternat.Med.397821.

3.Mansi, C, Rakesh, KS, Inderbir, S, Deepak K, Inderjit, SS andSandeep A. 2020.Formulation and Evaluation of Essential Oils based Liquid Herbal Hand Wash. Research J. Pharm. and Tech. 13(4):1917-20.

4.Fadil, M, Farah,IhssaneA, Haloui, T andRachiq, S. 2015. Optimization of parameters influencingthehydrodistillation of *Rosmarinusofficinalis* L. by response surface methodology. Journal of Materials and Environmental Science.6(8): 2328–36.

5.Frank, AS. 1997.Infrared Spectroscopy. In: Handbook of Instrumental Techniques For Analytical Chemistry, 1st ed.; Sherman, H.; Prentice Hall PTR, New Jersey, 247-283.

6.Franelyne,PC,Agnes, LC andMaryJho-Anne, TC. 2016. GC–MS analysis of bioactive compounds present in different extracts of an endemic plant *Broussonetialuzonica* (Blanco) (Moraceae) leaves.Asian Pacific Journal of Tropical Biomedicine.6(11): 957-961.

7.Kaur, C andKapoor, HC. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol.37(2): 153-161.

8.Chang, CC, Yang, MH, Wen, HM andChern, JC.2002.Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis. 10(3): 178-182.

9.Lee, HS. 1992.Antioxidant activity of browning reaction product isolated from storage-aged orange juice. J Agric Food Chem.40(4):550–2.

10.Chakraborthy, GS. 2009.Free radical scavenging activity of *Costusspeciosus* leaves. Indian J.Parm. Educ. Res.43(1):96–8.

11.Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucose amine. Jpn. J Nutr.44:307-l5.

12.Prieto, P, Pineda, M and Aguilar,M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal.Biochem.269(2):337–41.

13.Dinis, TCP, Madeira, VMC and Almeida, MLM. 1994.Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315(1): 161-69.

14.Hedge, JE andHofrelter, BT. 1962.In: Carbohydrate chemistry. 17 Eds.Whistler RL, Be Miller JN, Academic press, NewYork.

15.Lowry, OH, Rosebrough, NJ, Farr, AL and Randall, RJ. 1951.Protein measurement with folin phenol Reagent.J. Biol. Chem. 193(1): 265-75.

16.Yemm,EW,Cocking,EC and Ricketts,RE. 1955.The determination of amino acids with ninhydrin.Analyst.80(948):209-14.

17.Bauer, AW, Kirby, WMM, Sherris, JC andTurck,M. 1966.Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol.45(4):493- 96.
18.Mosmann, T. 1983.Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J.Immunol.Methods.65(1-2): 55–63.
19.Fellah, S, Diouf, PN, Petrissans, M, Perrin, D, Romdhane, M andAbderrabba,M.2006.Chemical composition and antioxidant properties of *Salvia officinalis* L. oil from two culture sites in Tunisia. Journal of Essential Oil Research, 18(5): 553-56.
20.Halliwell, B. 1996. Antioxidants in human health and disease.Annual Review of Nutrition.16: 33-50.