Chemical Composition and Antimicrobial Activity in Cold Press Oil of Fennel, Anise, White and Black Mustard Seeds

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ABSTRACT

In this study, the cold press oil components and antimicrobial activities of fennel (Foeniculum vulgare) and anise (Pimpinella anisum) and white mustard (Sinapis alba) and black mustard (Brassica nigra) species seeds, which are widely used by the people for alternative medicine, were determined. F. vulgare, P. anisum, S. alba and B. nigra species seeds were obtained from cultivated areas in central Anatolia in Turkey. The oil was extracted by using a screw press (MP-001 Cold Press, Turkey), and the volatile oil components and fatty acid components in these oils were analysed by GCMS and total phenolic content, total flavonoid content and antioxidant activities by DPPH and FRAP (%) method were determined. Antimicrobial activities of obtained oils were investigated by using minimum inhibitory concentration (MIC) test by against 18 different species microorganisms. In the GCMS results, F. vulgare and P. anisum oils were found to be the most abundant components which were anethole (89.74%, 88.95%, respectively). According to these results, the plants oils didn't show any antimicrobial activities against tested microorganisms. However especially white and black mustard oils showed strong antioxidant activity when compared with artificial antioxidants.

Keywords: Cold Press Oils, Gs-Mc, Antioxidant Activity and Antimicrobial Activities.

INTRODUCTION

The medicinal using of non-wood forest products have become widespread with the development of alternative medicine as complementary to modern medicine. These plants can be easily purchasable as a product of volatile oils, cold press oils, solvents extracts, or dry tea. However, it is a fact that their components adversely affecting people during the use of these products depending on their amounts.^{1,2} On the other hand, if these products, do not show antibacterial properties, are kept for a long time by the end user, they can cause excessive damage due to the contamination of harmful bacteria. Therefore, it is important to determine the contents of these products and know their properties for standardization.²

Foeniculum vulgare (fennel) and Pimpinella anisum (anise) are famous and common used plants having the carminative effect.³ 45 Also, Sinapis alba (white mustard) and Brassica nigra (black mustard) are used as a spice in cooking and are used for diuretic, appetizing, and atrit in alternative medicine.³

In this study, oils of *F. vulgare*, *P. anisum*, *S. alba* and *B. nigra* seeds were obtained by using cold-press machine. The obtained oils were chemically analysed with GC-MS and tested against 18 microorganism by the Minimum Inhibitory Concentration (MIC) test. Furthermore, it was determined antioxidant activity, total phenolic and total flavonoid contents.

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MATERIALS AND METHODS

Plant Samples and Oil Extraction

F. vulgare, P. anisum, S. alba and B. nigra seeds were obtained from cultivated areas in central Anatolia in Turkey. The cold-press oil of seeds was extracted by using a screw press (MP-001 Cold Press, Turkey).⁴

Determination of Antimicrobial Activities

Antimicrobial activities were determined by MIC test with a slight modification against the 18 different microorganism.⁶ Oils were sterilized by filtration using $0.45~\mu m$ filters (Millipore). Two-fold dilutions of the oils were prepared ranging from $100~\mu g/mL$ to $0.195~\mu g/mL$ by using 96-well micro-titration plate.

GC-MS Analysis

Samples was analysed by GCMS QP 2010 Ultra (Shimadzu) equipped with Rtx-5MS capillary column (30m·0.25 mm; coating thickness 0.25 μ m). For the identification of chemical components, both fatty acid and essential oil Wiley Data library were used. Experimental conditions were applied according to Wiley Data library matching requirements.

Determination of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activities

Total Phenolic Content and Total Flavonoid Components were determined according to literature studies with slight modifications. ^{5-7,8} The total phenolic contents

were calculated as micrograms of gallic acid equivalent (GAE) obtained from standard gallic acid graph ($R^2=0.9965$) and the total flavonoid contents were calculated as micrograms of catechin equivalent obtained from standard catechin graph ($R^2=0.9965$). Antioxidant activities of cold press oils were determined by FRAP (%,) and DPPH (%) methods. For this purpose, 10% oil- methanol solution were prepared and tested according to literature studies with slight modifications. $^{5-9,10}$

RESULTS AND DISCUSSION

The components, which observed more than 3 percent, were accepted as the main components in the oils. GC-MS results were scanned in both the essential oil and fatty acid database, and their main components are shown in Table 1 and 2. The MIC test results of oils are given in Table 3. The most abundant component of the fennel and anise oils to according to essential oil database is similar comparison with previous studies. 11,12 It was seen that methyl linoleate is also a common component of the four cold-press oils according to results of fatty acid database scanning. However, it was found that essential oils of these species had antimicrobial activity in the previous studies, but any antimicrobial activities weren't observed in cold press oils in this study. 11,12,13,14.

Table 1: Main components in essential oil scanning										
White Mustard	%	Black Mustard	%	Anise	%	Fennel	%			
Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methyl]-, methyl ester	48.7	Di-(9-Octadecenoyl)- Glycerol	42.16	Anethole	89.74	Anethole	88.95			
Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	42. 08	9,12-octadecadienoyl chloride, (Z,Z)-	41.4	Butanoic acid, 2-methyl-, 2-methoxy-4-(2- propenyl)phenyl ester	3.19	D-Limonene	3.41			
		Hexadecanoic acid, 1-(hydroxymethyl)-1,2- ethanediyl ester	13.2							

Table 2: Main components in fatty acid scanning										
W. Mustard	%	B. Mustard	%	Anise	%	Fennel	%			
Methyl linoleate	68.19	Methyl erucate	38.23	Methyl elaidate	60.44	Methyl elaidate	66.44			
Methyl oleate	15.79	8,11,14-Docosatrienoic acid, methyl ester	23.72	Methyl linoleate	20.01	Anethole	14.18			
Hexadecanoic acid, methyl ester	10.51	11-Eicosenoic acid, methyl ester	15.82	Anethole	10.84	Methyl linoleate	10.28			
		Methyl linoleate	10.13	Hexadecanoic acid, methyl ester	3.7	Hexadecanoic acid, methyl ester	3.88			

Table 3: MIC values for cold press oil samples																		
	B. subtilis DSMZ 1971	C. albicans DSMZ 1386	E. faecalis ATCC 29212	E. aerogenes ATCC 13048	E. durans*	E. faecium*	E. coli ATCC 25922	K. pneumonia*	L. monocytogenes*	L. innocua*	P. aeruginosa DSMZ 50071	P. fluorescens P1	S. enteritidis ATCC 13075	S. infantis*	S. kentucky*	S. typhimurium SL 1344	S. aureus ATCC 25923	S. epidermidis DSMZ 20044
Anise	-	-	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-
Fennel	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W.Mustard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. Mustard	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[&]quot;-": No inhibition observed for the volumes of oils tested

^{*} Isolated from food samples and identified at the Department of Biology, Faculty of Science, and Ankara University.

Table 4: Total Phenolic Contents and Total Flavonoid Contents									
GAE/ML	W. Mustard	B. Mustard	Anise	Fennel					
Total Phenolic	167,78	142,86	480,62	333,21					
Total Flavonoid	12,09	23,43	16,47	17,8					

Table 5: Antioxidant activities of cold pressed oils											
Method	B. Mustard	W. Mustard	Fennel	Anise	ВНА	внт	TOC				
Frap (%)	23,85	8,92	0,64	3,12	10,66	2,51	2,76				
DPPH (%)	89,25	94,24	39,64	45,34	94,17	88,73	77,46				

The total phenolic contents and total flavonoid contents of cold-press oils are given in Table 4. Besides, anti-oxidant activities results of cold-press oils are given in Table 5. The highest total phenolic content was found in anise oil, and also, the maximum total flavonoid amount was found in black mustard oil. On the other hand, black and white mustard oils exhibited very strong anti-oxidant activities in comparison to artificial antioxidant while anise and fennel showed low antioxidant activities. Ghanem *et al.* reported that different solvent extractives of fennel showed high antioxidant activities.⁵ For this reason, it will be said that non-antioxidant materials are obtained in cold press oil extraction process.

CONCLUSION

It can be clearly said that *F. vulgare* and *P. anisum* have significant potential as raw material to obtain high quality anethole for pharmaceutical industry. Fennel, anise, white and black mustard cold press oils have rather high total phenolic content, and also, white and black mustard cold press oils have strong antioxidant activities. Besides,

it may be recommended that these cold press oils are used with the purpose of increasing the shelf-stable of food and medicines due to their preservative properties against microorganisms.

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CONFLICT OF INTEREST

None

ABBREVIATION USED

GC-MS: Gas chromatography-mass spectrometry; W. Mustard: White Mustard; B. Mustard: Black Mustard; DSMZ: German Collection of Microorganisms and Cell Cultures; ATCC: American Type Culture Collection; ID: Identity; MIC: Minimum Inhibitory Concentration; GAE/ML. gallic acid equivalence/milliliters;

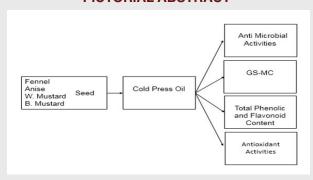
FRAP: The Ferric Reducing Ability of Plasma; DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

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PICTORIAL ABSTRACT



SUMMARY

- Antimicrobial activities of the fennel, anise, white and black mustard cold pressed oils were investigated by using MIC test by against 18 different species microorganisms.
- The chemical components of the cold pressed oils were determined with GC-MS.
- Total phenolic, total flavonoid and anti-oxidant activities of oil samples were investigated.

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