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Research Article

GC-MS ANALYSIS OF PHYTOCOMPONENTS AND TOTAL ANTIOXIDANT ACTIVITY OF HEXANE EXTRACT OF SINAPIS ALBA

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ABSTRACT

To characterize the phytochemical constituents from Sinapis alba by using GC-MS method. Ten grams of the powdered sample mixed with 40ml of n-Hexane. This fraction was taken for GC-MS analysis. The analysis was carried out on a GC clarus 500GC system with a column packed with Elite-1(10% dimethyl poly siloxane, 30x0.25mm ID x 1EMdf) the compounds are separated using with Helium as carrier gas at a constant flow (1ml/min).Sample extract (2HL) injected into the instrument was detected by Turbo gold mass detector (Perkin Elmer) with the aid of Turbo mass 5.1 software. The GC-MS analysis provided peaks of different phytochemical compounds namely 1, 3, 5- Cyclo-heptatriene, octane, Hexanal, Heptanal, Furan, 2-pentyl octanal, Nonanal, Decanal, 4- Undecene,2-Tridecenal,2,4-Decadienal,2-Undecenal,2-Pentene,1-ethoxy-4,4-dimethyl,7-Tetradecene,Diethylphthalate,3-octadecenePentadecene,3-Hexadecene,3,7,11,15-Tetramethyl-2-hexadecen-1-ol,n-Butyl myristate,Sulfurous acid,2-prophl tride cylester,1-Hexadecanol,9,12-octadecadicn-1-ol,1-Hexadecanol,9,12-octadecadicn-1-ol,Hexadecanoic acid,butylester,Ethanone,2-cyclohexyl-1-(1H-imidazol-2-yl).Total Antioxidant activity was evaluated by Phosphomolybdenum assay method.

Keywords: Ascorbic acid equivalent antioxidant capacity; GC-MS, Optical density.

INTRODUCTION

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and Fruits are considered as one of the main sources of biologically active compounds. As estimate of the World Health Organization (WHO) states the around 85-90% of the World's population consumes traditional herbal medicines[1]. The mustard plant is native to Europe and Asia. Black mustard seeds are usually grown in Canada, GreatBritian, Hungary, United States, Argentina, India and Pakistan. This strong herb belongs to the Cruciferae family[2,3]. Mustard has stimulant, irritant, antibacterial, anti-fungal, anti-inflammatory, diaphoretic, emetic and aphrodisiac properties[4].

The herb (mustard oil) contains isothiocyanates known for their anti-cancer quality, especially for gastrointestinal and colorectal cancer[5]. Moreover, mustard oil works as an appetizer as it stimulates digestive juices.Besides; regular consumption of mustard seeds reduces the frequency of migraines. Mustard benefits in the treatment of cough, cold, flu and so on[6]. Furthermore, this herb lowers blood pressure, relieves the symptoms of rheumatoid arthritis, reduces the severity of asthma, stimulates digestion and helps in the treatment of certain skin diseases. It is used for hair growth too[7]. Plus, it helps prevent atherosclerosis and diabetic heart disease. Due to its purgative quality, mustard can be used to get rid of accidental poisoning by having water mixed with powdered black mustard seeds[8].

In terms of nutritional benefits of mustard, it is rich in vitamins, minerals, antioxidants and phytonutrients. This spice contains magnesium, selenium, iron, calcium, manganese, potassium, phosphorus, vitamin B complex, etc.[9,10]. It is worth nothing that the hot taste of this spice is released when it is crushed and mixed with

water, whole mustard seeds have no aroma. This herb can be used both internally as well as externally[11]. With this background, the present investigation to identify phytoconstituents and antioxidant activity of hexane extract of sinapis alba seeds.

MATERIALS AND METHODS

Plant Material

Seeds were collected form Thanjavur District of Tamilnadu. The botanical identity of the plant of was confirmed by Dr. JohnBrito, Rapinet Herbarium. St. Joseph's College, Thiruchirappalli.

Preparation of Extract

The powder (1kg) was extracted with Hexane at room temperature for 48h. The extracts were filtered and concentrated under reduced pressure in a rotary evaporator. The extracts were subjected to GC-MS analysis and Total Antioxidant activity.

GC-MS ANALYSIS

Instrument Details

Make: PerkinElmerClarus 500
Software: Turbo mass ver5.2.0
Column Type: Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane)
Column length: 30m
Column id: 250µm

GC Conditions

Oven Program: 50ºc@7ºc/min to 220ºc(2min)@7ºc/min to 270ºc(10min)
Injector temp : 270ºc
Carrier gas : Helium @ flow rate 1ml/min
Split ratio : 1:20

MS Conditions

Mass Range: 40-600amu
Type of Ionization: Electron Ionization (EI)
Electron Energy: 70ev
Transfer line and source temperature: 200ºc, 180ºc
Library : NIST 2005
Sample Injected: 2.0 microlitres.

Total Antioxidant

Phosphomolybdenum assay method

The total antioxidant of the extracts was assessed by the phosphomolybdenum reduction assay. The reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (600 mM) mixed with the extracts diluted in methanol at the concentrations of 5, 10, 25, 50, 100 µg/mL. The samples were incubated for 60 min at 90°C and the absorbance of the green phosphomolybdenum complex was measured at 695 nm. For reference, the appropriate solution of ascorbic acid was used and the reducing capacity of the extracts was expressed as the ascorbic acid equivalents[12,13].

Calculation

(%) inhibition = Absorbance of control – Absorbance of sample/ Absorbance of control ×100.

RESULTS AND DISCUSSION

GC-MS STUDY

The GC-MS study of Sinapis alba has shown many phytocheminals which contributes to the medicinal activity of the plant (Table 1 & 2). The major components which present Hexadecanoic acid, octadedanoicacid. The other compounds like 2-undecenal, 9, 12-octadecadien-1-oe, 1, 3, 5-cycloheptatriene, octane, Hexanal present in the Sinapisalba. Table shows mass spectrum and structure of Hexadecanoic acid 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol which is suggested acid ester and Fatty ester compound and is used as an anticancer, anti-inflammatory, antioxidant and antimicrobial.

Total Antioxidant Activity

The results obtained from the total antioxidant activity of sinapis alba have shown in Table -3. The antioxidant activity showed the inhibition percentage is 43 % and 65% respectively. The results observed from hexane extract of sinapis alba shows higher antioxidant potential. The results were compared to the standard ascorpic acid, it was only slight difference has been noted. The WHO estimated that 80% of the population of developing countries still relies on traditional medicine, mostly plant drugs for their primary health care needs. Hence, there is an urgent need to study the screening of antioxidant properties of herbs which will be helpful in the treatment of several diseases [12]. Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidants may be synthetic or natural. Synthetic antioxidants such as BHT and BHA have recently been reported to be dangerous for human health. Thus, the search for effective, non-toxic natural compound with antioxidative activity has been intensified in recent years[13]. On the basis of our results, sinapis alba seeds appears to have potential for treatment of oxidative stress related diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies in vivo.

CONCLUSION

The GC-MS study showed many phytochemicals, Hexadecanoic acid, Octadecanoic acid, 2-Undecenal,9,12-octadecadien-1ol,1,3,5 Cycloheptatrieneoctane,Hexanal,Hexadecanoic acid, Octadecanoic acid which contributes the activities like antimicrobial, antioxidant, anti-inflammatory and other activities. This investigation has helped to evaluate Total antioxidant of Sinapisalba.

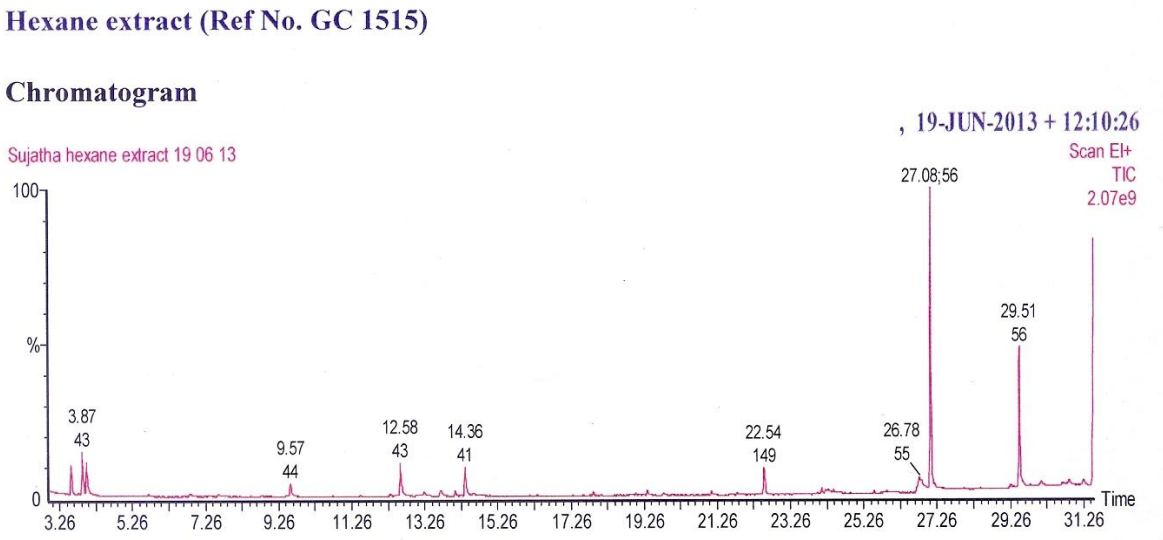
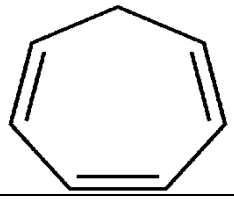
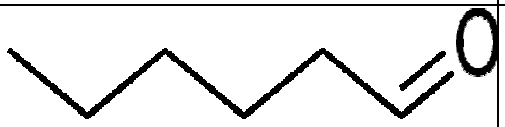
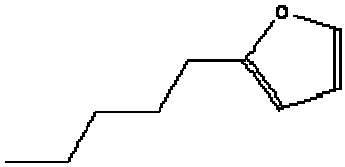
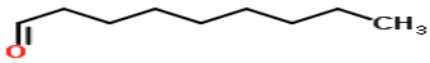
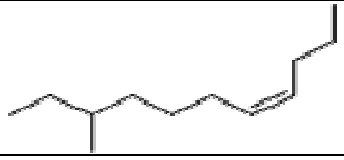


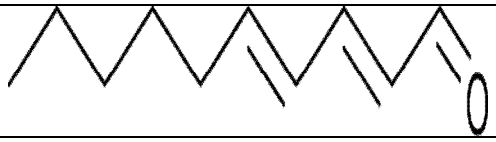
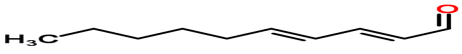
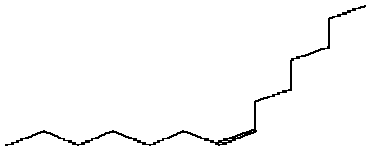
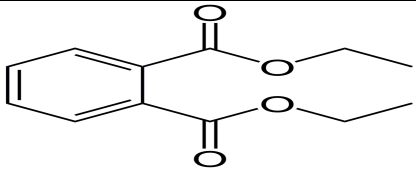
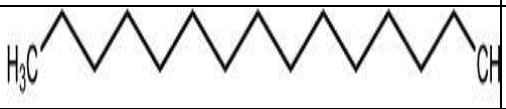
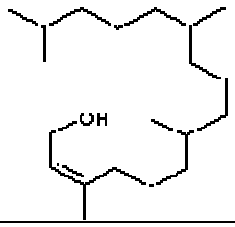
Fig. 1:

Table 1: Phyto-components identified for Sinapis alba

Compound Name	RT	Peak Area %	Molecular Formula	Molecular Weight
1,3,5 – Cycloheptatriene	3.57	3.5781	C7 H8	92
Octane	3.87	3.8145	C8H18	114
Hexanal	3.99	3.0550	C6H12O	100
Heptanal	5.70	0.3789	C7 H14O	114
2-Heptenal, (E) -	6.81	0.7336	C7 H12O	112
Furan, 2-pentyl-	7.33	0.2718	C 9H14O	138
Ocatanal	7.61	0.5440	C8 H16O	128
Nonanal	9.57	2.2125	C9 H18O	142
Decanal	11.49	0.1303	C10 H20O	156
4-Undecene, 9-methyl-, (Z)-	12.31	0.2731	C12 H24	168
2-Tridecenal, (E)-	12.58	5.2300	C13 H24O	196
2,4-Decadienal, (E,E)-	13.24	1.0014	C10H16O	152
2,4-Decadienal	13.68	1.8805	C10H16O	152
2-Undecenal	14.36	5.3208	C11H20O	168
2-Pentene, 1-ethoxy-4,4-dimethyl-	14.59	0.2302	C9 H18O	142
7-Tetradecene, (Z)-	16.13	0.0939	C14 H28	196
Diethyl Phthalate	18.08	0.0605	C12H14O4	222
3-Octadecene, (E)-	19.03	0.1513	C18 H36	252
Pentadecane	19.34	0.5602	C15 H32	212
3-Hexadecene, (Z)-	19.79	0.4521	C16H32	224
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	21.80	0.1653	C20H40O	296
n- Butyl myristate	24.28	0.6870	C18H36O2	284
Sulfurous acid, 2-propyl tridecyl ester	24.43	0.2673	C16 H34 O3S	306
1-Hexadecanol	25.54	0.2719	C16H34O	242
9,12-Octadecadien-1-ol,(Z,Z)-	26.78	5.4215	C18H34O	266
Hexadecanoic acid, butyl ester	27.08	40.8680	C20 H40O2	312
Octadecanoic acid, butyl ester	29.51	20.9745	C22H44O2	340
Ethanone, 2-cyclohexyl-1-(1H-imidazol-2-yl)-	31.25	1.3717	C11H16N2O	192

Table 2: Biological activity of hexane extract of sinapsis alba

S. No	Compound Name	Structure	Function
1	1,3,5-cycloheptatriene		No activity reported
2	Octane		no activity reported
3	Hexanal		Antibacterial, antiseptic, anesthetic, surfactant, neurolytic.
4	Heptanal		Antibacterial, antiseptic, anesthetic, surfactant
5	2-Heptanal, (E)-		used in pharmaceutical
6	Furan, 2-pentyl-		no activity reported
7	Octanal		anti-bacterial agent, food additive
8	Nonanal		food additives
9	Decanal		no activity reported
10	4-Undecene, 9-methyl-, (Z)-		food additives

11	2-Tridecenal, (E)-		no activity reported
12	2,4-decadienal, (E,E)-		no activity reported
13	2,4-Decadienal		no activity reported
14	2-Undecenal		pesticides, flavors, and fragrances
15	2-pentene, 1-ethoxy-4,4-dimethyl-	-	no activity reported
16	7-tetradecene, (Z)-		Use as flavors, fragrances and alcoholic beverages
17	Diethyl Phthalate	 -	Antioxidatnt, antimicrobial
18	3-Octadecene, (E)-		no activity reported
19	Pentadecane		No activity reported
20	3-Hexadecene, (Z)-		used in cosmetics for its fragrance
21	3,7,11,15-tetramethyl-2-hexadecen-1-ol		no activity reported
22	n-butyl myristate		used as plasticizers, antimicrobial activity
23	Sulfurous acid, 2-propyl tridecyl ester		No activity reported

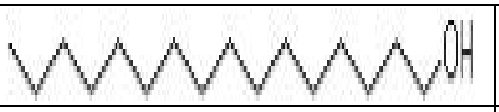
24	1-hexadecanal		Antiviral, diuretic, antianemic, insectifu
25	9,12-Octadecadiene-1-ol,(Z,Z)-		No activity reported
26	Hexadecanoic acid, butyl ester		Antioxidant, antimicrobial
27	Octadecanoic acid, butyl ester		Antioxidant, pesticide, antimicrobial, lubricant
28	Ethanone, 2-cyclohexyl-1-(1H-imidazol-2-yl)-	-	Antimicrobial

Table 3: Antoxidant activity of Sinapis alba

Concentration	Antioxidant Activiy
0.5	43
1	69

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