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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Analysis of Methanolic and Benzene Extracts of Aerial Part of *Raphanus sativus* by Gas Chromatography-Mass Spectrometry (GC-MS)

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ABSTRACT
Raphanus sativus L. is an edible plant belongs to Brassicaceae family was selected for this study to determine its phytocompounds in aerial part by GC-MS analyses. The fresh plant of Raphanus sativus was collected and cleaned with pure water. The aerial parts were separated and dried under shade then ground into powder. The dry powder of aerial part of Raphanus sativus was extracted with methanol and benzene by using Soxhlet apparatus. The extracts were filtered and kept in oven at 40-50°C until the solvent completely evaporated from it and obtained dark brown residues. The methanolic and benzene extracts of aerial part of Raphanus sativus were used for GC-MS analyses. The GC-MS analyses showed that the presence of sixty-seven phytocompounds in methanolic extract and thirty-five phytocompounds in benzene extract of aerial part of Raphanus sativus. In the GC-MS chromatogram, the highest peak area 20.04% for n- Hexadecanoic acid was observed in methanolic extract of aerial part of Raphanus sativus. The phytocompound B is (2-ethylhexyl) phthalate with the highest peak area of 35.02% was observed in the GC-MS chromatogram of benzene extract. The results of this study confirmed that the presence of phytocompounds in methanolic and benzene extracts of aerial part of Raphanus sativus. So, the present study concluded that it may be useful for further detailed study to identify the novel drugs from aerial part of Raphanus sativus.
Keywords: *Phytocompounds, Methanol, Benzene, Aerial part, Raphanus sativus.*

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INTRODUCTION

Plants have been utilized as medicines for thousands of years [1]. Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous and medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use [2]. Modernmedicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening [3]. India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurveda and Unani [4]. Ayurveda, each cell is considered to be inherently an essential expression of pure intelligence hence called self-healing science [5]. Public interest for the treatment with complementary and alternative medicine is mainly due to increased side effects by using synthetic drugs, lack of curative treatment for several chronic diseases, microbial resistance, and emerging diseases, etc., [6]. In the modern time, the Western medicinal system is reached almost at the top because of validated research and advanced techniques. There is an urgent need to validate, the basic principles as well as drugs used in the ayurvedic system of medicine with the help of advanced research methodology. Therefore, advancements in the ongoing research methodology are highly required for the promotion of Ayurveda. Plants are living chemical factories for the biosynthesis of a huge array of secondary metabolites. In fact, these metabolites that form the basis for many commercial pharmaceutical drugs, as well as herbal remedies derived from medicinal plants. The different chemical constituents in medicinal plants possess biological activities that can improve human health via the pharmaceutical and food industries, but they also represent important value in perfume, agrochemical and cosmetic industries [7]. Many of the secondary metabolites such as alkaloids, terpenoids, and phenylpropanoids are being considered for drug development [8].Secondary metabolites generally, but not always, occur in relatively low quantities in specialized cells or tissue and their production may be

widespread or restricted to particular families, genera or even species [9, 10, 11]. The secondary metabolites exert long-term effects on plant growth and survival under stressful environments [12, 13]. There are three major groups of secondary metabolites in plants based on their biosynthetic pathway such as nitrogen containing compounds like cyanogenic glycosides, alkaloids and glucosinolates, phenolic compounds like flavonoids and phenyl propanoids and terpenes like isoprenoids [14]. In recent years, the interest for the study of organic compounds from the plants and their activity has increased [15].The phytochemical compounds could be relevant to their nutritional incidence and their role in health and disease [16]. The combination of an ideal separation technique – Gas Chromatography (GC) with the best identification technique – Mass Spectrum (MS) made GC-MS, which is an ideal technique for qualitative and quantitative analysis ofvolatile and semi volatile compounds. This technique has proved to be a valuable method for the analysis of nonpolar compound and volatile oils, fatty acids, lipids and alkaloids [17]. *Raphanus sativus* L. (Radishe) is a root vegetable crop belongs to the family of Brassicaceae. Radishes have different skin colors like red, purple, black, yellow, and white through pink, while its flesh is typically white. In addition, the edible root of radish varies in its flavor, size, and length throughout the world. The roots and leaves of radishes consist of vital nutritional values and diverse secondary metabolites with antioxidant properties. When compared with roots, the leaves possessed higher levels of proteins, calcium and ascorbic acid whereas the total phenol contents were two-fold higher in leaves than roots which are corresponded with the free radical scavenging ability [18]. Leaves are used as an expectorant. The tender shoots and leaves are used as mild laxative. Root decoction is used to control vomiting, flatulence and fever. Roots are chewed to treat tooth ache. Fruits decoction is used to treat difficult and painful urination, piles and also used as brain tonic. Seed paste is given to treat epilepsy, diarrhea and dysentery. Seed decoction is given along with paste of long pepper to treat abdominal disorders [19]. It has anticancer, antimicrobial, antidiabetic, diuretic, antifertility, hypertensive, nephroprotective, gastroprotective and hepatoprotective properties and also used to treat gynaecological disorders and jaundice [20]. GC-MS analyses of methanolic extract of leaves [21] and petroleum ether extract of seeds [22] of *Raphanus sativus* were reported. But, there is no GC-MS study on methanolic and benzene extracts of aerial part of *Raphanus sativus*. So, the present study was aimed to analyze the phytocompounds of methanolic and benzene extracts of aerial part of *Raphanus sativus* by using Gas Chromatography-Mass Spectrometry (GC-MS).

MATERIAL AND METHODS

Collection of plant material

The plant *Raphanus sativus* was collected freshly from Swamimalainear Kumbakonam, Thanjavur District, TamilNadu,India during the months between December 2020 and February 2021.The plant was identified by Dr. R. Murugan, Associate Professor and Head, Department of Botany, Government Arts College (Autonomous), Kumbakonam - 612 002,TamilNadu,India.

Preparation of plant extracts

The aerial part was separated from the collected plants and it was cleaned using pure tap water and then dried under shade for 10-15 days. The dried plant materials were ground well into powder. About 100 g of dry powder was extracted with methanol and benzene at appropriate temperature by soxhlet apparatus. The extractions were continued for 48 hours. The extracts were then filtered by using cheese cloth and finally Whatman No.1 filter paper. The extracts were kept in an oven at 40 °C till the solvents were completely evaporated from it and then dark brown residues were obtained. The residues were kept in air tight containers separately and stored at 4°C until the time of use.

GC-MS Analysis

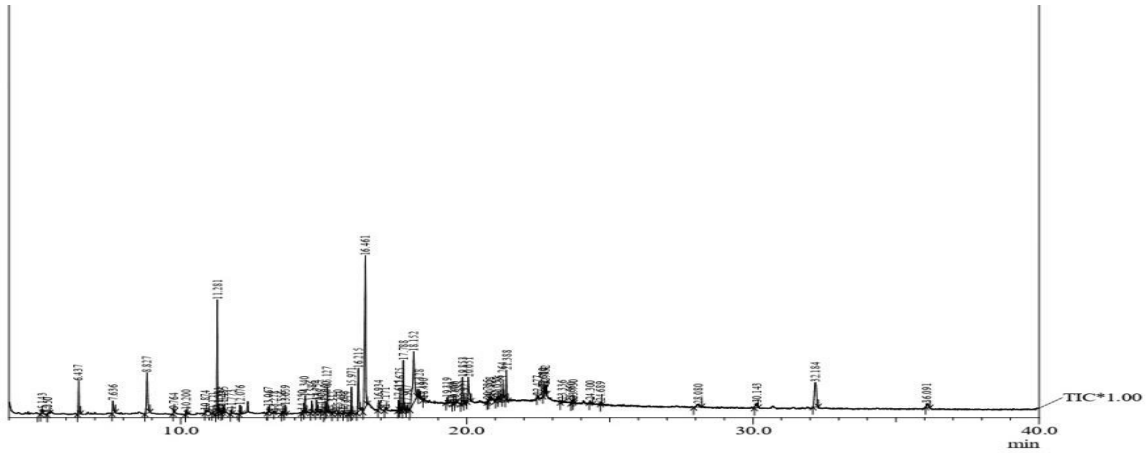
Methanolic and benzene extracts of aerial part of *Raphanus sativus* were used for phytochemical analysis by using the instrument Perkin Elmer Clarus 500. The data were obtained on a Capillary Column Elite-5MS and the following procedure was followed. Helium (99.999%) was used as the carrier gas with a flow rate of 1ml/min in the split mode (10:1). An aliquot of 1µl of methanol solution of sample was injected into the column with the injector temperature at 270°C. GC oven temperature started at 110°Cand holding for 2 min and it was raised to 200°C at the rate of 10°C/min without holding. Holding was allowed at 280 °C for 9 min with program rate of 5 °C / min (50 °C @8 °C / min to 150 °C (5 min)@ 8 °C /min to 250 °C (10 min)). GC interface and Ion source temperature was maintained at 200 °C. The mass spectrum of compounds in the samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 40-450 amu (atomic mass units). A scan interval of 0.5 second and fragments from 40 to 450 Da were maintained. Interpretation of mass spectra of the extracts of aerial part of *Raphanus sativus* were conducted using the database of National Institute of Standard and Technology [NIST] library is having more than62,000 spectral patterns. The spectrum of the compound was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of compounds. The name, molecular weight and structure of compounds of the extracts of aerial part of *Raphanus sativus*

were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area with the total area. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library using the Turbomass version 5.2.0.

RESULTS

The screening of the phytocompounds of methanolic and benzene extracts of aerial part of *Raphanus sativus* was carried out by GC-MS analyses. The identified compounds of the aerial part of *Raphanus sativus* with their name, retention time(RT), molecular formula(MF),molecular weight (MW) and peak area percentage were represented in Tables 1 and 2. The methanol extract of aerial part of *Raphanus sativus* showed sixty seven phytocomounds listed in Table 1 and their corresponding peaks were observed in GC-MS chromatogram (Figure 1).The phytocompunds such as S-Methylmethanethiosulphonate(0.38%);2-Bromo-1,1,3-trimethylcyclopropane(0.26%);1,5-Anhydro-6-deoxyhexo-2,3-diulose(3.02%);Benzofuran,2,3-dihydro- (1.61%); 2-Methoxy-4-vinylphenol (5.54%); Piperidine-2-carboxylic acid(0.28%);N-Phenethyl-2-methylbutylidenimine(0.37%);3-Nitrobenzyliodide(0.20%);Heptanoic acid, anhydride (0.08%); 1,4-Benzenedicarboxylic acid, dimethyl ester (8.03%);Methyl 2-formyl-4- pentenoate(0.30%); Dimethyl benzene-1,3-dicarboxylate (0.11%); 2-Hydroxy-1-(1'-pyrrolidiyl)-1- buten-3-one (0.47%); 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7A-trimethyl-(0.31%); Hexadecanoicacid (0.73%); Cyclohexane,eicosyl-(0.50%);Propyltrifluoroacetate(0.04%);7-Oxabicyclo[4.1.0]heptan-2-one,1,5,5-trimethyl-6-[2-(2-methyloxiranyl)ethenyl]- (0.17%); 7-Phenyl-3,4,5,6(2H)-tetrahydroazepine (0.68%); Benzene, (cyclohexylthio)-(0.28%); Tridecanoic acid(1.21%),6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2 (0.90%);3-Butylindolizidine(1.00%); Oxalic acid, ethyl neopentylester(0.24%); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (0.80%);2-Pentadecanone,6,10,14-trimethyl-(1.82%);2-Decen-1-ol(0.12%);4-Hexen-1-ol,2-isopropenyl-5-methyl-,acetate(0.23%); Methanesulfonic acid, trifluoro-, cyclohexylidenemethyl ester (0.14%); 3-Penten-2-one, (E)- (0.26%); Hexadecanoic acid, methyl ester (1.74%); 7,10,13-Hexadecatrienoic acid,(z,z,z)-(4.69%);n-Hexadecanoicacid(20.04%);9H-Pyrido[3,4-b]indole(1.23%);9,10-Anthracenediol,(0.29%);cis-13,16-Docasadienoicacid(0.64%);8,11,14-Docosatrienoicacid, methylester (1.54%); Phytol(3.97%); Eicosanoicacid, methylester (0.34%); Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl]cyclopropyl]methyl]-methylester (11.15%), Octadecanoic acid (0.65%); 7-Octen-2-ol, 2,6-dimethyl- (0.11%); 2-(Dimethylamino)ethyl vaccenoate (0.46%); 2-(2-Methoxyethyl) cyclohexanone(0.12%);Benzenamine,2-[(1-methyl-4-piperidinyl)oxy]-(0.13%);1,5-Pent-2-ene-3-methyl-5-(2,6-dimethylhepthyl)olide(3.32%);3,7-Dimethyl-1-octylmethylphosphonofluoridate(0.20%);Methyl5-(2-phenylpropionyl)hexanoate(3.66%);1-Propanone,1-(1-adamantyl)-3-dimethylamino- (0.21%); 2- (Dimethylamino)ethyl (8z,11z,14z)-icosa-8,11,14-trienoate(0.42%); Phosphonicacid, dioctadecylester (0.47%); 11-Tridecen-1-ol(0.28%); Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester (1.24%), Bis(2-ethylhexyl) phthalate (2.28%); 1-Stearoyl-1h-1,2,4-triazole(0.05%);cis-2-Phenyl-1,3-dioxolane-4-methyloctade (0.35%);Oleic Acid (0.13%); 9,12,15-Octadecatrienoic acid, (z,z,z)- (0.44%); Chloroacetic acid, heptylester (0.28%); Benzeneacetic acid, Alpha., 3,4-tris[(trimethylsilyl) oxy]-, trimethylsilyl ester(0.06%); Bicyclo[2.2.1] heptan-2-one,4,7,7-trimethyl-,(1S)-(0.23%);Undec-10-ynoicacid,butyl ester (0.34%); 3-Chlropropionic acid, nonyl ester (0.16%); Alpha.-Tocopheryl acetate(0.70%);1-Heptatriacotanol (1.11%);Gamma.-Sitosterol(5.95%);andAlpha.-Tocopherol(0.97%)wereidentified in methanolic extract of aerial part of *Raphanus sativus*.Twenty seven phytocompounds were identified in benzene extract of aerial part of *Raphanus sativus* listed in Table 2 and the peaks were observed in GC-MS (Figure 2). The phytocompounds Benzene,(1-butylhexyl)-(0.17%);Benzene,(1-methyldecyl)-(0.20%); Benzene, (1-pentyloctyl)- (0.16%); 2-Pentadecanone, 6,10,14-trimethyl- (0.27%); 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione(0.40%);Hexadecanoicacid,ethylester(0.16%); Heptadecane (0.27%); Phytol (0.37%); Docosane (1.29%); Tetracosane (4.99%);3-Butylcycloheptanone (0.33%); Hexacosane (10.94%); Eicosane(10.96%); Bis(2-ethylhexyl) phthalate (35.02%); Octacosane (0.30%); Nonacosane (9.26%); 3-Methylhexacosane (0.29%); n-Nonacosane (5.73%); Hexatriacontane (3.12%); Octacosane,2-methyl-(0.29%);Celidoniol,deoxy-(5.75%); Behenicalcohol (0.25%); Tetratetracontane (1.78%);Pentatriacontane(2.59%);2-Methylhexacosane(0.61%);Pregnane-3,11,20,21-tetrol,cyclic20,21-[(1,1-dimethylethyl(0.30%) and Gamma-Sitosterol (1.71%) were identified in benzene extract of aerial part chromatogram of Raphanus sativus.

Figure1.GC-MS Chromatogram of methanolic extract of aerial part of *Raphanus sativus*



Retention Time (Min)

Table:1.List of identified phytocompounds in the methanolic extract of aerial part of *Raphanus sativus* by GC-MS analysis

Name of compound	MF	MW	Peak Area %	RT	Nature of compound	Activity*
S-Methylmethanethiosulphonate	$C_2H_6O_2S_2$	126	0.38	5.143	Sulfur	Antibacterial
2-Bromo-1,1,3-trimethylcyclopropane	$C_6H_{11}Br$	162	0.26	5.350	-	-
1,5-Anhydro-6-deoxyhexo-2,3-diulose	$C_6H_8O_4$	144	3.02	6.437	Glycoside	Anti-inflammatory,Antioxidant
Benzofuran,2,3-dihydro-	C_8H_8O	120	1.61	7.636	-	Anti-inflammatory, Anti-HIV, Anticancer, Antibacterial, Antifungal, Antidiabetic, Hypolipidemic, Antihypertensive, Antilipidemic
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	5.54	8.827	Phenolic	Ant- inflammatory, Antioxidant, Antimicrobial
Piperidine-2-carboxylicacid	$C_6H_{11}NO_2$	129	0.28	9.764	Aminoacid	-
N-Phenethyl-2-methylbutylidenimine	$C_{13}H_{19}N$	189	0.37	10.200	-	-
3-Nitrobenzyliodide	$C_7H_6INO_2$	263	0.20	10.874	-	-
Heptanoicacid,anhydride	$C_{14}H_{26}O_3$	242	0.08	11.121	Fattyacid	Analgesic
1,4-Benzenedicarboxylicacid,dimethylester	$C_{10}H_{10}O_4$	194	8.03	11.281	-	-
Methyl2-formyl-4-pentenoate	$C_7H_{10}O_3$	142	0.30	11.331	-	-
Dimethyl benzene-1,3-dicarboxylate	$C_{10}H_{10}O_4$	194	0.11	11.425	-	-
2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-one	$C_8H_{13}NO_2$	155	0.47	11.495	Tricarbxylicacid	Insecticidal
2(4H)-Benzofuranone, 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-	$C_{11}H_{16}O_2$	180	0.31	11.773	-	-
Hexadecanoicacid	$C_{16}H_{32}O_2$	256	0.73	12.076	Fattyacid	Antioxidant
Cyclohexane,eicosyl-	$C_{26}H_{52}$	364	0.50	13.067	-	-
Propyltrifluoroacetate	$C_5H_7F_3O_2$	156	0.04	13.278	-	-

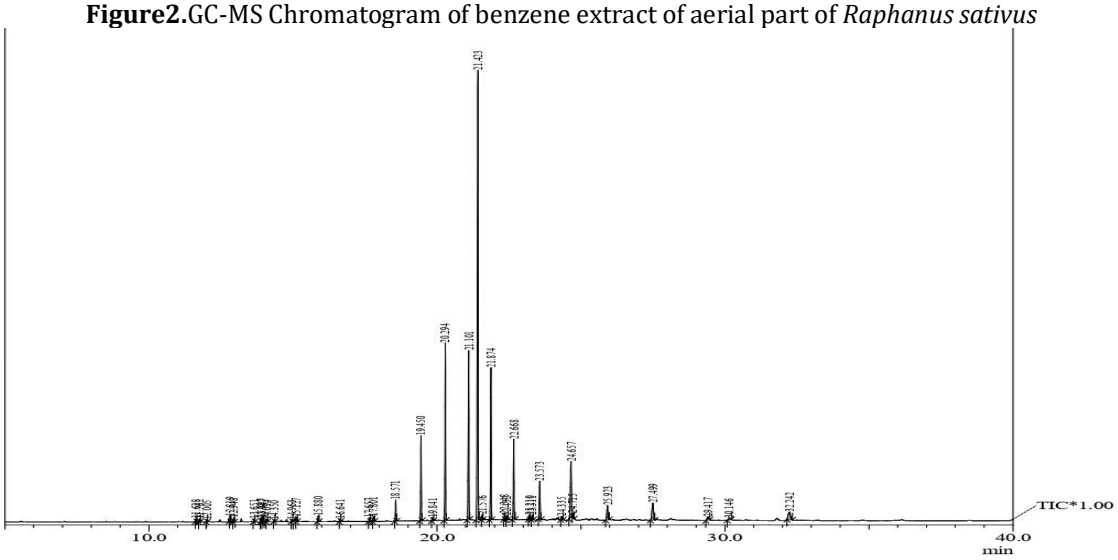
7-Oxabicyclo[4.1.0]heptan-2-one,1,5,5-trimethyl-6-[2-(2-methyloxiranyl)ethenyl]-	$C_{14}H_{20}O_3$	236	0.17	13.556	-	-
7-Phenyl-3,4,5,6(2h)-tetrahydroazepine	$C_{12}H_{15}N$	173	0.68	13.659	-	-
Benzene,(cyclohexylthio)-	$C_{12}H_{16}S$	192	0.28	14.250	-	-
Tridecanoicacid	$C_{13}H_{26}O_2$	214	1.21	14.340	Fatty acid	-
6-Hydroxy-4,4,7a-trimethyl- 5,6,7,7a-tetrahydrobenzofuran	$C_{11}H_{16}O_3$	196	0.90	14.586	-	Antioxidant
3-Butylindolizidine	$C_{12}H_{23}N$	181	1.00	14.754	-	-
Oxalicacid,ethylneopentylester	$C_9H_{16}O_4$	188	0.24	14.908	-	-
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.80	15.060	Terpenol	Antioxidant, Antimicrobial
2-Pentadecanone,6,10,14-trimethyl	$C_{18}H_{36}O$	268	1.82	15.127	Terpene	Antimicrobial, Antiosteoporotic
2-Decen-1-ol	$C_{10}H_{20}O$	156	0.12	15.312	Fatty acid	-
4-Hexen-1-ol,2-isopropenyl-5-methyl-, acetate	$C_{12}H_{20}O_2$	196	0.23	15.510	-	-
Methanesulfonicacid,trifluoro-,cyclohex	$C_8H_{11}F_3O_3S$	244	0.14	15.698	Alkylsulfonic acid	-
3-Penten-2-one,(e)-	C_5H_8O	84	0.26	15.855	-	-
Hexadecanoicacid,methylester	$C_{17}H_{34}O_2$	270	1.74	15.971	Fatty acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic,5-Alpha reductase inhibitor
7,10,13-Hexadecatrienoicacid,(z,z,z)-	$C_{16}H_{26}O_2$	250	4.69	16.215	-	-
n-Hexadecanoicacid	$C_{16}H_{32}O_2$	256	20.04	16.461	Fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide,Antiandrogenic,5-Alpha reductase inhibitor
9H-Pyrido[3,4-b]indole	$C_{11}H_8N_2$	168	1.23	16.934	-	-

9,10-Anthracenediol	$C_{25}H_{34}O_4S$	430	0.29	17.171	-	-
Cis-13,16-Docasadienoicacid	$C_{22}H_{40}O_2$	336	0.64	17.615	Fatty acid	Anti-borreliae
8,11,14-Docosatrienoic acid, methylester	$C_{23}H_{40}O_2$	348	1.54	17.675	-	-
Phytol	$C_{20}H_{40}O$	296	3.97	17.788	Diterpene	Antimicrobial, Anticancer, Diuretic, Anti-inflammatory
Eicosanoic acid, methylester	$C_{21}H_{42}O_2$	326	0.34	17.907	Fatty acid	-
Cyclopropaneoctanoicacid,2-[[2-[(2-ethylcyclopropyl) methyl]cyclopropyl]methyl]-methylester	$C_{22}H_{38}O_2$	334	11.15	18.152	Fatty acid	-
Octadecanoic acid	$C_{18}H_{36}O_2$	284	0.65	18.328	Fatty acid	Cancerpreventive, Insectifuge, Hypocholesterolemic
7-Octen-2-ol,2,6-dimethyl-	$C_{10}H_{20}O$	156	0.11	18.490	-	-
2-(Dimethylamino)ethylvaccenoate	$C_{22}H_{43}NO_2$	353	0.46	19.319	-	-
2-(2-Methoxyethyl)cyclohexanone	$C_9H_{16}O_2$	156	0.12	19.509	-	-
Benzenamine, 2-[(1-methyl-4-piperidinyl)oxy]-	$C_{12}H_{18}N_2O$	206	0.13	19.600	-	-
1,5-Pent-2-ene-3-methyl-5-(2,6-dimethylhepthy)olide	$C_{15}H_{26}O_2$	238	3.32	19.853	-	-
3,7-Dimethyl-1-octylmethylphosphonofluoridate	$C_{11}H_{24}FO_2P$	238	0.20	19.921	-	-
Methyl5-(2-phenylpropionyl)hexanoate	$C_{16}H_{22}O_3$	262	3.66	20.051	-	-
1-Propanone, 1-(1-adamantyl)-3-dimethylamino-	$C_{15}H_{25}NO$	235	0.21	20.735	-	-
2-(Dimethylamino)ethyl(8Z,11Z,14Z)-icosa-8,11,14-Trienoate	$C_{24}H_{43}NO_2$	377	0.42	20.808	-	-
Phosphonicacid,dioctadecylester	$C_{36}H_{75}O_3P$	586	0.47	21.055	-	-
11-Tridecen-1-ol	$C_{13}H_{26}O$	198	0.28	21.126	-	-

Hexadecanoicacid, 2-hydroxy-1-(hydroxymethyl)ethylester	$C_{19}H_{38}O_4$	330	1.24	21.264	Fatty acid	Antioxidant
Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	2.28	21.388	Phthalic acid	Antipyrine
1-Stearoyl-1h-1,2,4-triazole	$C_{20}H_{37}N_3O$	335	0.05	22.477	-	Antifungal
Cis-2-phenyl-1, 3-dioxolane-4-methyloctade	$C_{28}H_{40}O_4$	440	0.35	22.680	-	-
OleicAcid	$C_{18}H_{34}O_2$	282	0.13	22.714	Fatty acid	Cytotoxicity, Antioxidant, Antimicrobial
9,12,15-Octadecatrienoicacid,(Z,Z,Z)-	$C_{18}H_{30}O_2$	278	0.44	22.752	Fatty acid	Antiarrhythmic,Cardioprotective
Chloroaceticacid,heptyl ester	$C_9H_{17}ClO_2$	192	0.28	23.336	Carboxylic acid	Antioxidant
Benzeneaceticacid,alpha.,3,4-tris[(trimethylsilyl)oxy]-,trimethylsilylester	$C_{20}H_{40}O_5Si_4$	472	0.06	23.660	-	-
Bicyclo[2.2.1]heptan-2-one,4,7,7-trimethyl-,(1S)-	$C_{10}H_{16}O$	152	0.23	23.730	-	-
Undec-10-ynoicacid,butyl ester	$C_{15}H_{26}O_2$	238	0.34	24.300	-	-
3-Chlropropionicacid,nonylester	$C_{12}H_{23}ClO_2$	234	0.16	24.689	Acrylicacid	-
Alpha-Tocopherylacetate	$C_{31}H_{52}O_3$	472	0.70	28.080	Aceticacid	Wound healing
1-Heptatriacotanol	$C_{37}H_{76}O$	536	1.11	30.143	-	Anti-hypercholesterolemia
Gamma-Sitosterol	$C_{29}H_{50}O$	414	5.95	32.184	Phytosterol	Reduces hyperglycemia
dl-Alpha-Tocopherol	$C_{29}H_{50}O_2$	430	0.97	36.091	VitaminE	Antioxidant, Anticancer,Cardiovascular disease, Alzheimer's disease,Wound healing

RT - Retention Time, MF – Molecular Formula,MW- Molecular Weight,*Dr. Duke's Phytochemical and Ethnobotanical Database

Figure2.GC-MS Chromatogram of benzene extract of aerial part of *Raphanus sativus*



Retention Time(Min)

Table:2. : Identified phytocompounds in the benzene extract of aerial part of *Raphanus sativus* by GC-MS analysis

Name of compound	MF	MW	Peak Area %	RT	Nature of compound	Activity*
Benzene,(1-butylhexyl)-	$C_{16}H_{26}$	218	0.17	11.638	Aromatic	-
Benzene,(1-methyldecyl)-	$C_{17}H_{28}$	232	0.20	13.651	Aromatic	-
Benzene,(1-pentyloctyl)-	$C_{19}H_{32}$	260	0.16	14.963	Aromatic	Antimicrobial
2-Pentadecanone,6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.27	15.127	Aliphaticacyclic	Antibacterial, Adjuvent

7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.40	15.880	Oxaspiro	Antioxidant
Hexadecanoic acid, ethylester	$C_{18}H_{36}O_2$	284	0.16	16.641	Fatty acid ethylester	Antimicrobial
Heptadecane	$C_{17}H_{36}$	240	0.27	17.657	Alkanehydrocarbon	Antioxidant,Antimicrobial
Phytol	$C_{20}H_{40}O$	296	0.37	17.801	Diterpene	Antioxidant,Autophagy, Apoptosis, Antinociceptive,Anti-inflammatory,Immune-modulating,Antimicrobial
Docosane	$C_{22}H_{46}$	310	1.29	18.571	Alkane	Antibacterial
Tetracosane	$C_{24}H_{50}$	338	4.99	19.450	Alkanehydrocarbon	Cytotoxic
3-Butylcycloheptanone	$C_{11}H_{20}O$	168	0.33	19.841	-	-
Hexacosane	$C_{26}H_{54}$	366	10.94	20.294	Aliphaticsaturated hydrocarbon	Antimicrobial
Eicosane	$C_{20}H_{42}$	282	10.96	21.101	Acyclic saturated hydrocarbon	Anti-Inflammatory, Analgesic, Antipyretic
Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	35.02	21.423	Phthalateester	Apoptosis inhibitor
Octacosane	$C_{28}H_{58}$	394	0.30	21.576	Alkane	Larvicidal, Mosquitocidal, Antibacterial
Nonacosane	$C_{44}H_{90}$	618	9.26	21.874	Alkane	Antimicrobial
3-Methylhexacosane	$C_{27}H_{56}$	380	0.29	22.432	Biogenicaliphatic hydrocarbon, Volatile	Antibacterial
n-Nonacosane	$C_{29}H_{60}$	408	5.73	22.668	Alkane	Antibacterial
Hexatriacontane	$C_{36}H_{74}$	506	3.12	23.573	Alkane	Antibacterial
Octacosane,2-methyl-	$C_{29}H_{60}$	408	0.29	24.335	Branchedalkane,Aliphaticacyclicco mpound	Antimicrobial
Celidoniol,deoxy-	$C_{29}H_{60}$	408	5.75	24.657	Volatile oil,Alkane	Chemical communication of insects, Antimicrobial
Behenicalcohol	$C_{22}H_{46}O$	326	0.25	24.715	Saturated fatty alcohol	-
Tetratetracontane	$C_{44}H_{90}$	618	1.78	25.923	Alkane	Antibacterial
Pentatriacontane	$C_{35}H_{72}$	492	2.59	27.499	Aliphatic acyclic compound	-
2-Methylhexacosane	$C_{27}H_{56}$	380	0.61	29.417	Cuticular hydrocarbons	Antidiabetic, Anticancer, Antimicrobial, Hypocholesterolemic
Pregnane-3,11,20,21-tetrol,cyclic20,21-[(1,1- dimethylethyl) boronate]	$C_{25}H_{43}BO_4$	418	0.30	30.146	-	-
Gamma-Sitosterol	$C_{29}H_{50}O$	414	1.71	32.242	Phytosterol	Reduces hyperglycemia

RT - Retention Time, MF – Molecular Formula, MW- Molecular Weight,*Dr. Duke's Phytochemical and Ethnobotanical Database

DISCUSSION

In recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs like morphology, microscopy and physicochemical tests are the first step towards establishing the identity and the degree of purity of such materials should be carried out before any experiment is under taken. Currently, a number of modern drugs have been isolated from natural sources. Ethnobotanical research has increased considerably in the last few years and is presently considered a subject of great interest. There is a growing awareness in correlating the active principles from the medicinal plants with their biological activities [23]. The plant *Raphanus sativus* is an important vegetable and its members are rich in phytochemicals [24, 25], and have potential medicinal properties including antimicrobial, antifungal, antimutagenic, antioxidant and antitumor [26].The variations in phenolic and flavonoid contents of *Raphanus sativus* are attributed to the differences in species, chemodiversity, breeding condition, ontogenetic status, stage of maturation, degradation, and post-harvest handling [27]. The diversity of plant bioactive compounds derived from the infinite combinations of fundamental functional groups or carboxylic groups such as alkyls, hydroxyls, alcohols, steroids, aldehydes, benzyl rings that originate compounds with peculiar chemical and physical

characteristics such as solubility, melting point, and reactivity [28].Similarly, in the present study many phytocompounds were identified in the methanol and benzene extracts of aerial part of *Raphanus sativus*. The identified compounds possess biological and pharmacological properties were predicted from Dr.Duke's Phytochemical and Ethnobotanical Databases. The identified phytocompounds from the methanolic and benzene extracts of aerial part of *Raphanus sativus* possess anti-inflammatory, antioxidant, wound healing, antimicrobial, antiarrhythmic, antipyretic, anti- HIV, anticancer, antihypertensive, antilipidemic and anti-borreliae properties were reported. S-Methyl methane thiosulfonate, asulfur containing volatile organic compound was observed in the aerial part of *Raphanus sativus* which was reported to possess antibacterial activity. Similarly, S-Methylmethanethiosulfonate compound produced by plants and bacterial species, has recently been described to be an efficient anti-oomycete agent with promising perspectives for the control of the devastating potato late blight disease [29].In this study,1,5-Anhydro-6-deoxyhexo-2,3-diulose was determined in the aerial part and reported to possess anti-inflammatory and antioxidant activities. Similarly, the above-mentioned compounds were also found in the aerial part of *Delphinium glaucum* which is used as preservative [30]. In the present study, the identified compound Benzofuran, 2, 3-dihydro- reported to possess anti-inflammatory, anti-HIV, anticancer, antibacterial, antifungal, antidiabetic, hypolipidemic, antihypertensive and antihyperlipidemic activities. The researchers reported that the compound Benzofuran, 2, 3-dihydro- is a potential antileishmanial drug [31]. In this study, the phenolic compound 2-Methoxy-4- vinyl phenol was identified in aerial part of *Raphanus sativus* and reported to possess anti-inflammatory, antioxidant and antimicrobial properties. In addition, the2- Methoxy-4-vinylphenol compounds have previously been reported from other plant sources with their strong antioxidant activity [32].In this study, some fatty acid compounds such as Hexadecanoic acid; n- Hexadecanoic acid; Hexadecanoic acid, methyl ester and Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethylester were observed in the aerial part of *Raphanus sativus*.Similarly the presence of Hexadecanic acid in the chloroform extracts of *Albizia adianthifolia* and *Pterocarpus angolensis* was reported by using GC-MS analyses [33]. In this study, Hexadecanoic acid, methylester was observed and the compound was reported to possess antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, hemolytic and 5-alpha reductase inhibitor activities. Similarly, n-Hexadecanoic acid was reported in the plant parts of methanolic extracts of *Momordica cymbalaria* by GC-MS analysis [34].The compound hexadecanoic acid, methyl ester was also reported in aerial part of *Fluggea leucopyrus* [35].Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethylester compound possess medicinal properties and it was also reported in seed extract of *Anthonothamacrophylla* [36].In this study, the compound 3,7,11,15-Tetramethyl-2-hexadecen-1-olwasobservedinmethanolic extract of aerial part of *Raphanus sativus* and it is reported to possess antioxidant and antimicrobial activities. Similarly, it was also reported in the leaf of *Solanum xanthocarpum* [37]. In this study, phytocompound phytol was observed in the extract of aerial part of *Raphanus sativus*. Phytol was proven to exhibit antioxidant and antinociceptive effects [38,39]. Phytol is a precursor of vitamin E and vitamin K was reported to possess cytotoxic activity against breast cancer cell lines [40,41].Similarly, the compound phytol was reported in *Aristolochia ktysagathra* and the phytolconstitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and chronic inflammatory diseases [42]. Fatty acids in plants react with alcohol in an esterification reaction to form esters [43].The compound octadecanoic acid was observed in the aerial part of *Solanum khasianum* [44].Gamma-Sitosterol is a phytosterol was previously reported to possess antihyperglycemic activity by increasing insulin secretion in response to glucose[45].Benzene extract of aerial part of *Raphanus sativus* showed that the presence of thirty five phytocompounds. Among these, the five compounds such as, Benzene,(1-pentylheptyl)-;Benzene, (1-butyloctyl)-; Benzene, (1-propylnonyl)-; Benzene, (1- ethyldecyl)- and Benzene,(1-pentyloctyl)- were observed and reported to possess antimicrobial properties. Heptadecane was reported to possess antioxidant and antimicrobial properties. Similarly, these compounds were reported in leaf and root extracts of *Solanum khasianum* and exhibited antibacterial activity [46]. In this study, the compound hexacosane was observed in the benzene extract of aerial part of *Raphanus sativus* and it is reported as antimicrobial agent. Similarly, the compound hexacosane was also reported in methanolic extract of fruit of *Lagenariabreviflora* [47]. Further, the phytocompound eicosane and nonacosane were also observed in the benzene extract of aerial part of *Raphanus sativus.* The phytocompound eicosane was reported to possess anti-inflammatory, analgesic and antipyretic properties .Similarly, eicosane was reported in leaves of *Tamarindus indica* [48] and noacosane was in methanolic extract of *Leucaslavandulaefolia* [49]. In this study, the compound Celidoniol, deoxy- was observed in the aerial part of *Raphanus sativus.* The compound Celidoniol, deoxy- was reported to possess chemical communication of several insects and possess antimicrobial property. The Celidoniol, deoxy- was also reported for its potential bioactive chemical compound [50]. In the presentstudy, the phytocompound 2-Methylhexacosane was observed in the benzene extract of aerialpart of *Raphanus sativus* and it was reported to possess antidiabetic, anticancer, antimicrobial and hypocholesterolemic properties. Similarly,

the presence of compound 2-Methylhexacosane was reported in the leaves of *S.chamaecyparissus* [51]. The uses of medicinal plants and phytomedicines have led to need for the analysis of plant compounds. In this study, the GC-MS technique was used for the analysis of secondary metabolites in aerial part of *Raphanus sativus.* Similarly in the previous studies, the GC-MS technique was used for the analysis of phytocompounds in leaf, fruit and stem of *Aervalanata*, leaf and stem of *Marsilea minuta* [53] and leaf and root of *Mimosa pudica*[54] and reported that the presence of many phytocompounds. So, the GC-MS was used in this study to identify the phytocompounds of aerial part of *Raphanus sativus*.

CONCLUSION

The GC-MS analyses of the present study revealed that the presence of phytocompounds in the methanolic and benzene extracts of aerial part of *Raphanus sativus*. The phytoconstituents in the aerial part of *Raphanus sativus* may be attributed to the medicinal properties. In future, the isolation and purification of above mentioned phytocompounds from the aerial part of *Raphanus sativus* with further *in vivo* pharmacological study may be useful in the preparation of novel drugs for treating many diseases.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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