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CHEMICAL COMPOSITION OF *FAGOPYRUM ESCEULENTUM* MOENCH SEED THROUGH GC-MS

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GC-MS, *Fagopyrum esceulentum* Moench, Phyto-constituent, Retention indices

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ABSTRACT: Medicinal herbs are a precious heritage for us and since long is a part of traditional as well modern system of medicine. These herbs provide less toxic and more effective drug molecules which are helpful in various ailments. In the present work, we have focused on an edible herb which bears a large number of medicinal properties. *Fagopyrum esceulentum* Moench (Buckwheat) has a long tradition of being used as food and medicine which is endorsed with abundant of nutrients like flavonoids, polyphenols, proteins, vitamins, phytosterols, carotenoids, and minerals, *etc.* in it. In the present study, chemical and pharmacological investigation of buckwheat seed ethanol extract were carried out for determining its usefulness in human health. The GC-MS analysis was performed and based on Mass Spectra fragmentation pattern and retention indices, phytoconstituents were identified. Major constituents identified were 9-octadecenamide, n-hexadecanoic acid, ethyl linolate, 9-octadecenoic acid (z), 2, 3-dihydroxypropyl ester, ergost-5-en-3-ol (3.beta.24r), gamma-sitosterol, lupeol, fumaric acid. These phytoconstituents show various pharmacological activities like anti-inflammatory, antioxidant, antibacterial, anticancer, anti-hyperglycemic, dyslipidemic and cardiovascular activities.

INTRODUCTION: *Fagopyrum esculentum* Moench., commonly known as 'Buckwheat' or 'Kuttu' is a herb belonging to Polygonaceae family. It can easily grow in poor soil and can bear acidic environment. It mainly grows in the countries in the northern hemisphere of world [1]. The composition of buckwheat (carbohydrate, crude fat, crude fiber and crude ash content, *etc*.) resembles with wheat; therefore it is referred to as pseudocereal [2]. Usefulness of buckwheat is attributed to its prosperity in medicinally active components.

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Along with its curative potential, it also has many prophylactic effects [3]. It is a gluten-free grain which makes it a suitable substitute for celiac disease patients [4]. Buckwheat proteins have a unique amino acid composition with several biological activities.

Besides high-quality protein, buckwheat also has several other medicinally active components like starch, trace elements, dietary fibers, phenols, phytosterols, D-Chiro-inositol derivative, imino-sugars, fatty acid, and minerals, *etc*. [3] Flavonoids which are also known as nature tender drugs are present in a huge amount in buckwheat [5]. Consumption of buckwheat reduces risk of diabetes because of its high content of magnesium. It also cures the condition because of chiro-inositol present in it [6, 7]. Buckwheat alters cholesterol metabolism thus helpful in avoiding gallstones [8].

The high fiber content and plant lignans make buckwheat helpful in reducing risk related to colon cancer, breast cancer, and cardiovascular problems. Postmenopausal consumption of its grains is beneficial for a person with high cholesterol, high blood pressure, and cardiovascular problems [7]. To amplify the use of buckwheat for the improvement of human health, there is a need to further explore its chemical components. GC-MS is a fusion of two analytical techniques namely Gas Chromatography (GC) and Mass Spectroscopy (MS). GC separates different components of the sample into pulses of pure chemicals by flowing an inert gas (mobile phase), which carries the sample through a stationary phase fixed in column [9].

As the sample exits the end of the GC column, it is fragmented by ionization, and the fragments are sorted by mass to form a fragmentation pattern. These spectra of compounds are collected by the mass spectrometer, which identifies and quantifies the components according to their retention behavior and mass-to-charge ratio (m/z) [10]. Mass Spectra and retention time (RT) of a component known as the molecular fingerprint are used as the identifying characteristic of that component. Retention time (RT) and the fragmentation pattern (mass spectra) of every component is matched to commercial libraries like NIST and Wiley collections. In the case of complex mixtures, Kovatz retention index (RI) has to be used because Mass Spectral matching alone has been found insufficient for compound matching and recognition. RI is the most popular dependent variable in GC-MS studies because of its excellent reproducibility and accuracy. RI criterion is independent of the chromatographic column conditions and problems caused, during the injection of volatile and thermally unstable compounds, problems in a stationary phase like overloading, bleeding and trapping of the solutes. Present work is a GC-MS analysis of the chemical composition of buckwheat and their corresponding health benefits.

MATERIALS AND METHODS:

Material: *F. esculentum* seeds procured from local market Hisar, Haryana. Analytical grade ethanol and petroleum ether (60-80 ºC) were purchased from Hi-media, Mumbai, India. Alkane-series was formed Sigma-Aldrich India.

Methods:

Authentication of Plant: Authentication of the seed was done by Dr. Sunita Garg, Emeritus Scientist, CSIR-NISCAIR, New Delhi, India (letter no. NISCAIR/RHMD/Consult/2016/2984-11). A voucher specimen has been deposited in Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana for future reference.

Preparation of Sample for GC-MS: The seeds of *F. esculentum* were dried under shade and powdered coarsely using pestle and mortar. Coarsely powdered seeds were defatted with petroleum ether (60-80 ºC) and extracted by continuous hot percolation using Soxhlet assembly at room temperature for 7 h with 95% v/v ethanol. The extract was filtered; the solvent was recovered using rotary evaporator at 45 °C under reduced pressure. A semi-solid mass was obtained which was kept in a desiccator for further use.

Instrumental Conditions: Chemical analysis of ethanol extract of buckwheat was carried out using a Shimadzu GC-2010 attached with an autosampler (AOC-20i). Helium (>99.999%) was used as the carrier gas with an Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: cross bond 5% diphenyl / 95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 μm (film thickness). The temperature was programmed from 100 °C (3 min), then further increased to 280 °C at a ramp rate of 10 °C/min (19 min hold). The flow rate of carrier gas was 1.21 ml/min, the linear flow velocity of 40.9 cm/s and the split ratio was 10:1. The data was processed on GC solutions software for composition. GC-MS instrument was equipped with the mass selective detector, having ion source temperature of 230 °C, interface temperature of 260 °C, a solvent cut time of 2.50 min threshold of 1,000 eV and mass range of 40 to 650 m/z. The identity of the components was assigned by comparing their GC retention times with those of authentic samples as well as with known components of the standard composition of the fragmentation pattern with that reported in NIST and Wiley computer libraries.

RESULTS: GC-MS analysis of *F. esceulentum* seed extract revealed the presence of 42 components **Table 1**.

TABLE 1: CHEMICAL COMPONENTS ANALYSED THROUGH GC-MS

Retention Time	Retention Indices	Area Percent	Name	Molecular Formula	Molecular Weight	Pharmacological Action	Ref.
4.947	929	0.17	1,2-Cyclopentanedione	$C_5H_6O_2$	98	Prevents gastrointestinal tumor growth	11
5.353	951	0.54	Butane, 1,1-diethoxy-3-methyl	$C_9H_{20}O_2$	160	Flavouring agent	12
5.493	959	0.07	2-Heptenal, (E)	C_8H_{16}	112	Flavouring agent	13
5.963	984	0.10	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	Antifungal and insecticide	14
6.244	1000	0.06	2(3H)-Furanone, dihydro-4,4-dimethyl	$C_6H_{10}O_2$	114	Flavouring agent	15
7.013	1039	0.07	2-Pyrrolidinone, 1-methyl	$C_5H_9N_O$	99	Antioxidant and anticancer	16
8.156	1097	0.24	Methoxymethyl trimethylsilane	$C_5H_{14}OSi$	118	Coupling agent	17
9.095	1146	0.14	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	Anti-inflammatory, analgesic, antibacterial, antifungal	18
10.114	1200	0.11	Dodecane	-	-	No activity reported	19
11.254	1263	0.31	4-Isopropylcyclohexanone	$C_9H_{16}O$	140	Acaricidal	20
13.616	1399	0.17	Tetradecane	$C_{14}H_{30}$	198	Elephantiasis, asthma, throat disease, bronchitis	21
16.151	1560	0.16	Dodecanoic acid	$C_{12}H_{24}O_2$	200	Antioxidant	22
16.736	1599	0.34	Hexadecane	$C_{16}H_{34}$	226	Antimicrobial activity	23
17.261	1636	0.09	Diphenylmethanone	$C_{13}H_{10}O$	182	Antiviral, antimicrobial	24
17.861	1677	0.10	Pentadecafluorooctanoic acid, dodecyl ester	$C_{20}H_{25}F_{15}O2$	582	Antibacterial	25
18.756	1742	1.17	2-Ethoxycarbonyl syringic acid	$C_{12}H_{14}O_7$	270	Antidiabetic and antioxidant	26
19.035	1762	0.61	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	Antioxidant, cancer preventive	27
19.281	1780	2.24	9-Octadecenamide	$C_{18}H_{35}NO$	281	Anti-inflammatory	28
19.537	1799	0.29	Octadecane	$C_{18}H_{38}$	254	Anticorrosion agent, Antiseptic	21
21.779	1975	14.51	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant	29
23.264	2099	2.56	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	Cancer preventive, Antioxidant	27
23.556	2125	0.27	Methyl sterate	$C_{19}H_{38}O_2$	298	Anti-diarrhoeal, anti-proliferative, cytotoxic	27
23.973	2162	26.32	Ethyl linolate	$C_{20}H_{36}O_2$	308	Antibacterial agent	30
25.330	2285	0.42	Fumaric acid	$C_{17}H_{31}NO_4$	313	Food industry	11
25.651	2314	0.12	Palmitic acid monoethanolamide	$C_{18}H_{37}NO_2$	299	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor	18
26.506	2391	0.28	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340	No activity reported	31
27.317	2455	1.23	Fumaric acid	$C_{17}H_{31}NO_4$	313	Food industry	11
28.093	2509	0.53	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330 902	Pesticide, flavouring agent, Antioxidant.	32
28.293	2518	0.47	1,2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	Antimicrobial and antifouling agent	23
28.997	2518	0.24	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368	Detergents, floor polishing	11
30.053	2603	13.35	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	Anticancer	27
30.769	2692	0.43	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298	Antioxidant	27
30.912	2711	0.14	Squalene	$C_{30}H_{50}$	410	Antioxidant, skin hydrant, emollient	33
31.895	2844	0.07	Delta.-Tocopherol	$C_{27}H_{46}O_2$	402	Antioxidant	34
32.763	3124	0.70	Gamma.-Tocopherol			Antioxidant	34
34.621	3240	1.24	Ergost-5-en-3-ol, (3.BETA.,24R)-	$C_{28}H_{48}O$	400	Dyslipidemia and Cardiovascular problems	35
34.910	3265	0.29	Stigmasterol	$C_{29}H_{48}O$	412	Antioxidant, hypoglycemic and progesterone precursor, antimicrobial, anticancer, anti-arthritic, anti- asthamatic, anti-	32

						inflammatory, diuretic	
35.730	3336	7.66	Gamma.-Sitosterol	$C_{29}H_{50}O$	414	Reduces hypereglycemia	36
36.266	3383	1.32	Lupeol	$C_{30}H_{50}O$	426	Hypoglycemic	37
36.767	3427	0.10	9,19-Cyclolanost-24-en-3-ol, (3.beta.)	$C_{30}H_{50}O$	426	Anti-hyperglycemic	38
36.964	3444	0.20	Alpha.-Amyrin	$C_{30}H_{50}O$	426	Anti cancer	34
37.420	3484	0.50	Lup-20(29)-en-3-yl acetate	$C_{32}H_{52}O_2$	468	Anti-hyperglycemic	39

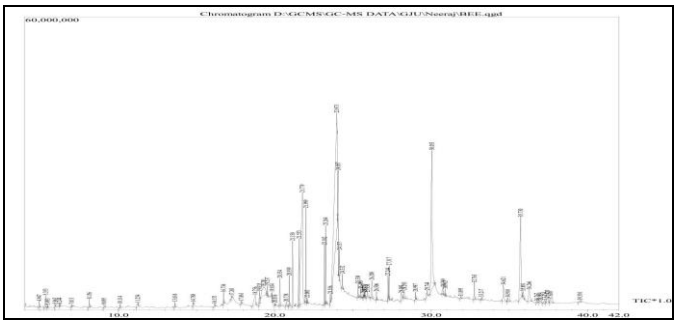

FIG. 1: GC-MS CHROMATOGRAM OF *F. ESCULENTUM* SEED EXTRACT

Fig. 1 shows the GC-MS chromatogram of extract. Major phytoconstituents identified are 9-octadecenamide, n-hexadecanoic acid, ethyl linolate, 9-octadecenoic acid (z), 2, 3-dihydroxy-propyl ester, ergost-5-en-3-ol, (3. beta., 24r), gamma-sitosterol, lupeol, fumaric acid, *etc*. have larger percentage area. Other components with lesser area percentage are butane, 1, 1-diethoxy-3-methyl, 4- isopropylcyclo-hexanone, hexadecane, tetradecanoic acid 1, 2-benzene dicarboxylic acid, and components with least percent area are methoxymethyl trimethyl-silane, methyl stearate, palmitic acid monoethanolamide, alpha.-amyrin. *etc*.

DISCUSSION: Presence of a large number of therapeutically active components explains the pharmacological potential of *F. esceulentum*. Majority of the phytoconstituents (hexadecanoic acid, dodecanoic acid, ethoxy carbonyl syringic acid, stigmasterol, heptadecanoic acid, *etc*.) are antioxidant and thus makes the herb a brilliant antioxidant to detoxify the body. *F. esceulentum* also possess anticancer potential as it posses antitumor agents like 1, 2 cyclopentanedione, 2-pyrrolidinone 1 methyl, octadecanoic acid, stigma-sterol, alpha-amyrin, *etc*. Herb also has antihyperglycaemic / anti-diabetic potential which is attributed to 2-ethoxy carbonyl syringic acid, gamma sitosterol, lupeol, stigmasterol, *etc*. In addition to above components various anti-bacterial, antifungal and anti-insect molecules like 2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-Hexadecane, hexadecanoic acid, 2-hydroxy -1-(hydroxymethyl)ethyl ester, ethyl linoleate, 1, 2-Benzenedicarboxylic acid, docosanoic acid, ethyl ester are also present.

CONCLUSION: To the best of our knowledge this is the first time when GC-MS is used to determine the chemical composition of *F. esculentum* extract. Further, every component of the extract can be easily separated and used for deriving new medicinal compounds.

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