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Determination of chemical composition of Panchgavya ghrita and garlic pill using gas chromatography and mass spectrometry (GC-MS)

MP Sakhare, NP Dakshinkar, NV Kurkure, SV Upadhye and GR Bhojne

Abstract
Panchgavya is a popular ayurvedic drug of human and Veterinary Medicine. Panchgavya is prepared with all five components of Indian cow derived products *viz*; cow milk, ghee, urine, dung juice and curd in equal proportions, its ghee based preparation is called as Panchgavya ghrita. Garlic (*Allium sativum*) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Gas chromatograph with a mass spectrometry were used to determine different compound present in the Panchgavya ghrita. After analysis, total fiften constituents, representing fatty acids were separated from the Panchgavya ghrita and total 12 constituents, were separated from the garlic pill.

Keywords: Panchgavya ghrita, garlic pill, *Allivum sativum*, gas chromatography, mass spectrometry, palmitic acid methyl ester, allyl trisulphide

Introduction
Panchgavya is a popular ayurvedic drug of human and Veterinary medicine. Panchgavya is prepared with all five components of Indian cow derived products *viz*; cow milk, ghee, urine, dung juice and curd in equal proportions, its ghee based preparation is called as Panchgavya ghrita and claimed to be useful against liver disorders, fever, inflammations, anemia and as a rejuvenator [1] and immunomodulatory effect in broiler chicks [2]. Panchgavya ghrita also had antimicrobial, immune booster, antidiabetic, anticancer, antiviral, antibacterial, antifungal, anticonvulsant, aphrodisiac property and acting as blood purifier and used as suitable medium to deliver medicines [3].

Garlic (*Allium sativum*) has acquired a reputation in different traditions as a prophylactic as well as therapeutic medicinal plant. Garlic has played vital dietary and medicinal roles throughout the history [4]. For centuries, fresh, and aged garlic, its powder and oil used as a nutritional, medicinal and spiritual remedy. In folk medicine, it used for treatment of many conditions including, joint, and tooth pain, cough, constipation, gynecologic disorder, infectious diseases, parasitic infestation, animal, and insect bites [5]. Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample (Figure 1). GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass. The further addition of mass spectrometer in it leads to GC-MS/MS [6]. In GC/MS analysis GC separates the components while MS identifies components in the mixture on the basis of molecular mass and fragmentation pattern [7]. The present work was carried out to quantify different compounds present in panchgavya ghrita and garlic pill sample using GC-MS.

Materials and Methods
The Panchgavya ghrita was procured from Go-Vigyan Anusandhan Kendra, Deolapar, Nagpur. The garlic pill sample was procured from Ranbaxy Private Limited, Mumbai.

GC-MS conditions
The oil of garlic pill and panchgavya was subjected to Gas chromatograph with a mass spectrometer (GC-MS/MS) instrument (Plate 3) by attempting following conditions: Column, 30-metre DB-WAX capillary column (0.25 mm i.d., film thickness 0.25 µm; Agilent

Technologies, USA). For GC-MS detection, electron ionization system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1.0 ml/min with a split ratio of 10:1. The oven temperature was operated according to the following oven temperature: 40 °C held for 1 min, raising at the rate of 20 °C min^{-1} up to 150 °C then, raising at the rate of 3 °C min^{-1}, hold for 0 min and raising at the rate of 20 °C min^{-1} up to 300 °C with 15 min held, injector temperature and volume 250 °C and 2µL, respectively. The total GC running time was about 36 min. The MS operating conditions were ionization voltage 70 eV, source temperature of 250 °C, inlet line temperature 280 °C, mass scan (m/z)-30-500. The mass spectra of compounds were identified by comparing the mass spectra obtained from their related chromatographic peaks with NIST mass spectral libraries.

Results and Discussion

The different compounds identified in Panchgavya ghrita along with their retention time and percent composition were shown in Table 1 and Fig.1.

Table 1: List of compounds identified in Panchgavya ghrita

Sr. No.	Retention Time (min)	Identified Compounds	Percent Composition
1	21.84	Phenol,2,4 bis-(1,1-dimethylethyl)-6 methyl	0.88
2	22.09	p-Benzoquinone	0.66
3	23.89	Eicosane,10-methyl	0.02
4	24.73	Benzo(b) dihydropyran	0.03
5	24.95	Ethyl iso-allocholate	0.82
6	25.51	Myristic acid methyl ester	0.03
7	25.71	Caproic acid methyl ester	1.28
8	26.14	Lauric acid methyl ester	2.43
9	27.75	Eicosanoic acid	0.84
10	29.94	Adipic acid, bis (2ethylhexyl) ester	2.56
11	30.96	Palmitic acid methyl ester	10.87
12	31.08	Butyric acid methyl ester	0.03
13	32.15	Stearic acid methyl ester	6.43
14	32.43	Oleic acid methyl ester	0.58
15	33.17	Undecane	0.01

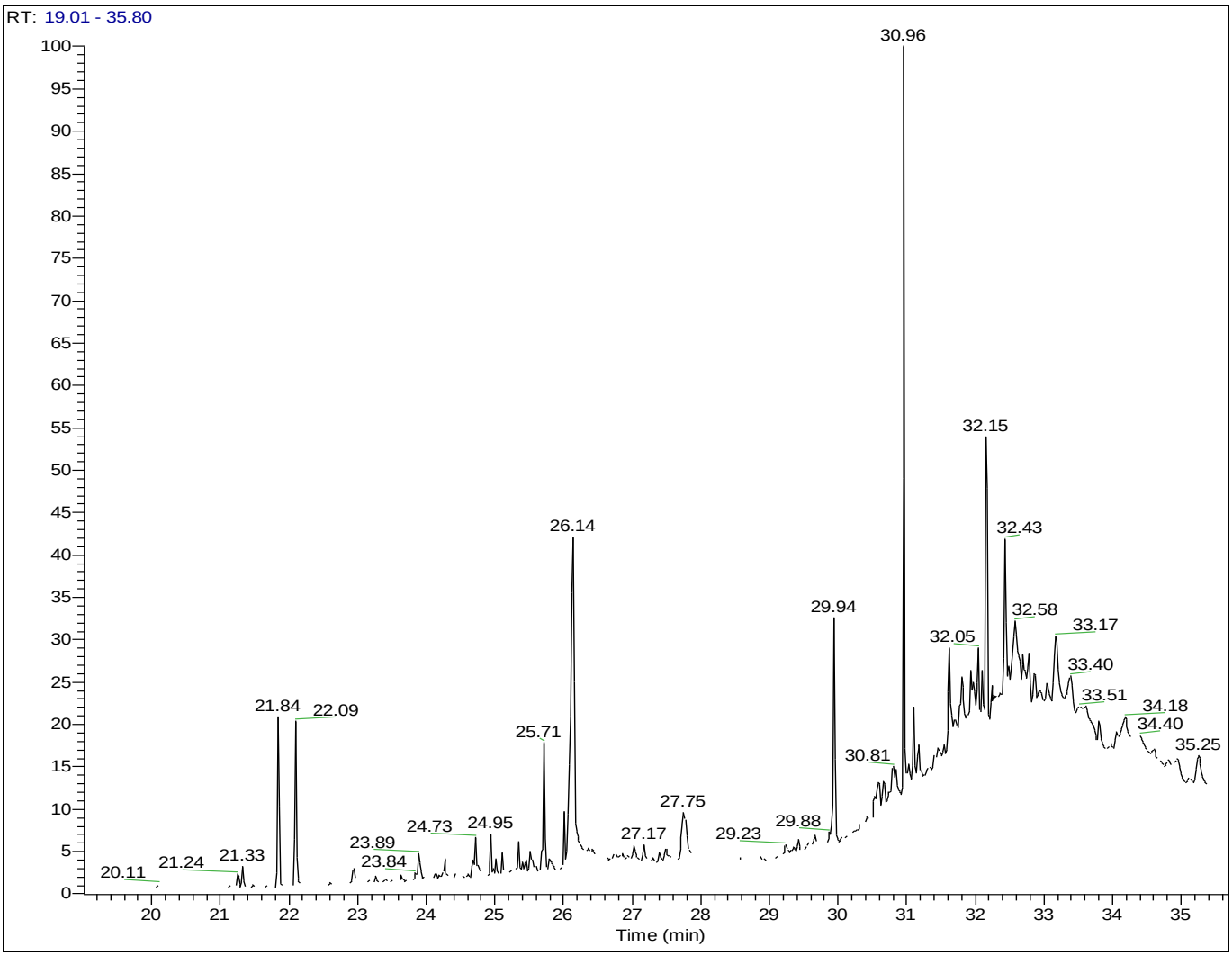

Fig 1: GC-MS spectra of Panchgavya ghrita components

After analysis, total fiften constituents, representing fatty acids were separated from the Panchgavya ghrita. The identified constituents with their percent composition were *viz;* Phenol,2,4 bis-(1,1-dimethylethyl)-6 methyl (0.88%), p-Benzoquinone (0.66%), Eicosane,10-methyl (0.02%), Benzo(b) dihydropyran (0.03%), Ethyl iso-allocholate (0.82%), Myristic acid methyl ester (0.03%), Caproic acid methyl ester (1.28%), Lauric acid methyl ester (2.43%), Eicosanoic acid (0.84%), Adipic acid, bis (2ethylhexyl) ester (2.56%), Palmitic acid methyl ester (10.87%), Butyric acid methyl ester (0.03%), Stearic acid methyl ester (6.43%), Oleic acid methyl ester (0.58%) and Undecane (0.01%).

The major identified compounds in Panchgavya ghrita were fatty acid which was in accordance with [8] who studied GC-MS spectra of cow ghee and found all fatty acid as butyric, capric, caprolyic, lauric, myrestic, palmetic, stearic, oleic, linolic and linoleic acid in it. The chemical compounds of garlic were investigated by gas chromatography mass spectrometry (GC/MS) and different compounds identified were shown in Table 2 and Fig 2.

Table 2: List of compound found in garlic pill using GC-MS

Sr. No.	Retention Time (min)	Identified Compounds	Percent Composition
1	10.21	Phenol,2,4 bis (1,1-dimethylethyl)	0.05
2	13.78	Estra	0.04
3	15.80	Propen-1-ol	0.12
4	16.38	Pthalic acid ester	0.01
5	17.36	Benzenedicraboxylic acid, bis (2methylpropyl)ester	0.62
6	17.70	Di (propenyl sulphide)	0.22
7	18.15	p-Cresol,2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)	1.74
8	18.32	Isoretinine a	1.22
9	19.71	Allyl trisulphide	43.83
10	23.23	Dimethyltetra sulphide	42.86
11	26.62	Ethyl thio-penta-1,5 dien-3-ol	6.98
12	32.46	All trans squalene	2.38

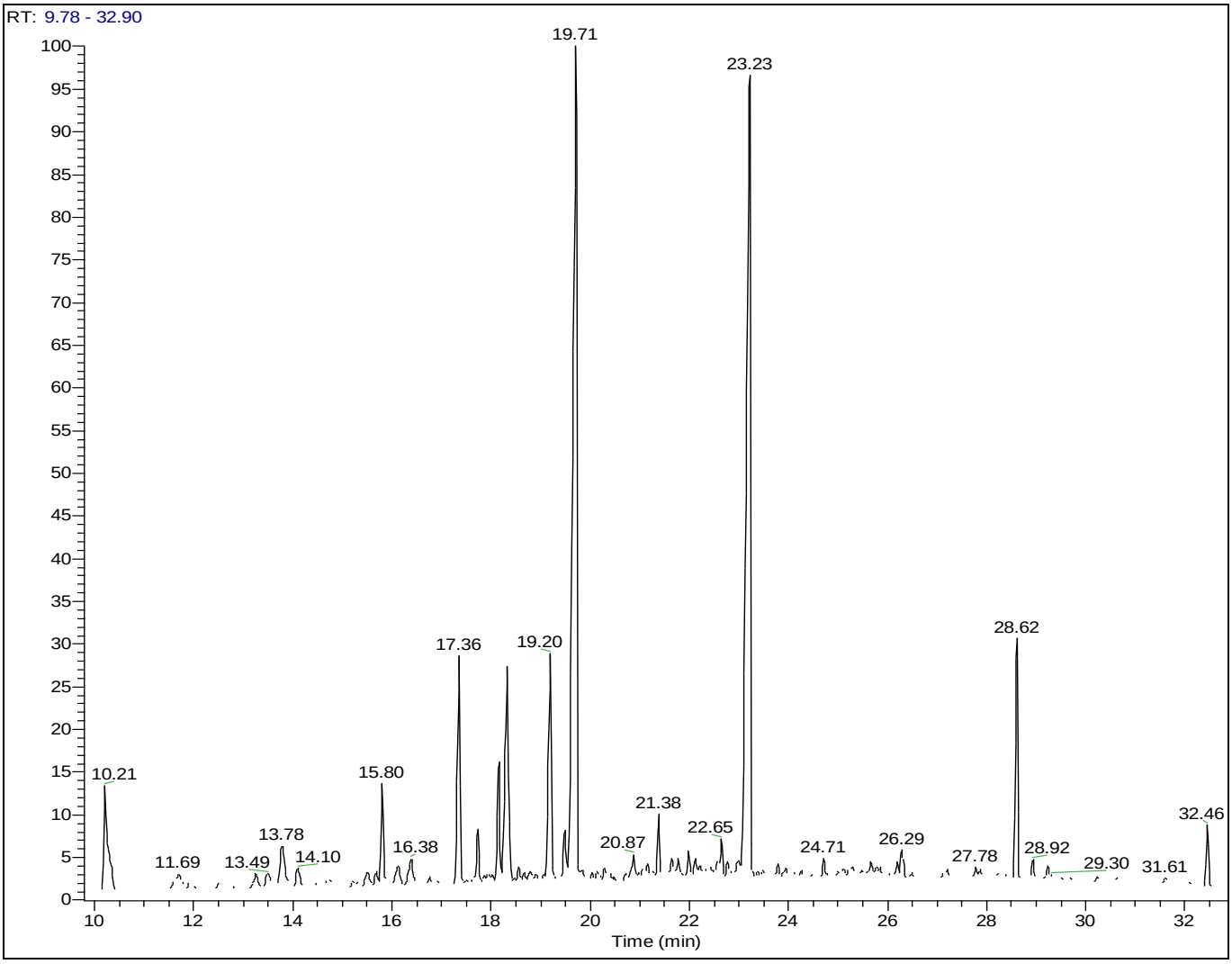

Fig 2: GC-MS spectra of garlic pill

A total of 12 constituents, were separated from the garlic pill. The identified constituents with their percent composition were viz; Phenol,2,4 bis(1,1-dimethylethyl) (0.05%), Estra (0.04%), Propen-1-ol (0.12%), Pthalic acid ester (0.01%), Benzene dicraboxylic acid and bis (2methylpropyl) ester (0.62%), Di (propenyl sulphide) (0.22%), p-Cresol,2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl) (1.74%), Isoretinine a (1.22%), Allyl trisulphide (43.83%), Dimethyltetra sulphide (42.86%), Ethyl thio-penta-1,5 dien-3-ol (6.98%) and All trans squalene (2.38%).

The major identified compounds were Allyl trisulphide (43.83%), Dimethyltetra sulphide (42.86%), Ethyl thio-penta-1,5 dien-3-ol (6.98%) and All trans squalene (2.38%). However, found the amount of allicin in aqueous garlic extracts varied from 35.6 to 44.5 mM [9]. The garlic oil and showed the presence of four compounds Diallyl monosulfide,

Trisulfide methyl 2 propenyl, Diallyl trisulfide, Dimethyl tetrasulfide and their GC retention times were 6.2minutes, 9.0 minutes, 5.9 minutes and 8.1 minutes respectively [10]. Author studied the various components of garlic and aged garlic extract, including allicin, S-allylcysteine (SAC) andvolatile metabolites of allicin were determined in breath, plasma and simulated gastric fluids by HPLC, gas chromatography (GC) or HPLC- and GC-mass spectrometry (MS) [11]. Allicin is an organosulfur compound of *Allium sativum* (garlic) with anti-fungal and antimicrobial properties [12]. The garlic and its oil have a potent anti-oxidant, anti-inflammatory, immunomodulatory, hepatoprotective, anti-atherosclerotic, antimicrobial and antineoplastic activities [13]. These activities assumed to be caused by the presence of hundreds of active ingredients such as diallyl, dimethyl and allylmethyl, mono to hexa-sulfides, and alliin [14].

Conclusion

Gas chromatography-mass spectrometry (GC-MS) is an important technique for qualitative and quantitative investigation of sample. GC-MS provides better sample identification, higher sensitivity, an improved range of analyzable samples, and rapid results.

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