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Research Article

GC-MS/MS ANALYSIS OF BROWN RICE

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ABSTRACT

Objective: The present study was undertaken to study the phytoconstituents quantitatively through gas chromatography-mass spectrometry (MS)/MS, as well as its antibacterial, antifungal activities.

Methods: The ethanol extract of brown rice was used for the study. 25gram of brown rice powder was used for the extraction process.

Results: Each peak in the chromatogram represents the signal created by a compound that elutes from the GC column into the detector. The size of the peaks is proportional to the quantity of the equivalent substances in the specimen analyzed.

The results obtained shows the highest peak for 6-(1-hydroxymethylvinyl)-4, 8a-dimethyl-3, 5, 6, 7, 8, 8a-hexahydro-1H-naphthalen-2-one, 26-Nor-5-cholesten-3β-ol-25-one, Propane, 1,1-diethoxy-, and stigmastan-3,5-diene. The remaining seven compounds (Stigmasteryl tosylate, Tetradecanedioic acid, 2H-1-Benzopyran-2-one, 6-acetyl-7-(acetyloxy)-4-methyl-, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl,-3,5,5tris (trimethylsiloxy) tetrasiloxane, Dodecanoic acid, ethyl ester, t-Butyl hydrogen phthalate) showed moderate levels of a peak area percent except 3, 4-Dihydroxy-α-(isopropylaminomethyl)-benzyl alcohol (isoproterenol) as it shows only 1.73 as peak area percent, which was found to be low compared to other compounds identified. No compound was present in trace amount. The range of retention time falls within the range of 4.79 to 35.15.

Conclusion: The antimicrobial property could be attributed due to the phytochemicals present in brown rice. Essential oil, sterols and various other bioactive constituents present in it would have been responsible for the antibacterial, antifungal activity. Brown rice is a whole grain which could be germinated to improve its overall health benefits. Brown rice extract finds applications in pharmaceutical preparations.

Keywords: Antibacterial, Antifungal, Brown rice, Gas chromatography-mass spectrometry/mass spectrometry.

INTRODUCTION

Rice is a very good economical food eaten by every south Indian the most commonly and the oldest crop. It is a unmilled rice, a form of whole grain, endowed with nutrients such as vitamins, minerals, fiber, iron, low fat available across the globe. Brown rice is able to reduce asthma, heart disease, so it is necessary to promote the consumption of brown rice so as to increase its demand. The best way to consume brown rice is by steaming it. Increased plasma glucagon like peptide levels are reported in humans after consuming glucose but not equivalent portions of complex carbohydrates like brown rice/barley [1]. Like white rice, brown rice lacks gluten, trans fat, cholesterol and contains only traces of fat, thiamin, niacin, folate, iron. Since, brown rice is an essential, energetic, healthy food for all, it was decided to study quantitatively through gas chromatography-mass spectrometry (GC-MS)/MS and also its activity against bacteria, fungi.

METHODS

Sample collection

The brown rice purchased from the supermarket at Chennai was powdered. 25 g of powdered sample was used for ethanol extraction. The extracted sample was used for phytochemical analysis through GC-MS/MS, anti-microbial activity.

Analytical method

GC-MS/MS was performed on a Scion 436-GC Bruker carrying Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% dipheny l95% dimethyl poly siloxane), 30 m × 0.25 mm ID × 0.25 m df. The column oven temperature program was as follows: 80°C hold for 2 minutes, Up to 160°C at the rate of 20°C/minutes no hold, up to 280°C at the rate of 5°C/minutes no hold, up to 300°C at the rate of 20°C/minutes 10 minutes hold, injector temperature 280°C, Total GC running time was 41 minutes. The inlet temperature was set at 280°C, source temperature 250°C; ionization mode, ionization at 70-eV ionization energy; For single scan analysis, the scan range was set from m/z 40 to 600; solvent delay: 0-3.5 minutes; and the injection volume was 2 µl. The GC-MS/MS was performed by the Institute of Crop Processing Technology, Tanjavur.

Antimicrobial assay

The antimicrobial activity was assessed by means of Kirby - Bauer technique [2].

RESULTS AND DISCUSSION

The results of compounds identified are shown in Table.1.

Totally 12 compounds were identified. The first compound identified was propane, 1, 1-diethoxy-, Cyclohexasiloxane, dodecamethyl-, 3-isopropoxy-1, 1, 1, 7, 7, 7-hexamethyl-3, 5, 5- tris (trimethylsiloxy) tetrasiloxane, 3,4-dihydroxy-α-(isopropylaminomethyl)-benzyl alcohol (isoproterenol), tetradecanedioic acid, dodecanoic acid, ethyl ester, t-butyl hydrogen phthalate, 2H-1-benzopyran-2-one, 6-acetyl-7-(acetyloxy)-4-methyl-, stigmasterol tosylate, stigmastan-3,5-diene, 6-(1-hydroxymethylvinyl)-4, 8a-dimethyl-3, 5, 6, 7, 8, 8a-hexahydro-1H-naphthalen-2-one, and 26-Nor-5-cholesten-3β-ol-25-one.

Their molecular weight, molecular formulae, peak area percent of the compound are as follows: Compound1: $C_7H_{16}O_2$, 132, 15.30. Compound 2: $C_{12}H_{36}O_6Si_6$, 444, 2.40, Compound 3: $C_{18}H_{52}O_7Si_7$, 576, 3.52. Compound 4: $C_{11}H_{17}NO_3$, 211, 1.73, Compound 5: $C_{14}H_{26}O_4$, 258, 8.25. Compound 6: $C_{14}H_{28}O_2$, 228, 2.75, Compound 7: $C_{12}H_{14}O_4$, 222, 2.27, Compound 8: $C_{14}H_{12}O_5$, 260, 5.03. Compound 9: $C_{36}H_{54}O_3S$, 566, 9.41. Compound 10:

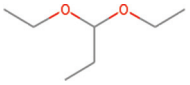
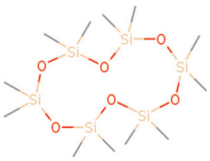
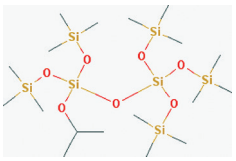
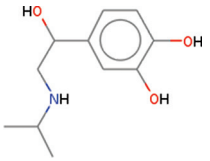
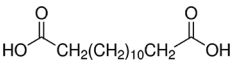
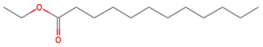
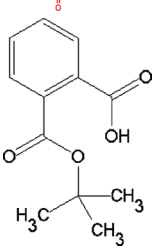
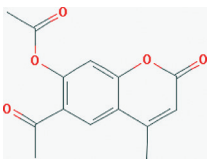
$C_{29}H_{48,}$ 396, 11.24. Compound 11:$C_{15}H_{22}O_{2,}$ 234, 22.17. Compound 12: $C_{26}H_{42}O_{2}$, 386, 15.92. The structures of the identified twelve compounds are given in Table.2. The phytoconstituents, antioxidant activity of brown rice was studied by Krishnaveni *et al*. [3,4]. The compound isoproterenol find pharmacological application. The Tetradecane dioic acid is applied in antiseptics, top-grade coatings, painting materials, corrosion inhibitor, surfactant, engineering plastics.The Dodecanoic acid, ethyl esters are used as food grade flavoring agent. The

Table 1: Bioactive components in ethanol extract of brown rice

S. No	RT	Name of the compound	MF	MW	PA%
1	4.79	Propane, 1,1-diethoxy-	$C_7H_{16}O_2$	132	15.30
2	6.63	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	2.40
3	8.25	3-isopropoxy-1, 1, 1, 7, 7, 7-hexamethyl-3, 5, 5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	3.52
4	10.34	3,4-dihydroxy-α-(isopropylaminomethyl)	$C_{11}H_{17}NO_3$	211	1.73
5	12.73	Tetradecanedioic acid	$C_{14}H_{26}O_4$	258	8.25
6	16.34	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	228	2.75
7	25.19	t-Butyl hydrogen phthalate	$C_{12}H_{14}O_4$	222	2.27
8	28.36	2H-1-Benzopyran-2-one, 6-acetyl-7 -(acetyloxy)-4-methyl-	$C_{14}H_{12}O_5$	260	5.03
9	31.70	stigmasterol tosylate	$C_{36}H_{54}O_3S$	566	9.41
10	32.34	Stigmastan-3,5-diene	$C_{29}H_{48}$	396	11.24
11	34.50	6-(1-Hydroxymethylvinyl)-4,8a- dimethyl-3, 5, 6, 7, 8, 8a-hexahydro- 1H-naphthalen-2-one	$C_{15}H_{22}O_2$	234	22.17
12	35.15	26-Nor-5-cholesten-3β-ol-25-one	$C_{26}H_{42}O_2$	386	15.92

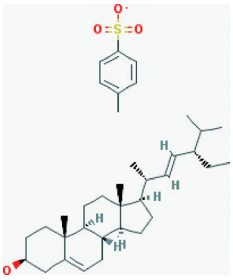
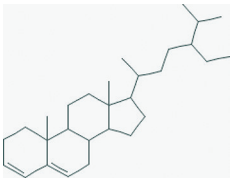
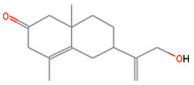
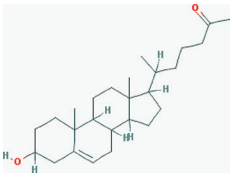
MF: Molecular formulae, MW: Molecular weight, PA: Peak area percent

Table 2: Structure of isolated compounds

S. No	Name of the compound	Structure
1	Propane, 1,1-diethoxy-	
2	Cyclohexasiloxane, dodecamethyl-	
3	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	
4	3,4-Dihydroxy-α-(isopropylaminomethyl)-benzyl alcohol (isoproterenol)	
5	Tetradecanedioic acid	
6	Dodecanoic acid, ethyl ester	
7	t-Butyl hydrogen phthalate	
8	2H-1-Benzopyran-2-one, 6-acetyl-7-(acetyloxy)-4-methyl-	

(*Contd*...)

Table 2: (*Continued*)

S. No	Name of the compound	Structure
9	stigmasterol tosylate	
10	Stigmastan-3,5-diene	
11	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3, 5, 6, 7, 8, 8a-hexahydro-1H-naphthalen-2-one	
12	26-Nor-5-cholesten-3β-ol-25-one	

compound 2H-1-Benzopyran-2-one, 6-acetyl-7-(acetyloxy)-4-methyl- is reported for its antitumor, [5,6], anti-HIV [7,8], anticoagulant [9,10], antimicrobial [11,12], antioxidant [13,14] and anti-inflammatory [15,16] activity.The compound Stigmasteryl tosylate is used as a precursor in the synthesis of cholesterol [17,18]. The Stigmastan- 3,5- diene identified have Antifungal, antibacterial activity[19]. The observed compound compound 6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one have Antibacterial activity. [20]. The identified compound 26-Nor-5-cholesten-3β-ol-25-one have antioxidant property. [21].

Antimicrobial activity

Table 3 shows the antimicrobial activity of brown rice. The antibacterial and antifungal activity of brown rice was found to be good. The activity against the particular microbe was measured by a zone of inhibition. The zone of inhibition observed was higher for *Candida albicans*, *Aspergillus niger*, *Escherichia coli* compared to *Staphylococcus aureus*. Toxin production occurs in brown rice according to the storage conditions like temperature, moisture, duration etc. Improper storage causes the growth of fungi, allowing the conversion of aminoacid tryptophan in rice to α- picolinic acid, a chemical that leads to itching, allergic reactions to persons. The quality and nutrient value, therapeutic properties are preserved by means of good storage conditions alone.

CONCLUSION

The high nutritional content of brown rice makes it a very good therapeutic agent in treating various diseases so as to keep us healthy. Our present GC-MS/MS study shows, phytochemicals that are effective in killing *C. albicans, A. niger, E. coli, S. aureus*. Further, this work can be extended to society by making a complete study with each component identified and also by comparing it with germinated brown rice. The present study was aimed and performed in an attempt to increase the consumption of brown rice containing increased phenolic compounds as there is a general assumption that phenolic compounds are present in rich amount in fruits and vegetables only.

Table 3: Antimicrobial activity of ethanol extract of brown rice

Sample used	Microbes tested	Zone of inhibition (mm)
Brown rice	*Escherichia coli*	11
	Staphylococcus aureus	9
	Aspergillus niger	13
	Candida albicans	14

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REFERENCES

1. Kothandam HP, Umamaheshwari P, Samuel DW. Hormone based therapy in Type 2 diabetes mellitus. Asian J Pharm Clinical Res 2012;5(4):20-24.
2. Bauer AW, Perry DM, Kirby WM. Single-disk antibiotic-sensitivity testing of staphylococci; an analysis of technique and results. AMA Arch Intern Med 1959;104(2):208-16.
3. Krishnaveni M, Dhanalakshmi R. Phytoconstituent study of brown rice. World J Pharm Res 2014;3(8):1092-9.
4. Krishnaveni M, Magesh P, George JS, Dhanalakshmi R, Ponraj K, Lavanya K, *et al*. Antioxidant potential of brown rice. Int J Pharm Sci Rev Res 2014;28(1):134-7.
5. Madari H, Panda D, Wilson L and Jacobs RS. Dicoumarol: unique microtubule stabilizing natural product that is synergistic with Taxol. Cancer Res, 2003; 63(6): 1214-20.
6. Kostova I, Synthetic and natural coumarins as cytotoxic agents, Curr

Med Chem Anticancer Agents 2005; 5(1): 9-46.
7. Takeuchi Y, Xie L, Cosentino LM and Lee KH. Anti-AIDS agents-XXVIII. Synthesis and anti-HIV activity of methoxy substituted 3',4'-di- O-(–)-camphanoyl-(+)-ciskhellactone (DCK) analogues. Bioorg Med Chem Lett 1997; 7: 2573-8.
8. Shikishima Y, Takaishi Y, Honda G, Ito M, Takfda Y, Kodzhimatov O K, Ashurmetov O, Lee KH. Chemical constituents of Prangos tschiniganica; structure elucidation and absolute configuration of coumarin and furanocoumarinderivatives with anti-HIV activity. Chem Pharm Bull 2001; 49(7): 877-80.
9. Manolov I, Maichle-Moessmer C and Danchev N. Synthesis, structure,toxicological and pharmacological investigations of 4-hydroxycoumarin derivatives. Eur J Med Chem 2006; 41(7): 882-90.
10. Jung J, Kin J and Park O. Simple and cost effective syntheses of 4 hydroxycoumari n. Synth.Commun 1999; 29: 3587-95.
11. Ostrov DA, Hernandez Prada JA, Corsino PE, Finton KA, Le Nand Rowe TC. Discovery of novel DNA gyrase inhibitors by high-throughput virtual screening. Antimicrob Agents Chemother 2007; 51(10): 3688-98.
12. Musiciki B, Periers AM, Laurin P, Ferroud D, Benedetti Y,Lachaud S, Chatreaux F, Haesslein JL, Ltis A, Pierre C, KhiderJ, Tessol N, Airault M, Demassey J, Dupuis-Hamelin C, Lassaigne P, Bonnefoy A, Vicat P and Klich M. Improved antibacterial activities of coumarin antibiotics bearing 5',5'-dialkylnoviose: biological activity of RU 79115. Bioorg Med Chem Lett. 2000; 10: 1695-9.
13. Koshy L, Dwarakanath BS, Raj HG, Chandra R and Mathew TL. Suicidal oxidative stress induced by certainantioxidants. Indian J Exp Biol. 2003; 41(11): 1273-8.
14. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE and Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Curr Pharm Des. 2004;10(30): 3813-33.
15. Ghate M, Manohar D, Kulkarni V, Shobha R and Kattimani SY. Synthesis of vanillin ethers from 4-(bromomethyl) coumarins asanti-inflammatory agents. Eur J Med Chem, 2003; 38(3): 297- 302.
16. Kontogiorgis CA, Hadjipavlou-Litina DJ. Synthesis and anti inflammatory activity of coumarin derivatives, J Med Chem. 2005; 48(20): 6400-8.
17. Partridge JE, Shannon LM, Gumpf DJ, Colbaugh P. Glycoprotein in the capsid of plant viruses as a possible determinant of seed transmissibility. Nature 1974; 247: 391-2.
18. Khripach VA, Zhabinskii VN, Konstantinova OV, Antonchick AP, Schneider B. Synthesis of 26-2H(3) brassinosteroids. Steroids 2002; 67: 587-95.
19. Boligon AA, Janovik, V, Frohlich JK, Spader TB, Froeder ALF, Alves SH, Athayde ML. Antimicrobial and cytotoxic activities of leaves, twigs and stem bark of Scutia buxifolia Reissek. Nat. Prod. Res. 2012; 26: 939-44.
20. Tg Siti Amirah Tg Kamazeri, Othaman Abd Samah, Muhammad Taher, Deny Susanti, Haitham Qaralleh. Antimicrobial activity and essential oils of Curcuma aeuroginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. Asian pacific journal of Tropical Medicine 2012; 202-9.
21. Meenakshi VK, Gomathy S. Chamundeswari KP. GC-MS analysis of the simple ascidian Microcosmus exasperatus Heller 1878. Inter. J. ChemTech. Res. 2012; 4: 55-62.