



Study on Ethanolic Extract of *Pitchavari*: A Native Medicinal Rice from Southern Peninsular India

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ABSTRACT

Southern peninsular India is bestowed with a large number of medicinal rice varieties, used for different ailments in folklore practices. *Pitchavari* is a native medicinal rice variety cultivated in traditional way in Tamil Nadu. The Indian *materia medica* documented that *pitchavari* is used for diarrhoea in cattle. There is a recent focus on native varieties of rice by the scientific community because rice is now considered as a potential functional food. This study is aimed to identify different phytochemicals present in this variety through GC-MS method. The ethanolic extract was used for the study. The ethanolic extract showed that *pitchavari* possesses 15 different phytochemicals having therapeutic values. *Cis-13-Octadecenoic acid* and *n-hexadecanoic acid* were the two major phytochemicals present in this variety.

Keywords: *Pitchavari*, medicinal rice, GC-MS, phytochemicals, *Cis-13-Octadecenoic acid*, *n-hexadecanoic acid*.

INTRODUCTION

Rice belongs to the genus *Oryza*, of grass family *Gramineae* (*Poaceae*) to the tribe *Oryzaceae*. There are wild as well as cultivated varieties of rice. The genus *Oryza* is distributed throughout the tropics and subtropics of the world whereas *O. glaberrima* is confined to western tropical Africa. The rice kernel is the seed of the *Oryza* plant. There are large numbers of rice varieties exists in the world. There are more than two lakhs varieties of rice identified in India.¹ The origin of rice is a debatable subject, however, molecular based evolutionary study suggests that rice domesticated in Yangtze Valley of China at about 8200-13500 years ago.²

Rice is the major staple food of 34 countries across the globe. Rice eating population are predominantly seen in countries in Asia, Pacific Region, North America, South America, and Africa. Rice provides 20 % of world dietary energy supply and is the major source of carbohydrates, protein, minerals, and vitamins to the humankind.³ Annual global rice production is estimated to be 467 Million MT (FAO 2010).

The world health organization (WHO) estimates that about 80% of the world population depends on plant extracts as primary health care measures.⁴ Natural resource and compounds isolated from plants contributes about 50% of modern medicine active compounds.⁵ Due to rapid urbanization and change in life style, the lifestyle related diseases are rampant among world urban population. All around the world there is a great attention towards alternative holistic therapies. "Food as medicine" and "medicine as the food" is the key emphasis in food and pharmaceutical industries across the globe. With the invention that oxidative stress is the causative agent for many of life style related diseases, science looked into the

vistas of nutritionally efficient, physiologically active natural compounds with less toxicity.⁶ The science and mankind has identified and agreed to explore the possibilities of a better medicine for everyday use, which lead them to arrive to a conclusion that food is the only medicine that can have wide possibilities to keep away from different diseases.⁷

Different demographic studies revealed that health care cost of aging population are expensive, in addition, there is a steady increase in life expectancy in world population.^{8,9} Japanese solution to this problem has led to the birth of Functional Food.^{10,11}

In Indian context, this basic concept of functional food had existed in the ancient Vedic scripture, the *Ayurveda*, and also in other Indian traditional systems of medicine such as *Siddha* and *Unani*.¹²

The functionality of the food is attributed by its nutritional richness, mineral (micro and macro) content, vitamins, bio active compounds and also its functional, antioxidant and other physiologically active therapeutic properties.¹³ There is a great interest in plant and plant derived phytochemicals as food source because of its divergent nutritional, functional, antioxidant and other therapeutic properties.^{14,15}

There is a revival of ancient foods which were being used by the primitives as well as native people. Rice and its different forms including by its by-products were being used by the human since time immemorial for different purposes which include ritual, medicinal and other food preparations.^{16,17}

A lot many Indian medicinal plants were screened for evaluating different therapeutic values. A recent study on *njavara*, a medicinal rice got significant attention towards

the nutritional significance of Indian medicinal rices.¹⁸ During our literature review we have observed that even though there are a large number of medicinal rices exists, an exhaustive bio-prospecting has not been done to validate the prospectable phytochemicals present in such rice's. The Indian *materia medica* documented that *Pitchavari* medicinal rice is having a curative property for diarrhoea in cattle's. This variety is cultivated and is used in many traditional food preparations in Tamil Nadu State of India. In order to understand the phytochemicals present in *Pitchavari*, we have undertaken this study. The ethanolic extract was used for the gas chromatography - mass spectrographic study.

MATERIALS AND METHODS

Rice study material

Pitchavari medicinal rice variety was a generous gift from Sri. Sithar G, a traditional farmer and a nature cure promoter from Thanjavure District of Tamil Nadu.

Sample Preparation

50 grams of the medicinal rice variety was cleaned and pulverized using pestle and motor. 2 grams of the well homogenized powdered rice was taken in a centrifuge tube. 20 ml of ethanol (chromatography grade) was added in the tube mixed for 10 min using Cyclomixer. The mixture was then centrifuged at 10000 rpm for 10 minutes. The resultant supernatant was filtered using 0.2 micron filter. The filtered supernatant is stored in a glass vial. 1 ml of the filtered supernatant is used for GC-MS analysis. 1 μ l of sample is injected into the GC column syringe in split mode.

Main instruments & Chemicals and reagents used

GC-MS analyser, instrument used Thermo fisher GC-MS/MS quantum TSQ XLS, column used DB-5MS(30m \times 0.25mm \times 0.25 μ m) pestle and motor, CM-101 Remi-Cyclomixer, Centrifuge Remi C-24 BL, 0.2 micron filter & Glass wares, Ethanol (Chromatography Grade) Distilled Water

Gas Chromatographic conditions

Column used was DBS-MS capillary column (30m \times 0.25mm \times 0.25 μ m), carrier gas helium with flow rate 1.0ml/min, initial column temperature 80°C kept for 2 minutes, and heated to 150°C with 40°C / min then to 240°C with 4°C /min, then to 255°C with 2°C/min and then 285°C with 4°C/min keeping for 3 min, split injection mode with split ratio of 10. Total run time was 44.25 min

MS Conditions

Transfer line temperature was 220°C, ion source was kept at 220°C, solvent cut at 5 min, electron energy is 70ev and mass range (m/z) is 50-650.

GC-MS analysis

1ml of sample prepared freshly was kept in auto injector vial and 1 μ l of sample is injected into the GC column using

syringe in split mode. The injection temperature was kept at 290°C. The sample was injected with split mode with a split ratio of 10. Initial Column temperature of 80°C keeping for 2 minutes and heated to 150°C with 40°C /min then to 240°C with 40°C/min then to 255 with 20°C/min and then 285 with 40°C/min keeping for 3 mints. A 30m \times 0.25mm \times 0.25 μ m thickness of DB5-MS capillary column 5% phenyl-95% dimethyl polysiloxane cross bonded liquid phase capillary column was used with 99.9995% helium as carrier gas with a flow rate of 1.0 ml/min in a split mode. The total GC cycle run time consisted of 44.25 minutes. The MS was operated in the scan mode from m/z 50 to 650. Each compound was identified by the presence of selected ions and their ratio, and by comparing the MS spectra to the reference spectra in the National Institute of Standards and Technology (NIST, ver.2.0f, 2008) mass spectral database.

Data Processing

The compounds of unknown nature were identified by computer through searching and matching NIST libraries. The compound were quantified using peak area normalization

RESULTS AND DISCUSSION

Major compounds present in the ethanolic extract of *pitchavari* rice by GC-MS method (Figure 1) were Propanoic acid, 2-methyl-(decahydro-6a-hydroxy-9a-methyl-3methylene-2, 9-dioxoazuleno (4,5-b) furan-6-yl-O-methyl-ester), n-Hexadecanoic acid (Figure 2), Cis-13-Octadecenoic acid, Oleic acid, Octadecanoic acid (Figure 3), Cis-13-eicosenoic acid, Eicosane,2-cyclohexyl, Tetradecane, 2, 6, 10- trimethyl, 2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-(all-E), Ethanol, 2-(octadecyloxy)-, a-Dglucopyranoside, O-a-D,glucopyranosyl-(1.fwdarw.3)-a-D-fructofuranosyl, N-(2-(piperazyl)-N'-(2-thiophosphatoethyl)-1, 3-propanamine, Ethyl iso-allochololate, 9, 10-secocholesta-5, 7, 10(19)-triene-3, 24, 25-triol (3a,5Z,7E)- and (+)-a-Tocopherol acetate.

The variety was found in high amount of n-Hexadecanoic acid (31%) and Cis-13-Octadecenoic acid (61%). Other relatively high compounds were Octadecanoic acid (1.3%) a-D-glucopyranoside, O-a-D,glucopyranosyl-(1.fwdarw.3)-a-D-fructofuranosyl(0.83%), Tetradecane,2,6,10-trimethyl (0.57%) and Eicosane,2-cyclohexyl (0.53%) respectively (Table 1 & 2).

Rice and it's by - products are being used by the man since time immemorial. There are a numerous varieties of rice exists in the world. Famous Indian rice scientist Dr. Richaria opinioned that during *vedic* period, India were having about four lakhs varieties of rice varieties. Many of The Indian native cultivars have lost its germplasm due to much lesser yield, ignorance about preserving valuable natural resources and also mandated Green Revolution.

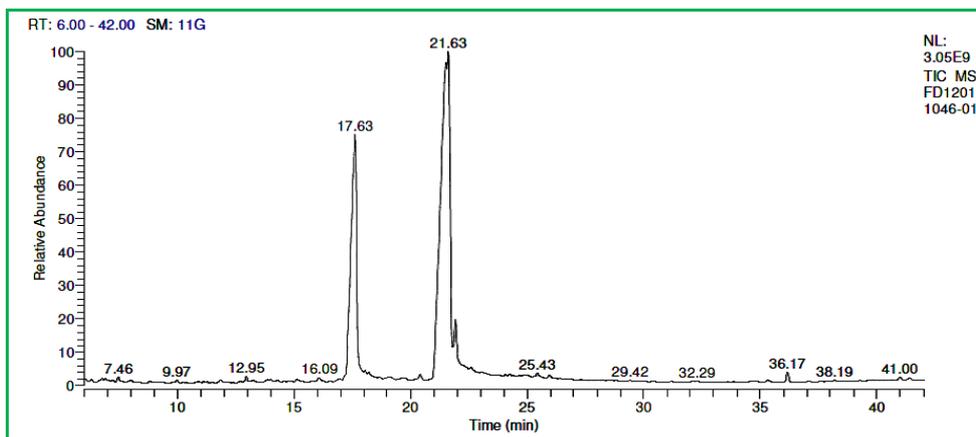


Figure 1: Chromatogram of ethanolic extract of *Pitchavari* rice by GC-MS method

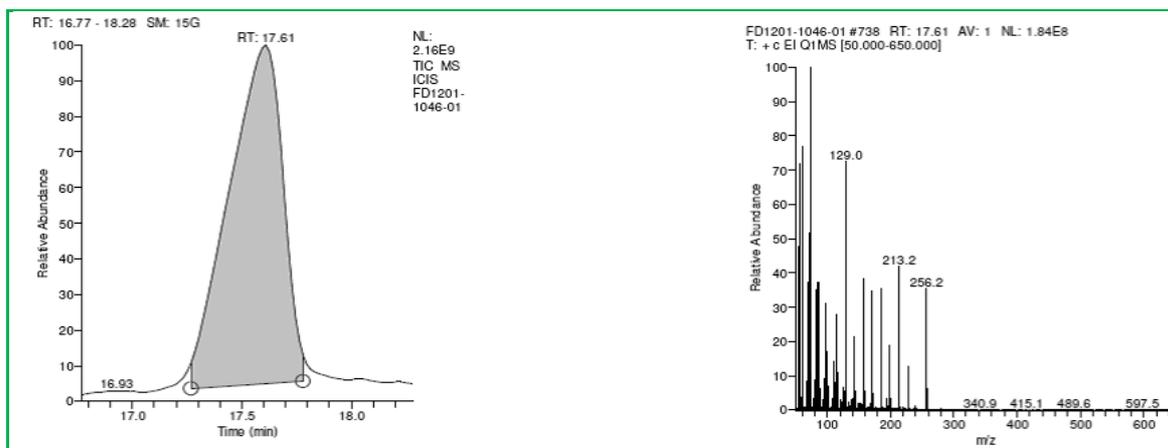


Figure 2: n-hexadecanoic acid RT peak and Mass spectrum

Table 1: Phytochemicals identified in the ethanolic extract of *Pitchavari* rice using GC- MS method

S.No	RT	Name of the compound	Molecular formulae	Molecular weight	Area In (%)
1	16.07	Propanoic acid,2-methyl-(decahydro-6a-hydroxy-9a-methyl-3methylene-2,9-dioxazuleno(4,5-b) furan-6-yl-O-methyl-ester)	C ₁₉ H ₂₆ O ₆	350	0.47
2	17.63	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	31.14
3	21.63	Cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	61.83
4	20.4	Oleic acid	C ₁₈ H ₃₄ O ₂	282	0.38
5	21.94	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	1.3
6	25.43	Cis-13-eicosenoic acid	C ₂₀ H ₃₈ O ₂	310	0.42
7	6.78	Eicosane,2-cyclohexyl	C ₂₆ H ₅₂	364	0.53
8	9.97	Tetradecane,2,6,10- trimethyl	C ₁₇ H ₃₆	240	0.57
9	36.17	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-(all-E)	C ₃₀ H ₅₀	410	0.49
10	11.85	Ethanol,2-(octadecyloxy)-	C ₂₀ H ₄₂ O ₂	314	0.19
11	5.69	α-Dglucopyranoside,O-α-D,glucopyranosyl-(1.fwdarw.3)-α-D-fructofuranosyl	C ₁₈ H ₃₂ O ₁₆	504	0.83
12	7.46	N-(2-(piperazyl)-N'-(2-thiophosphatoethyl)-1,3-propanamine	C ₁₁ H ₂₇ N ₄ O ₃ PS	326	0.25
13	25.96	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	0.32
14	41	9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol(3a,5Z,7E)-	C ₂₇ H ₄₄ O ₃	416	0.28
15	43.45	(+)-α-Tocopherol acetate	C ₃₁ H ₅₂ O ₃	472	0.54

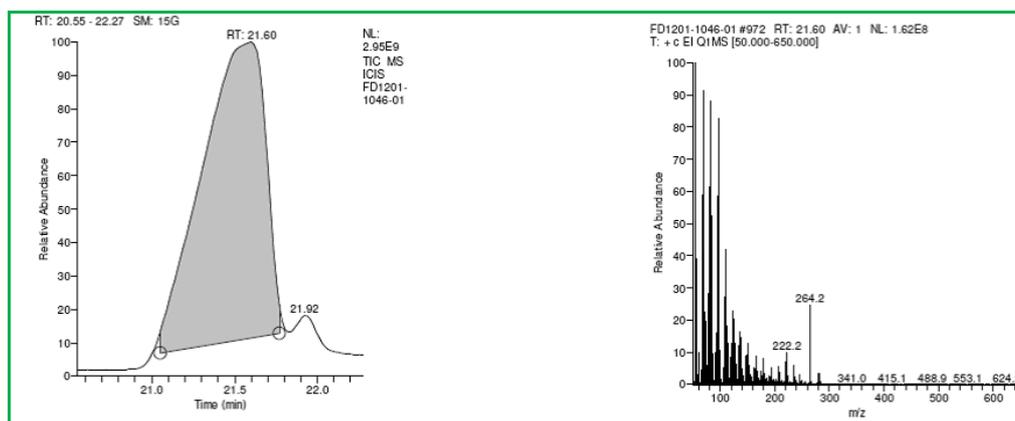


Figure 3: Cis-13-Octadecenoic acid RT peak and Mass spectrum.

Table 2: Biological activity of major phytochemicals identified in medicinal rice pitchavari

S.No	% of area	Name of the compound	Nature of the compound	Biological Activity of the compound
1	0.47	Propanoic acid, 2-methyl-(decahydro-6a-hydroxy-9a-methyl-3methylene-2,9-dioxazuleno(4,5-b) furan-6-yl-O-methyl-ester)	C ₁₉ H ₂₆ O ₆ Carboxylic Acid	Antimicrobial
2	31.14	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂ Fatty Acid	Antioxidant, Flavor, Hypocholesterolemic Hemolytic 5-Alpha reductase inhibitor Nematicide, Pesticide, Lubricant, Antiandrogenic,
3	61.83	Cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂ Fatty Acid	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
4	0.38	Oleic acid	C ₁₈ H ₃₄ O ₂ Stearic Acid	Anti inflammatory, anti androgenic, anticancer, preservative and hypocholesterolemic
5	1.3	Octadecanoic acid	C ₁₈ H ₃₆ O ₂ Fatty Acid	Flavour, nematicide
6	25.43	Cis-13-eicosenoic acid Or paullinic acid	C ₂₀ H ₃₈ O ₂ an omega-7 fatty acid	A rare omega 7 fatty acid. Reported only in Sapindaceae Family <i>Paullinia elegans</i> (Sapindaceae).
7	0.53	Eicosane,2-cyclohexyl	C ₂₆ H ₅₂ Aliphatic Hydrocarbon	
8	0.57	Tetradecane,2,6,10- trimethyl	C ₁₇ H ₃₆ Alkenes	
9	0.83	a-Dglucopyranoside,O-a-D,glucopyranosyl-(1.fwdarw.3)-a-D-fructofuranosyl	C ₁₈ H ₃₂ O ₁₆ Sugar Moity	
10	0.54	(+)-a-Tocopherol acetate	C ₃₁ H ₅₂ O ₃ Provitamin Precoursour of Vit E	Antioxidant

A large number of Indian villager's uses traditionally acquired knowledge for treatment of various ailments by using locally available plants and plant products. Rice is part of human culture and is having a significant place in traditional rituals and other medicinal preparation. There are many varieties of medicinal rice used locally, however, no exhaustive studies have been found undertaken to validate and explore the therapeutic values of many such varieties. "Food as Medicine" and "Medicine as the Food" is the theme of many of food and pharma industries across the world. "Rice – a functional food" is the new trend in the economic front.

Technological advancement also attempted to alleviate the malnutrition by developing rice varieties like "Golden Rice¹⁹" having functional properties. Indian *ayurveda* treatise is the main focus by the scientific community as well as multinational food and pharma industries. Preservation of national germplasm and resources with traditionally acquired knowledge is of paramount significance. Bio-prospecting of such varieties will cater the basics to the scientific communities for further studies.

CONCLUSION

Pitchavari is a medicinal rice documented in Indian *Materia Medica*, and this variety was subjected to GC-MS analysis using the ethanolic extract. The ethanolic extract was possessing 15 phytochemicals of therapeutic importance. The *pitchavari* rice was found to have 31% of n-Hexadecanoic acid which is having noted biological activity as an antioxidant, nematicide, flavouring compound, a potent anti-androgenic agent, a haemolytic 5-alpha reductase inhibitor. Hexadecanoic acid is otherwise known as palmitic acid, a common fatty acid present in the oil from palm trees and presence of relatively high concentration in this variety is of great pharmaceutical importance. Presence of this compound in relatively high concentration may be the reason for the stickiness while cooking the same could be the reason for the typical flavour for the dishes prepared out of this variety. *Cis-13-Octadecenoic acid* is reported to have therapeutic importance in the treatment of dopaminergic cell loss and the motor sequelae of Parkinson disease.²⁰. The quantity of *Cis-13-Octadecenoic acid* in higher side is not found reported elsewhere in any Indian medicinal rice varieties. This rice variety being a low yield cultivar, it is not an economically friendly variety to the farming communities. The medicinal claim as envisaged in *material medica* needs to be scientifically validated with further studies.

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