



GC-MS and ICP-OES Analysis of Secondary Metabolites From Traditional Rice Varieties of Andhra Pradesh, India

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Abstract: The popular varieties such as Navara, Parimala Sanna, Bahurupi, Sugandhi, and Indrani are commonly cultivated in Andhra Pradesh. Traditionally germinated red, black and brown rice has been used in Asian traditional medicine for centuries. Hence the present study is aimed to find out the metabolites from selected varieties by using standard methods. Qualitative analysis revealed the presence of a greater number of metabolites from aqueous extracts. The quantitative analysis highlighted the significant levels of flavonoids, proteins, carbohydrates, ascorbic acid and fatty acids from Parimala Sanna and Navara varieties. The GCMS analysis of Parimala Sanna aqueous extract revealed that the presence of 1,2,2-trichloroethane, 1,1-difluoro ethane, 1,2,5-oxadiazole, 4 methyl 4-pentane 2-one, formic acid, ethyl ester, 3-hydroxy-2-butanone with higher percentage. ICP OES analysis showed that the Sugandhi variety is richer in Iron, Sodium, Potassium, Sulphur and Copper than that of other varieties. This study will be helpful for isolation and identification of active principles from these rice varieties.

Keywords: *Oryza sativa*, Phytochemicals, GC-MS, ICP-OES, Secondary metabolites, Medicinal properties

Oryza sativa (Poaceae) is the most widely consumed staple food for a large part of world human populations in Asia. Traditional colored rice varieties are particularly known for their high dietary fiber, starch, flavonoids, phenols, and other beneficial compounds, that are believed to contribute to reducing the incidence of non-communicable diseases such as cardiovascular disease, diabetes, cancer, and stroke (Bhattacharyya and Roy 2018). De-husked rice has higher protein, fiber, minerals, and vitamins compared to non-medicinal varieties. It is well-suited for use in traditional Ayurvedic therapy and is known for facilitating the transfer of bioactive compounds from medicinal herbs and maintaining heat during topical body massage (Deepa et al., 2007).

Paddy is cultivated in an area of about 155 million hectares with a production of about 596 million tonnes that provides 22 per cent of the world's supply of calories and 17% of the proteins. India ranks second in production with 131 million tonnes of paddy next to China with average yield per hectare whereas, India extends from 8 to 35°N latitude and from sea level to as high as 3000 meters. Rice is a short-day plant that needs a hot, humid climate and an assured supply of water. The average temperature required throughout the life period of the crop ranges from 21 to 37°C. The crop requires a high temperature for blooming, in the range of 26.5 to 29.5°C at the time of tillering than ripening. Soils having good water retention capacity with a good amount of clay and organic matter are ideal for rice cultivation. Clay or clay loams are most suited for rice cultivation, such soils are capable of holding water for long and sustain crops. Rice being a semi-

aquatic crop grows best under submerged conditions. Rice plants are able to tolerate a wide range of soil reactions, but it does have a preference for acidic soils. It grows well in soils having a pH range between 5.5 and 6.5. It can be grown on alkali soils also, after treating them with gypsum (Ranking analytics 2024). Cultivars with growth duration of 150 to 210 days are usually photoperiod sensitive and planted in the deep water areas. Temperate day length are two environmental factors affecting the development of the rice plant that can be divided into three phases. Rice grains have been acquiring more attention from nutritionists, consumers, and health consultants in the past few years due to their greater importance in their biological activity, nutritional value, and substantial impact on human health. Due to its higher digestibility, nutritional quality, potential health, and biological activity, rice is categorized as the queen of cereal (Verma et al., 2017). Moreover, a large percentage of the Asian population heavily relies on rice as their primary energy source of food, mainly carbohydrates and a small amount of protein, recent studies have highlighted the nutritional value of rice protein (Juliano 1985 and Chaudhary and Tran 2001). The traditional varieties are a viable source of various agricultural properties as well as sources of many bioactive non-vital nutrients such as vitamin D, calcium, thiamine, riboflavin, glutamic acids, and high in fiber (Bhat and Riar 2015; Verma et al., 2020). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is an analytical technique used to determine nutritional elements in a sample (Bulcka and Wagner 2016).

Medicinal rice like Njavara contains high levels of minerals and carbohydrates and is used in treating various diseases such as arthritis, muscle wasting, and neurological disorders. Phytochemicals are bioactive compounds with antioxidant properties that are essential for safeguarding the body from diseases by delaying or inhibiting cellular damage. Medicinal plants also exhibit significant antioxidant potential due to their diverse phytoconstituents. In contrast, concerns have been raised about the potential negative health effects of synthetic antioxidants, leading to strict restrictions on their use. As a result, there is a growing trend to replace synthetic antioxidants with naturally occurring ones (Ankanna and Savithamma 2011). Rice bran have several pharmacological activities including immunomodulatory, antidiabetic, anti-inflammatory, antioxidant, anticancer, cardiovascular protective, anti-hyperlipidemic, hepatoprotective, nephroprotective, and antimicrobial, making them potential components to act as a nutraceutical with therapeutic value (Sen et al., 2020). Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample (Uma and Balasubramaniam 2012). It plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active compounds (Hethelyi et al., 1987). Furthermore, phytochemical compounds tend to accumulate in the pericarp and bran of the rice kernel. Studies have shown that rice varieties with color pigments, such as black rice, are rich in anthocyanin and other polyphenolic compounds (Muntana and Prasong 2014). The Indian Materia Medica, an Ayurvedic treatise, mentions several medicinal rice varieties in India, highlighting the need to document and conduct research on these Rice varieties (Das and Oudhia 2003). The scientific data on the nutritional and medicinal properties of popular rice varieties in India, such as Navara, Parimala Sanna, Bahurupi, Sugandhi, and Indrani, are scanty. Therefore, the present study aimed to screen primary and secondary metabolites in these rice varieties were carried out by using standard methods both qualitative and quantitatively.

MATERIAL AND METHODS

Procurement of raw materials: The study included five representative rice varieties (Fig. 1), procured from diverse regions of India. Navara rice was cultivated in Kerala, while the other varieties Parimala Sanna, Bahurupi, Sugandhi and Indrani were obtained from farmers in Andhra Pradesh.

Sample Preparation: The samples were carefully cleaned to eliminate small sand particles and impurities before being ground into a fine powder. This powder was then sifted



Fig. 1. Selected rice varieties

through a 0.5 mm metallic mesh to obtain a crude fine powder suitable for phytochemical screening using standard chemical tests and nutritional properties were estimated by ICP OES method and GCMS.

Extraction: Extractions were carried out using polar solvents such as water and methanol, as well as nonpolar solvents including benzene and hexane. For aqueous extraction, 5g of each rice powder was dissolved in 100 ml of distilled water and boiled in a water bath for 30 minutes. The mixture was filtered, and the filtrate was stored for further processing. The remaining solvents were used for extraction by macerating 5g of each powder with 100 ml of each solvent for 24 hrs, followed by filtration and storage (Peeriga and Banoth 2016 and De Silva et al., 2017).

Phytochemical Screening

Metabolite name	Reference
Carbohydrates	McCready et al., 1950
Sugars	Duboise et al., 1956
Lipids	Jayaraman 1981
Proteins	Lowry et al., 1951
Tannins	Van-Burden and Robinson 1981
Saponin	Obadoni and Ochuko 2002
Lignin	Gibbs 1974
Tannins	Trease and Evans 1985
Anthocyanins	Paris and Moyse 1969
Leucoanthocyanins	Paris and Moyse 1969
Flavonoids	Peach and Tracey 1956
Steroids	Gibbs 1974
Tannins	Trease and Evans 1985
Glycosides	Harborne 1973
Alkaloids	Gibbs 1974
Phenols	Gibbs 1974
Emodines	Harborne 1973
Ascorbic acid	Fujita et al., 1935

Screening for metabolites from selected rice varieties:

This was done as per standard procedure (Table 1).

Statistical analysis: Statistical analysis was performed using KyPlot version 2.0 beta 15 (32 bit).

RESULTS AND DISCUSSION

The phytochemical study of different rice varieties revealed that the aqueous extract of all rice grain varieties contained a greater number of compounds, primary metabolic compounds like carbohydrates, proteins, reducing sugars, ascorbic acid and fatty acids; and secondary metabolites like alkaloids, flavonoids, glycosides, terpenoids, steroids, tannins, and coumarins (Table 1). The hexane extract showing fewer compounds compared to methanol and benzene. The methanolic extracts showed moderate primary and secondary compounds in Parimala Sanna and Sugandhi, whereas, Navara showed more compounds but Indrani and Bahurupi having fewer compounds. Benzene extracts of all varieties possess proteins and Bahurupi showed carbohydrates, fatty acids and reducing sugars but Indrani had fatty acids only. In addition to primary metabolites alkaloids, tannins and glycosides presence in Parimala Sanna whereas Sugandhi and Indrani indicated almost equal compounds but Navara revealed lower compounds. Hexane extracts of all varieties contained tannins and alkaloids but in addition to these, Parimala Sanna had fatty acids.

The aqueous extracts showed more solubility of secondary metabolites than other solvents such as methanol, benzene and hexane. Among all tested varieties

Navara and Parimala Sanna have a greater number of secondary metabolites followed by Sugandhi, Bahurupi and Indrani. Primary metabolites like proteins, reducing sugars and carbohydrates were present in a greater number of solvents. Whole grain rice has a rich nutritional profile and medicinal properties (Carlos et al., 2007) and plays a crucial role in human health and provides numerous health benefits (Valarmathi et al., 2014).

Parimala Sanna has higher content of flavonoids, carbohydrates and ascorbic acid than the other varieties (Table 2) and Sugandhi only high protein content. The nutrients and phytochemicals present in rice bran are comparable to those found in other whole grain cereals like corn, oat and wheat, providing disease protection and immune system support (Baris and Yilmaz 2011). Certain traditional rice varieties such as Kattuyanam, Mapillai Samba, Navara, Karunguruvai, Kavuni, Kichadi Samba, Illupaipoo Samba, Kalana Mak, Karudan Samba and Seeraga Samba can help treat various human ailments and beneficial physiological changes in the body (Kowsalya et al., 2022). Many rice varieties are used in medical purposes such as atropine as anticholinergic and morphine as analgesic. A small amount of alkaloids is present in black rice while it is completely absent in Gobindobhog rice (Bhattacharyya and Roy 2018). The bran and germ regions of rice are the nutrient-dense whereas outer layers and sprouting parts of the grain are rich in antioxidants, phytochemicals and other beneficial compounds (Ghasemzadeh 2018). The bioactive compounds concentrated in the bran regions of rice have numerous health benefits, including reducing inflammation

Table 1. Preliminary screening of phyto-metabolites from selected rice varieties

	Bahurupi				Parimala Sanna				Sugandhi				Indrani				Navara			
	A	M	B	H	A	M	B	H	A	M	B	H	A	M	B	H	A	M	B	H
Carbohydrates	+	-	-	-	+	+	-	-	+	+	+	-	+	+	-	-	+	+	-	-
Proteins	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
Reducing sugars	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
Fats	+	-	+	-	+	-	-	+	+	-	-	-	+	-	+	-	+	-	-	-
Alkaloids	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	-	+	+	-	-	-	+	+	-	-	-	+	-	+	+	-	-
Tannins	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
Glycosides	+	-	+	-	+	+	+	-	-	+	+	-	+	-	+	-	+	+	+	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	+	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
Terpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascorbic acid	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

A=Aqueous, M=Methanol, B= Benzene, H= Hexane; '+' indicates Presence, '-'Indicates Absence

Table 2. Quantitative estimation of metabolites from selected rice varieties

Compounds	Flavonoids	Proteins	Carbohydrates	Lipids	Ascorbic acid
Bahurupi	0.12±0.01	0.8±0.14	18.8±0.63	0.12±0.1	6.5±0.20
ParimalaSanna	0.13±0.01	1.33±0.04	20.6±0.18	0.01±0.01	6.5±0.06
Sugandhi	0.02±0.01	1.75±0.30	11.11±0.52	0.05±0.02	0.43±0.04
Indrani	0.10±0.01	1.2±0.02	9.7±0.42	0.06±0.01	13±0.20
Navara	0.02±0.01	0.84±0.01	20.2±0.17	0.05±0.01	6.5±0.12

± indicates the SE of three samples

Table 3. GCMS analysis compounds in Parimala Sanna rice

Name of the compound	RT	Area %	MF	MW (g/mol)	Biological activity
1,2,2 trichloroethane	4.29	1.16	C ₂ HCl ₃ F ₂	168	Anesthetic, Analgesic, Sedative
2,2- dimethoxy propane	4.29	1.16	C ₂ H ₄ F ₂	64.05	Analgesic, Sedative
1,2,5 oxadiazole	3.77	0.59	C ₂ H ₂ N ₂ O	70	Anti-inflammatory, Anti-bacterial, Anti-viral, Anti-cancer
4 methyl 1,4 pentane 2 one	3.63	0.69	C ₆ H ₁₀ O	98	Anti-inflammatory, Anti-bacterial, Anti-cancer, Antioxidant
Formic acid	3.25	0.77	C ₃ H ₄ O ₂	72	Anti-inflammatory, Anti-bacterial, Antiseptic, Antifungal
Ethyl ester (Ethyl acetate)	3.25	0.77	H ₃ COOR	88.12	Analgesic, Antioxidant
3 hydroxy 2 butanone	3.09	0.75	C ₄ H ₈ O ₂	88	Anti-inflammatory, Anti-bacterial, Antifungal

RT: Retention time, MF: Molecular formula, MW: Molecular weight

and improving cardiovascular health (Penny et al., 2002). Traditional rice contains a range of bioactive compounds, which are present in small quantities in various fractions of the grain. These bioactive compounds exhibit diverse biological activities (Rondanelli et al., 2019).

Sugandhi showed a greater number of nutritional properties like iron, sodium, potassium, sulphur and copper than the other varieties, but Navara recorded higher volume of phosphorus and in than the other varieties. Rice is a good source of essential minerals that contains significant amounts of magnesium, phosphorus and calcium as well as trace elements like iron, zinc, copper and manganese (Verma et al., 2017 and 2020). Traditional rice is an essential nutritious food and is an excellent source of vitamin-D, calcium, thiamine, riboflavin, glutamic acid and fibre (Bhat and Riar, 2015). The rice bran contains a range of nutrients, including vitamin B9, essential amino acids and micronutrients (Goffman and Bergman 2004). The GCMS analysis revealed that a total 60 compounds were identified in aqueous extract of Parimala Sanna (Table 3). Among these 1,2,2-trichloroethane, 1,1-difluoro ethane, 1,2,5-oxadiazole, 4 methyl 4-pentane 2-one, formic acid, ethyl ester, 3-hydroxy-2 butanone showed higher percentage. Chandra Sekar and Bhagavathy (2023) reported the same results in Rice varieties like Karuppu kavuni, Mappillai samba and Seeraga samba of south India. ICP-OES analysis revealed that, among all rice varieties, Sugandhi are rich iron, sodium, potassium, sulphur and copper (Table 4). Valarmathi et al. (2014) reported the same results in Kavuni

Table 4. ICP-OES identification of nutrient components in Parimala Sanna

Nutrients	mg/ Kg dry wt
Sodium (Na)	1.34
Potassium (K)	1.44
Phosphorus (P)	0.26
Sulfur (S)	0.04
Calcium (Ca)	0.19
Magnesium (Mg)	0.03
Copper (Cu)	24.5
Manganese (Mn)	54.3
Iron (Fe)	172.5
Zinc (Zn)	33.9

in traditional rice variety of Tamil Nadu. These findings are in line with previous reports that emphasize differences in the activities of extracts obtained from the same plant using different solvents.

CONCLUSION

Rice varieties of Parimala Sanna and Sugandhi have many bioactive compounds that may help to regain immune power and maintain various metabolic reactions inside the body to dominate over a wide range of stress generated ailments due to their free radicals. Results indicate that the presence of various types of phytochemicals in aqueous extracts. Water is the best solvent for nutritional and pharmaceutical preparations from rice varieties.

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