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A STUDY ON PHYTOCHEMICAL EVALUATION OF TRADITIONAL RICE VARIETY OF TAMIL NADU - 'MAAPPILLAI SAMBA' BY GC-MS

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ABSTRACT

Rice (*Oryza sativa L.*) is the grain of life, for most of the Asians. In the olden times lakhs of traditional varieties were in vogue. But, after the intervention of high yielding varieties, the traditional varieties eroded and lost their identity. Now the new era has come, in which the traditional varieties are gaining more importance. Among the recently identified traditional varieties, Maappillai Samba is one of the varieties with red pericarp, grown widely among the farmers in Tamil Nadu. The phytochemical analysis of this variety reveals the presence of phytosteroids, flavanoids, terpenoids, tannins, saponins, carbohydrates and cardioglycosides. The GC-MS study shows the existence of more than 50% of the steroidal active compounds, viz stigmasterol, β- sitosterol and cyclolanostanol that helps in improving the Male Fertility. Also, it contains several compounds which reduces the cholesterol levels and possesses anti-cancer and anti-diabetic activity.

KEYWORDS: Traditional Paddy Variety, Maappillai Samba, Phytochemical Screening, GC-MS study, Male Fertility and Steroids

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INTRODUCTION

Rice, the life of most of the Asians, was cultivated from the time immemorial, and more than 4,00,000 rice varieties existed around the world, before the intervention of the high yielding varieties. These traditional varieties were used for medicinal purpose by traditional healers of different countries like, India, Srilanka, China, Nepal, Japan and many other countries. Hence, these varieties were named after their medicinal properties it inherits. In Chhattisgarh region, the variety "Laicha" was named as "Laicha" because of its unique property to prevent the skin infection [1]. Ancient records speak of the existence of rice varieties of curative value for various ailments, as detailed in Ayurvedic treatise of the 15th and 16th century AD. The varieties 'Njavara' and 'Gathuran' were used in the treatment of arthritis, whereas varieties 'Kalama', 'Pundarika', 'Panduka', 'Sugandhalaka', 'Kardamaka', 'Maetunaka' and 'Mahasali' have different medicinal properties. Surveys made in the 1970s and 1980s in Chhattisgarh have led to the identification of several traditional varieties of rice with medicinal utility. 'Aalcha' for the treatment of pimples, Maharaji, which gives strength and stamina to ladies immediately after delivery, 'Baisoor' for epilepsy, and 'Laicha' for pregnant women to deliver healthy children are a few examples[2]. But with the advent of hybrid revolution, farmers are gladly accepting the new varieties for higher yield. Thus millions of years of genetic diversity and germplasm heritage are vanishing forever[3]. Maappillai Samba is a pigmented traditional rice variety of Tamil Nadu. Traditionally it is given to newly wedded bridegroom to increase the natural sexual potency in alternate to synthetic viagra. This study was planned to explore the phytoconstituents of this rice variety to validate this traditional practice.

MATERIALS AND METHODS

The Maappillai Samba variety was collected from the village of Kuruvadippatti, Thanjavur. Tamil Nadu, India. The paddy was shelled and the brown rice obtained was pulverized and the ethanolic extract was used for phytochemical screening and GC-MS study.

i. Qualitative Phyto-chemical Analysis

To 10 ml of ethanol, two grams of rice powder were added and soaked for 24 hours. After filtration the extract was used for photochemical screening. The phytochemical contents, namely alkaloids, tannins, saponins, cardioc glycosides, flavanoids, terpenoids and steroids in the extracts was determined according to the standard procedures[4-6].

ii. GC-MS study

Preparation of sample for GC-MS analysis

Twenty grams of the powdered brown rice sample was soaked in ethanol at 1:2 ratio for 12 h. The extract was then filtered through filter paper and concentrated to 1ml by flushing nitrogen gas into the solution. The extract was then filtered through Whatmann filter paper No. 41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. 2µl was employed for GC-MS analysis[7]. The ethanol was selected as an extraction solvent as most of the phytochemicals in Maappillai Samba found to be polar in nature.

Instrumentation

The GC – MS analysis was carried out on a GC Clarus 500 Perkin Elmer, Carrier gas: 1ml per min, Split: 10:1, Detector: Mass detector: Turbomass gold-Perkin Elmer, Software: Turbomass 5.2, Sample injected: 2µl, Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), L × I.D. 30 m × 0.25 mm, df 0.25 µm, Oven temperature Programme: 110° C with 2 min hold ,Up to 200° C at the rate of 10 ° C/min without hold, Up to 280 ° C at the rate of 5° C / min with 9 min hold, Injector temperature 250° C, Total GC running time 36 min, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450,Solvent Delay: 0-2 min, Total MS running time: 36 min[8].

Interpretation of compounds

In the MS Programme, NIST version 2.0 library database of the National Institute of Standard Technology (NIST) having more than 2,00,000 patterns was used for identifying the chemical components of the Maappillai Samba rice variety. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components were ascertained.

RESULTS AND DISCUSSION

The result of the preliminary phytochemical screening was carried out on the ethanolic extract of the sample and revealed the presence of a wide range of phytoconstituents including alkaloids, glycosides, saponins, flavonoids, tannins, steroids supporting the reason for its wide range of biological activities as showed in table I. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. Flavanoids, on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity[9-11]. Saponin has the property of precipitating and coagulating red blood cells[12].

Table I
Phytochemical screening of Maappillai Samba rice extract

S.No.	Phytochemicals	Test	Observation
1.	Steroids	Libermann's Sterol Test	+
2.	Flavonoids	With Concentrated Hydrochloric Acid and Magnesium salt	+
3.	Terpenoids	With chloroform and concentrated Sulphuric Acid	+
4.	Tannins	With 0.1% ferric chloride	+
5.	Alkaloids	With Mayer 's reagent	+
7.	Cardiac glycosides	Libermann-Burchard's Test	+
8.	Saponins	Foam Test	+

Note: + : Positive

The components present in the ethanol extract of brown rice of Maappillai Samba rice was identified by GC-MS (Fig.1).The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of Maappillai Samba rice are presented in Table 1. Fourteen compounds were identified in the ethanol extract of Maappillai Samba rice. The prevailing compounds were hexadecanoic acid, ethyl ester (6.97%), 11-Octadecenoic acid, methyl ester (0.97%), Linoleic acid ethyl ester (7.94%), Ethyl Oleate (8.13%), Octadecanoic acid, ethyl ester (0.97%), Octadecane, 1-(ethenyloxy) (2.55%), Nonadecane (3.82%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (3.70%), Octacosane (4.24%), Heptacosane (4.31%), Ergost-5-en-3-ol, (3 α)- (8.13%), Stigmasterol (7.10%), β-Sitosterol (24.44%) and 9,19-Cyclolanostan-3-ol, 24-methylene-, (3α)- (16.74%).

Table 2
Phyto-Components identified in the Maappillai Samba rice sample by GC-MS

RT	Name of the compound	Molecular formula	Peak Area %	Biological Role**
13.00	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	6.97	Antioxidant, Wound healing, Diuretic and anti-rheumatism
14.33	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.97	Cancer preventive activity, Anti inflammatory, Hypocholestrolemic.
15.10	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	7.94	Anti inflammatory, Omega-3 fatty acids supplementation. Prevention of Cancer and Cardio Heart Disease.
15.18	Ethyl Oleate	$C_{20}H_{38}O_2$	8.13	Improves fertility, Anti coagulation and Anti oxidant.
15.53	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	0.97	Cancer preventive activity, Anti inflammatory, Hypocholestrolemic.
18.32	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	2.55	No Activity
19.73	Nonadecane	$C_{19}H_{40}$	3.82	Antibiotic and Antioxidant activity
20.28	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	3.70	Anti cancer and Hypocholestrolemic.
21.14	Octacosane	$C_{28}H_{58}$	4.24	Common cold, probiotics, antibiotic activity and osteoporosis. Rice growth stimulant and nutraceuticals.
22.54	Heptacosane	$C_{27}H_{56}$	4.31	Jaundice, intestinal worm infection, wounds, asthma and fever, malaria, syphilis, epilepsy, hemorrhoid, Stomach disorders - dysentery, constipation, diarrhea, stomach aches.
29.80	Ergost-5-en-3-ol, (3 α)-	$C_{28}H_{48}O$	8.12	Lowers blood cholesterol. Improves fertility
30.27	Stigmasterol	$C_{29}H_{48}O$	7.10	Anti cancer, Reduces cholesterol, and for Skin protection. Improves fertility
31.41	β-Sitosterol	$C_{29}H_{50}O$	24.44	Used for Colon cancer Heart disease, Hypocholestrolemic and Improves fertility
34.35	9,19-Cyclolanostan-3-ol, 24-methylene-, (3α)-	$C_{31}H_{52}O$	16.74	Used for Anti diabetic and Improves fertility

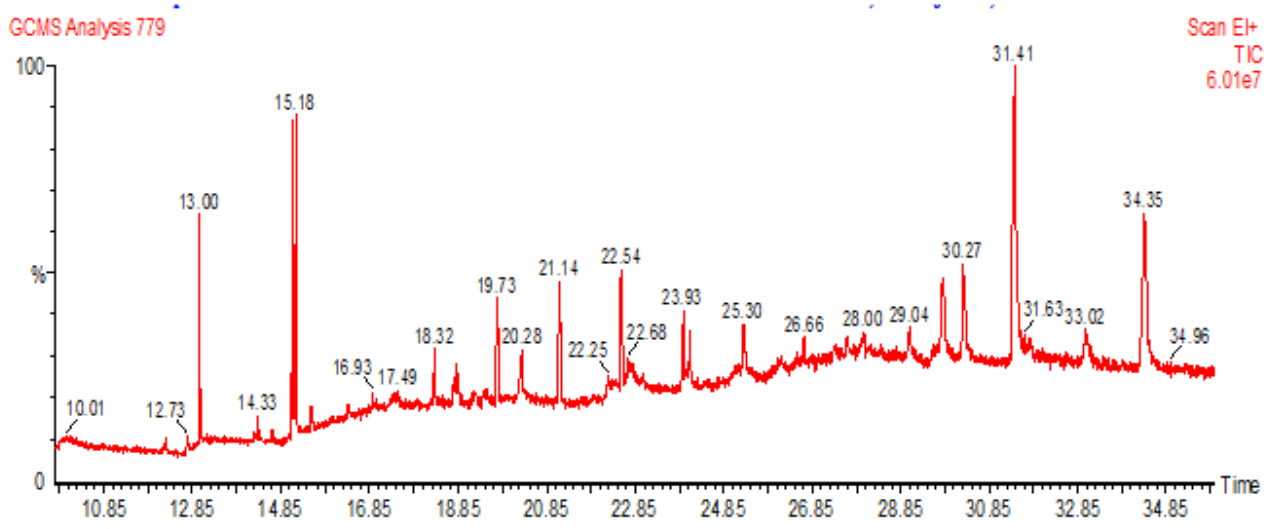

Figure 1
GC-MS Chromatogram of Maappillai Samba rice extract

Among the 14 compounds, the maximum 24.40% was observed as β- sitosterol. This component claimed as hypo cholesterolemic which improves the fertility as well as heals the colon cancer[13]. The result gives a scientific base, claimed by the traditional healers. Table 2 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the brown rice of Maappillai Samba rice. Among the identified phytochemicals, hexadecanoic acid has the property of antioxidant activity[14]. Stigmasterol is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids [15].

CONCLUSION

The study concludes that the qualitative analysis of Maappillai Samba rice variety showed vast amount of phytochemicals such as steroids, terpenoids, flavanoids, alkaloids, cardiac glycosides and reducing sugars. The GC-MS study also showed the vital sterols such as Stigmasterol, β-Sitosterol and Cyclolanostanol. The presence of these steroid derivatives confirms the traditional practice of this rice variety in improving the potence of the newly married men.

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