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PHYTOCHEMICAL STUDIES OF GERMINATED AND FERMENTED BLACK RICE SEED (*Oryza sativa*)

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ABSTRACT

Herbal medicine has been a big part of traditional health care for ages, with around 75-80% of people around the world depending on it for their basic health needs. Lately, there's been a rise in utilizing herbal extracts and products in developed countries, and sales are expected to hit over $20 billion. So, this study is all about checking out the phytochemical constituents and anti-diabetic effects of germinated and fermented black rice (Oryza sativa) and compare their efficacy. In this study, employed various method, such as collection and authentication of black rice seeds, preparing the fermented and germinated black rice, extracting phytochemicals using a hydroalcoholic solvent, and done some basic analysis on the phytochemicals. The result reveals that a variety of bioactive compounds were present, such as alkaloids, saponins, glycosides, reducing sugars, tannins, flavonoids, steroids, proteins, terpenoids, and fixed oils. In this study conclude that the hydroalcoholic extracts from both germinated and fermented black rice seeds reveals a impressive phytochemical activity, validating their traditional use in herbal medicine. Oryza sativa were promising as a natural antioxidant, anti-inflammatory, anti-diabetic, and even anti-cancer agent and their properties.

KEYWORDS: Herbal medicine, traditional health, Oryza sativa.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman 2001).

HERBAL medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now.

Plants have been used as medicines throughout history. Indeed, studies of wild animals showed that they also instinctively ate certain plants to treat themselves for certain illnesses. In Asia, the practice of herbal medicine is extremely well established and documented. As a result, most of the medicinal plants and their international recognition come from China and India.

Most of the developing countries have adopted a traditional medical practice as an integral part of their culture. Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.(krishnaraju et al.,2005)

This recent resurgence of interest in plant remedies has been spurred on by several factors (WHO et al., 2002, WHO et al., 2005, Calixto et al., 2000, Kong et al., 2003).

- The effectiveness of plant medicines.
- Source of direct therapeutic agents.
- Afforded by the people.
- Raw material base for the elaboration of more complex semi-synthetic chemical compounds.
- Models for new synthetic compounds.
- Taxonomic markers for the discovery of new compounds.
- The production, consumption and international trade in medicinal plants and phytomedicines are growing and expected to grow in future quite significantly.
- Renewable source.
- The preference of consumers for natural therapies, a greater interest in alternative medicines and a commonly held belief that herbal products are superior to manufactured products.(Nair et al., 2007)

Indian system of medicine

Ayurveda, meaning the "science of life," is said to be the oldest and most complete medical system in the world and dates back to 5000 B. C. There is no denying the benefits of Ayurvedic treatments that several Indians and others across the globe have experienced. The diagnostic and treatment procedures used are unique and are still valid today as are its foundational principles of panchamahabhutha (five basic elements of nature), tridosha (three humours) and prakrithi (individual constitution) (Venkatasubramanian et al., 2007).

Ayurveda has vast literature in Sanskrit and various Indian languages, covering various aspects of diseases, therapeutics, and pharmacy (Devet al., 1999)· the original source of Ayurveda is the Vedas and the texts known as the Samhitas, which are treatises on health care and describe medical procedures, including surgery and a form of massage of vital energy points (Ebadiet al., 2007). The earliest references to such plants are found in the Rig Veda and the Atharva Veda, dating back to the second millennium B.C.

The first recorded treatise fully devoted to the concepts and practice of Ayurveda; its primary focus was therapeutics (Charaka Samhita et al., 1949, Sharmaet al., 1981). This text sets out all the fundamental principles of Ayurveda but concentrates most of its attention on digestion (described as internal fire, or Agni). Another early classic, the Susruta Samhita, focuses on surgical techniques (Majumdar et al., 1971, Krishnamurthy et al., 1991).

The Astanga Hridayam, written in about 500 A.D., said about most of the detailed principles of Ayurveda, including the dosha and subdosha (Sharma et al., 1979).The Madhava Nidana (800-900 A.D.) was the next important milestone and it is the most famous Ayurvedic work on the diagnosis of diseases. As per Ayurveda, every material (dravya) is a manifestation of five elements (earth, water, fire, air and space) in different proportions. The material could be living as well as non-living things. Depending on the predominant combination of the elements, nature can be categorized into three doshas, namely vata, pitta and kapha.

Vata: Linked to the wind, the force that controls movement and the functioning of the nervous system in the body.

Pitta: The force of heat and energy, linked with the sun that controls digestion and all biochemical processes in the body.

Kapha: The force of water, influenced by the moon, the stabilizing influence that controls fluid metabolism in the body.

When balanced these three forces ensure that the body is healthy, but when they are "abnormal" or unbalanced, disease follows.

India has a rich cultural heritage of traditional medicines which chiefly comprised the two widely flourishing systems of treatments i.e. Ayurvedic and Unani systems since ancient times (Surana et al., 2008).

Role of 'WHO' in herbal medicine

To traditional health systems (including herbal medicine) as 'holistic' 'That of viewing man in his totality within a wide ecological spectrum, and of emphasizing the view that ill health or disease is brought about by an imbalance or disequilibrium of man in his total ecological system and not only by the causative agent and pathogenic evolution' 'WHO' probably implying that the indigenous system drugs (including herbal medicine) restore the imbalance leading to the cure of ill health or disease. However, it helped the inclusion of proven traditional remedies in national drug policies and regulatory approval by developing countries. In 1991 'WHO' developed guidelines for the assessment of herbal medicine, and the 6th International Conference of Drug Regulatory Authorities held in Ottawa in the same year ratified the same

The salient features of WHO guidelines are:

- Quality assessment: Crude plant materials or extract plant preparation and finalized product.

- Stability: Self life.
- Safety assessment: Documentation of safety based on experience and toxicological studies.
- Assessment of efficacy: Documented evidence of traditional use and activity determination (Animals and human).

Advantages

- Herbal medicine has a long history of use and better patient tolerance as well as acceptance.
- Medicinal plants have a renewable source, which is only hope for sustainable supplies of cheaper medicines for the growing world population.
- The cultivation and processing of medicinal herbs and herbal products is environmental friendly.
- Availability of medicinal plants is not a problem, especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.
- Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
- Sometimes herbal medicines prove more effective than the traditional prescribed medicines.
- The herbal medicine is costly. Herbs cost is much less when compared to the prescription medications.
- The herbal medicine is the non-existence of side effects. Also, they tend to offer long lasting benefits in terms of overall wellness.

Disadvantages

- The quality of herbal products may vary between batches, brands or manufacturers. This is much more difficult to prescribe the proper dose of an herbal medicine.
- Herbal medicines have the potential to cure many ailments, the curing period is usually longer duration when comparing to the conventional medication.
- Herbal medicines can cause allergic reactions in some cases. Before resorting to herbal medication, you need to ensure that you are not allergic to the particular herb that going to consume.
- There are many herbal remedies and medications that cause high blood pressure in the vessels of the lungs.

The main object to carry out the research in Indian medicinal plants

Plants have provided a large variety of potent drugs to alleviate suffering from diseases to mankind. In spite of spectacular advances in synthetic drugs in recent years, some of the drugs of plant origin have still retained their importance.

The use of plant-based drugs of the western world is increasing and this is because of the belief that many herbal medicines are known to be free from side effect.

A total of 122 biologically active compounds has been identified, derived only from 94 species of plants. A conservative estimate of the number of flowering plants occurring on the planet is 2,50,000. Of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated only Phytochemically.

Consistent findings should be carried out to discover a probable abundance of medicinal extracts in these plants (Turker and Usta 2008).

The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO 1996).

Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies (UNESCO1998).

The Pharmaceutical Research and Development Committee, Ministry of Chemicals, Government of India also underscores the importance of traditional knowledge (Mashelkar et al., 1998). The increasing use of traditional therapies demands more scientifically sound evidence for the principles behind such therapies and for effectiveness of medicines.

Recent advances in the analytical and biological sciences, along with innovations in genomics and proteomics can play an important role in validation of these therapies. Western scientific community views traditional medicines cautiously and stresses the concerns related to research, development and quality (Patwardhan et al., 2003) ; (Fabricant and Farnsworth et al., 2001).

PLANT PROFILE

ORYZA SATIVA

Botanical information

Botanical name: *Oryza sativa*

Family: *Poaceae*

Vernacular name

English: Black rice

Tamil: Karuppukavuniarisi

Manipur : Chak – hao

Italian: Risonero

Thai: kaoneowdom

Korean: Heukmi bap

Hindi: Kaalachaaval

Classification

Kingdom: Plantae – Plants

Superdivision: Embryophyta- Seed plants

Class: Liliopsida

Order: Poales

Family: Poaceae

Sub Family: Ehrhartoideae

Genus: Oryza

Species: *Oryza sativa* (Seed of grass species)

Description

Black rice, also known as **purple rice** or **forbidden rice**, is a range of rice types of the species *Oryza sativa*, some of which are glutinous rice.

There are several varieties of black rice available today. These include Indonesian black rice, Philippine heirloom balatinaw black rice and pirurutongblack glutinous rice, and Thai jasmine black rice. It is also known as *chak-hao* in Manipur, India and as “kavuniarisi” or “kavuni rice” in Tamil Nadu, India.

The bran hull (outermost layer) of black rice contains one of the highest levels of anthocyanin pigment found in food. The grain has a similar amount of fiber to brown rice and like brown rice, has a mild, nutty taste.

Black rice has a deep black color and usually turns deep purple when cooked. Its dark purple color is primarily due to its anthocyanin content, which is higher by weightthan that of other colored grains. Itis suitable for creating porridge, dessert, traditional Chinese black rice cake, bread, and noodle.[35]

Distribution

Oryza sativa (Black rice) is vibrant. The black rice was consumed in predynastic china and often referred to as “Emperor’s rice”. Since it was valued for its rarity and its limited availability and served as a tribute dish as per the belief, initially, black rice was introduced by Meitei king poiretonkhunthokpa during 38- 18 BC. Hence the name of the black rice which is cultivated in Manipur is named as chakhaopoireiton. However the origin of the black rice have not been clear.

Cultivation

- The demand for Black rice has been increasing in today's world because of their several biological activities due to the presence of antioxidants and phenolic compounds.
- Phenolics can donate hydrogen and act as decreasing agents. Phenolics also act as singlet oxygen quenchers and free radical hydrogen donors and due to these properties, phenolics have a protective effect on cell constituents against oxidative damage. Such antioxidant characteristics of phenolics have been shown in epidemiological studies to prevent cardiovascular, cancer, and nerve diseases. Other names of Black rice is forbidden rice, emperor's rice, and purple rice.

- Types of black rice cultivated:
- **Black Japonica Rice** - It is a combination of Black short-grain rice and mahogany medium-grain rice grown together in the same field. It has an earthy flavor with a mild, sweet spiciness.
- **Black Glutinous Rice** - It also known as Black sticky rice is short-grain rice with a sweet flavor an sticky texture. The grains are unevenly colored and are often used to make sweet dishes in Asia.
- **Italian Black Rice** - It is long-grain rice that combines Chinese Black rice with Italian rice. It has a rich, buttery flavor.
- **Thai Black Jasmine Rice** - It is medium-grain rice from Thailand that combines Chinese Black rice with jasmine rice. It has a subtle floral aroma when cooked.
- Here the example of cultivation of black rice, One local variety of Black rice is "Kalabati" which grows up to 5 to 6.5 feet in height. The duration of Kalabati is 150 days. The medium type of land is suitable for growing of Kalabati. The color of its leaves is a mix of green and purple colors. The number of tillers per plant is a minimum of 20 to 30. The total duration of the crop is 150 days so it can be called as long-duration type rice.
- After 2 to 3 months of its growth when the plant reaches a height of 2 to 3 feet, it is pruned once during its main vegetative period of growth to encourage more numbers of productive tillers which increases the total yield and productivity per unit area.
- The pruned green and purple mix leaves of the Black rice plant are used to feed the milking cows and buffaloes. There is an increase in the amount of production of milk is observed after giving Black rice leaves harvested after 2 to 3 months of crop growth as a highly nutritious (rich in antioxidants and iron) feed to cows and buffaloes.
- Rice Panicle is harvested after a period of 5 months starting transplanting from June to July till harvesting the Black rice grains from January to February. Low temperature during the winter season from September up to January is highly necessary to increase the number of productive tillers and total grain yield of Black rice per acre.
- The Black rice "Kalabati" (local Variety of Odisha) can only be grown once in a year during the Kharif season, Whereas Manipuri Black rice Chakhao is grown twice as well as thrice in a year due to shorter duration of crop growing period. It is transplanted under SRI (system of rice intensification) or Row transplanting. The traditional way of cultivating Black rice using the broadcasting process can consume 30-40 kg of seeds per acre.
- The seed rate is 5 kg/acre under SRI as recommended. Seedlings are transplanted by 25cm (row to row) x 25cm (plant to plant) to maintain 64,000 seedlings/acre. Although its yield is comparatively very low (12 to 15 quintal/acre), the price of its seed and rice is sold above 160 Indian rupees per Kilogram in the retail market all over India which is highest among all rice grown in India.

Chemical constituent of black rice

Rice is the main cereal crop preferred by majority of the world's population. It belongs to the family of grasses i.e. Poaceae. Two main fractions of rice are the hull & the caryopsis (20% of total grain weight).The outer covering (i.e. hull) consisting of two specialized leaves, lemma and palea, are responsible for covering the back and front portion of the seed. These fractions are rich in mineral silica and cellulose. Separation of hull results in obtaining the caryopsis which contains bran, endosperm and germ portion of the paddy.

The germ layer which is (2-3)% of the overall rice grain weight, comprises minerals, vitamins, fibers, proteins & phytochemicals and bran is removed from paddy by the process of milling resulting in conversion of brown rice to

white rice. The bran of black rice is a rich source of dietary fiber, essential amino acid, minerals, functional lipids, phenolic compounds, γ- oryzanols, tocopherols, tocotrienols, phytosterols, phytic acid and phytochemicals.

Thus, in case of black rice milling, lesser quantity of the bran is removed Endosperm of black rice is a good source of carbohydrate, predominantly starch & the outer sub aleurone layer constitutes mainly of protein. The main carbohydrate present in black rice is predominantly starch, which constitutes amylose & amylopectin. Amylose is simple and linear compound whereas amylopectin is branched. In non-glutinous variety of rice, (10-30) % starch is amylose while the major proportion is amylopectin.

On the other hand, glutinous rice contains amylose content in very less (<2%), very low (2–9%), low (10–20%), intermediate (20–25%), and high (25–33%) amount But, there may be compositional variations in the grain depending on variety, environmental conditions, processing, storage, management and location of production. Black rice is also an alternate choice for patients suffering from celiac disease as it is gluten free, contains low fat, sugar, cholesterol free and at the same time it is also a source of essential amino acids, free fatty acid, sterols, diglyceroidsreported that the amount of saturated fattyacid (73.40%) in black rice is more than unsaturated fatty acid (25.40%).

MATERIAL AND METHODS

Collection and authentication

Dried seeds of *Oryzasativa* were collected and authenticated Dr. P. Radha Research Officer (Botany) Sci II & i/c and Voucher (No: SSMCOP/110/4754) has been deposited in the department of pharmacognosy, SSM college of pharmacy, Jambai, Tamil Nadu, India.

Preparation of black rice

- ✓ **Fermentation of black rice**

Cooking of Rice Good quality commercially available black rice is selected for this study 200 gm of black Rice is taken in a container and washed 3 times properly and the water is drained out fully. The washed black rice is transferred to a 2-litre pressure cooker. 400 ml of RO water is added to black rice in the pressure cooker. The pressure cooker is closed with the lid and weight provided and kept on the gas stove. The rice is cooked for 15 minutes. The Pressure cooker maintains 15 lbs pressure (121 °C) for 15 minutes. The cooker is allowed to cool to room temperature without opening the lid of the Cooker. The Cooked and cooled black rice is Transferred to sterile conical flask and plug it with a cotton plug and kept it for further processing.

Fermentation of Cooked Black Rice Three numbers of 500ml conical flask with cotton plug were taken and sterilized using autoclaving at 15 lbs pressure (121°C) for 15 minutes.[34] 1 liters of RO water is taken in a 1-liter conical flask and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes [34]. 100 grams of cooked black rice prepared as per the above protocol is transferred to a 500ml sterile conical flask and 200 ml of sterile RO Water is added and it is cotton plugged. This conical flask is named as “T1”. Similarly, one more conical flask was prepared and named as “T2”.“T3”. Procedure control was prepared by adding 200 ml of sterile RO water without cooked b lack rice in the sterile conical flask and it is cotton plugged. This conical flask is named as “C”. The whole procedure was carried out under Laminar flow.

Similarly, three numbers of mud pots of 500 ml are taken and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.[34] 100 grams of cooked black rice prepared as per the 4.2.1 protocol is transferred to one of the sterile Mud pots and 200 ml of sterile RO Water is added and it is covered properly with the 10 sterile paper. This Mud pot is named as “M1”. Similarly, one more mud pot was prepared and names as M2,M3. Procedure control was prepared by adding 200 ml of sterile RO water without cooked black rice in the other sterile mud pot and it is covered with sterile paper. This mud pot is named as “MPC”. The whole procedure was carried out under Laminar flow. Both the above-mentioned conical flask of T1,T2& T3and the mud pot of M1, M2,M3 &MPC were kept in room temperature without disturbance.[40]

After 24 hours fermented rice removed from each pot and conical flask then dried properly in shadow and crushed using mortar and pestle then progressed for solubility.

Powder was dissolved in different solvent to find out its solubility by increasing polarity by trial and error basis. Then finally it was find out the powder was completely soluble in hydro alcohol in the ratio of (ethanol and water 70:30). This extract was used for both phytochemical and antidiabetic activity.

✓ **Germination of Black rice**

Germination of black rice involves soaking, draining, and sprouting the grains under controlled conditions to enhance their nutritional content and bioavailability. Here’s a step-by-step procedure:

Materials Needed:

- Black rice
- Distilled or clean water
- Strainer
- Damp cloth or paper towel
- Tray or germination container

Procedure

1. ***Selection of Black Rice***

- High-quality, unpolished black rice was chosen for the process. A pre-cooked or parboiled variety was avoided, as they may not germinate.

2. ***Washing and Cleaning***

- The black rice was rinsed thoroughly under running water to remove dirt, dust, and impurities.

3. ***Soaking***

- Soaked the black rice in distilled or clean water in a container.
- Maintained a water-to-rice ratio of **3:1** (three parts water for one-part rice).
- Let it soak for **12 to 24 hours** at room temperature (25-30°C).
- The water was changed every 8-12 hours to prevent fermentation.

4. ***Draining and Incubation***

- After soaking, the excess water was drained using a strainer.
- The soaked rice was Transferred onto a damp cloth or a germination tray.

- Covered it with a moist paper towel or another damp cloth.

5. Germination Process

- Kept the rice in a warm, dark place (temperature around **28-32°C**).
- Mist the grains with clean water every 6-8 hours to keep them moist but not waterlogged.
- Germination typically takes **24 to 48 hours**. After 48 hrs Small sprouts will begin to appear.

6. Stopping Germination

- Once the sprouts reach about **1-2 mm in length**, stopped germination.
- Rinsed the sprouted rice gently and drain well.

7. Storage

After 24 hours germinated rice removed from tray then dried properly in shadow and crushed using mortar and pestle then progressed for solubility.

Powder was dissolved in different solvent to find out its solubility by increasing polarity by trial and error basis. Then finally it was findout the powder was completely soluble in hydro alcohol in the ratio of (ethanol and water 70:30). This extract was used for both phytochemical and antidiabetic activity.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The hydroalcoholic solvent (70:30) of both fermented and black rice was used for preliminary phytochemical analysis by using standard procedures described by Gibbs (1974), Ayoolaet al.,(2008), Harborne (1973), Sofowora (1993) and Trease& Evans (1989).

ALKALOIDS

a. Mayer's test

The methanolic crude extract of black rice was mixed with Mayer's reagent (Potassium mercuric iodide solution). Formation of cream color precipitate indicates the presence of alkaloids.

b. Dragendorff's test

Methanolic black rice crude extract was mixed with Dragendorff's reagent (Potassium bismuth iodide solution). Formation of reddish-brown precipitate confirms the presence of alkaloids.

CARBOHYDRATES

a. Benedict's test

The Methanolic black rice crude extract was mixed with few drops of Benedict's reagent (alkaline solution containing Cupric citrate complex) boiled in water bath; a reddish-brown precipitate formation indicating the presence of sugar.

b. Fehling's test

Equal volumes of Fehling A (Copper sulphate in distilled water) and Fehling B (Potassium tartrate and Sodium hydroxide in distilled water) reagents were mixed with few drops of methanolic black rice extract and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

FATS

a. Stain test

The small quantity of methanolic crude extract of black rice was pressed between two filter papers; the stain on 1st filter paper indicates the presence of fixed oils.

b. Saponification test

To a small quantity of methanolic black rice crude extract, add few drops of 0.5 N of alcoholic Potassium hydroxide to which a drop of Phenolphthalein was added separately and heated in a water bath for 1 hour. The formation of soap indicated the presence of fixed oils and fats.

FLAVONOIDS

a. Shinoda test

The methanolic crude extract of black rice was mixed with few fragments of Magnesium ribbon and concentrated Hydrochloric acid was added drop wise. Development of pink scarlet color after few minutes indicates the presence of flavonoids.

b. Alkaline reagent test

The methanolic black rice crude extract was mixed with few drops of Sodium hydroxide solution. An intense yellow color was formed. Yellow color turned to colorless on addition of few drops of diluted acid, marked the presence of flavonoids.

GLYCOSIDES

a. Borntrager's test

200 mg of methanolic black rice extract was mixed with 2 ml of diluted Sulphuric acid and 2 ml of 5% aqueous Ferric chloride solution, boiled for 5 minutes which lead to oxidation of anthroquinones, indicating the presence of glycosides.

b. Kedde's test

The methanolic black rice extract was mixed with chloroform, one drop of 90% alcohol and 2 drops of 2% 3, 5 dinitro benzoic acid in 90% alcohol and made alkaline with 20% sodium hydroxide. A purple color formation, suggests the presence of glycosides.

c. Baljet test

To the methanolic black rice extract, sodium picrate is added. It shows yellow to orange color.

d. Legal test

The methanolic black rice crude extract is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline, pink or red color is produced.

PHENOLS

The extract (500mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour formation indicates the presence of phenolic compounds.

PROTEINS

a. Millon's test

The methanolic black rice crude extract was mixed with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), white precipitate appeared, which turned red upon gentle heating.

b. Ninhydrin test

The methanolic black rice crude extract when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3, trione hydrate), violet colour appears indicating the presence of amino acids and proteins in the extract.

SAPONINS

a. Froth test

Methanolic black rice crude extract was mixed in 1 ml of distilled water in a semi-micro tube, shaked well for 10 minutes. The formation of honey comb like froth (stable froth) indicates the presence of saponins.

b. Haemolysis test

0.2 ml methanolic black rice crude extract was mixed with 0.2 ml of blood (containing normal saline) and centrifuged. A red supernatant was thus resulted which was then matched with colorless control, suggesting the presence of saponins.

STEROIDS

a. Lieberman-Burchard test

Few ml of the extract is treated with 0.5 ml of CHCl3 followed by adding Conc. H2SO4 along the sides of the test tube. Formation of green color indicates the presence of steroids.

b. Salkowski test

The methanolic crude extract of black rice was mixed with chloroform and a few drops of conc. H2SO4, shaked well and allowed to stand for some time. Formation of red colour shows the positive test for steroids.

TANNINS

a. Gelatin test

Methanolic black rice extract was mixed with 1% gelatin solution (1 g of gelatin dissolved in 10 g NaCl w/v solution) containing 10% sodium chloride. Appearance of white precipitate is taken as positive test for tannins.

b. Ferric chloride test

To 5 ml of methanolic black rice crude extract, few drops of ferric chloride was added. Blue green color appeared, suggested the presence of tannins.

TERPENOIDS

A volume of 5 ml of the plant extract was mixed in 2 ml of chloroform and concentrated H2S04 was added to form a layer. A reddish-brown coloration of the interface was formed indicating the presence of terpenoids.

RESULT AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL SCREENING OF *Oryzasativa* (BLACK RICE)

Table 1: Preliminary phytochemical screening of both extracts of *Oryza sativa* (black rice).

S. No.	Phytochemical Constituents	Germinated seed grained with solvent ofhydro alcohol	Fermented seed grained with solvent of hydro alcohol
1.	Alkaloids	+	+
2.	Saponins	+	+
3.	Glycosides	+	+
4.	Reducing Sugars	+	+
5.	Tannins	+	+
6.	Flavonoids	+	+
7.	Steroids	+	+
8.	Proteins	+	+
9.	Terpenoids	+	+
10.	Fixed Oils & Fats	+	+

The preliminary phytochemical studies were reported in table: 1. The Hydro Alcohol extract showed the presence Alkaloids, and Carbohydrates, flavonoids, steroids, proteins and fixed oils etc. The literature studies show the alkaloids possess number of pharmacological activities.

SUMMARY AND CONCLUSION

In this study we done the phytochemical activity of germinated and fermented black rice with a hydroalcoholic solvent were shown to contain high phytochemical contents, corresponding to enhanced various activities such as anti-oxidant, antidiabetic, anti-inflammatory and anticancer more over these findings indicated that black rice seed should considered as a potential source of various activity for nutraceutical and pharmaceutical applications. Hence, future studies are required to explore the potential medicinal benefits.

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