International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Phytochemical and anti-bacterial screening of two traditional rice varieties, Chitti Muthyalu and Khetri Maharaj

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International Journal of Science and Research Archive, 2025, 14(02), 1643-1649

Publication history: Received on 10 January 2025; revised on 18 February 2025; accepted on 21 February 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.2.0510

Abstract

Rice is a staple crop for most of the world's population, with a rich history dating back to ancient times. In India, traditional rice varieties like Chitti Muthyalu and Khetri Maharaj are renowned for their distinct aroma and medicinal properties. However, their detailed potential for phytochemical and antimicrobial activities remains largely unexplored. This study investigated the methanolic seed extracts from two rice varieties for qualitative phytochemical composition and antibacterial properties. The preliminary phytochemical assessment revealed the presence of various bioactive compounds, including amino acids, alkaloids, proteins, carbohydrates, flavonoids, cardiac glycosides, saponins, tannins, and terpenoids. Antibacterial screening showed that both rice varieties exhibited significant activity against *Bacillus subtilis, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa*. These findings suggest that both rice varieties are rich in phytochemicals with potential antibacterial properties.

Keywords: Chitti Muthyalu; Khetri Maharaj; Methanolic Seed Extract; Phytochemicals; Anti-Bacterial Activity

1. Introduction

Rice is a dominant food for most Asian populations, providing essential nutrients and minerals, that are easily absorbed and utilized by the body. Its nutritional properties make it valuable food for healing various digestive, respiratory, and neuromuscular disorders (Bhattacharyya and Roy, 2018). Rice contains a range of beneficial compounds, including phenolics, tocopherols, tocotrienols, and γ-oryzanol, in addition to minerals, vitamins, and natural antioxidants. However, the concentration and composition of these bioactive compounds can vary significantly depending on factors such as rice variety, genetics, environmental factors, and their processing conditions (Kalita and Hazarika, 2022).

Rice grains play an important role in connecting the diet and health of the residing population in the world because; rice is one of the most produced and consumed cereals. Due to the introduction of high-yielding rice varieties, most traditional varieties are unaware to the current generations. Chitti Muthyalu is a short-grained aromatic rice variety, whereas Khetri Maharaj is a long-grained aromatic rice. Both rice varieties are traditional varieties with white grains. The present investigation aims to find the variable phytoconstituents and their antimicrobial properties of these two brown rice varieties. The findings reveal the presence of bioactive compounds such as alkaloids, carbohydrates, flavonoids, glycosides, anthraquinones, proteins, phenols, saponins, steroids, tannins, and terpenoids, exhibiting antibacterial activities.

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2. Materials and Methods

Sample collection: Whole grains of two rice varieties were collected from organic farmers in Kakinada, Andhra Pradesh, India. Small soil particles and other impurities were manually separated from the samples.

2.1. Preparation of Solvent Extracts

The cleaned grains were de-husked and made into fine powder using an electric grinder. The resultant powder was passed through a 0.5 mm metallic mesh and the obtained powder was stored in desiccators used to prepare solvent extracts. The phytochemical investigation was carried out according to formerly reported chemical tests (Durai *et al.,* 2016; Prabhavathi *et al.,* 2016; Bhattacharya and Roy, 2018).

2.2. Qualitative Analysis of Phytochemicals

Methanolic seed extracts were analyzed for phytochemical content using standard protocols, which included testing for the presence of alkaloids, flavonoids, phenols, carbohydrates, cardiac glycosides, saponins, tannins, terpenoids, quinones, and proteins.

2.3. Test for Alkaloids

A few drops of dilute HCl were added separately to the extracts and filtered. The filtrate was used for the alkaloid test.

2.4. Hagner's test

The methanolic extracts were treated with a Picric acid solution (Hagner's reagent). The appearance of a yellow precipitate confirms the presence of alkaloids.

2.5. Mayer's test

The extracts were treated with Potassium mercuric iodide solution (Mayer's reagent), and the formation of a cream-colored precipitate is a positive indicator of alkaloid presence.

2.6. Test for Carbohydrates

Small quantities of crude extracts were dissolved separately with 5 ml of distilled water and filtered. The filtrate was used for further tests

2.7. Molisch's test

Rice crude extracts were subjected to Molisch's reagent (alpha naphthol in 95% ethanol) and a few drops of concentrated HCl at the sides of the test tube. The formation of a violet ring at the junction indicates the presence of carbohydrates.

2.8. Fehling's test

Crude extracts were treated with Fehling's reagents A (Copper Sulfate in water) and B (Sodium potassium tartrate), the formation of red colour indicates the presence of carbohydrates.

2.9. Test for Glycosides

- **Borntrager's test**- Extract treated with diluted sulphuric acid, add solvent ether, shake, and filter. To the organic layer add ammonium solution, and the organic layer becomes pink to red.

- **Legal test**- The formation of red colour with Disodium nitroprusside in pyridine and sodium hydroxide, indicates the presence of glycosides.

2.10. Test for Phytosterols

Reflux the extracts with alcoholic KOH till complete saponification takes place and dilute the mixture with distilled water and extract it with ether. After evaporation of the ethereal extract, the residue was subjected to

- **Liberman Burchard's test-** To the extracts treated with chloroform add a few drops of glacial acetic acid and concentrated H_2SO_4 at the sides of the test tube, formation of red colour at the junction of two layers, and the upper layer shows green colour indicates the presence of phytosterols.
- **Salkowski test –**To the extracts add equal volumes of chloroform and sulphuric acid, the formation of red or violet colour indicates the phytosterols.

2.11. Test for Saponins

- **Foam test –** Dilute 1 ml of extract to 20 ml with distilled water and shake it gradually for 15 min, the formation of a 1 cm foam layer indicates the saponins presence.
- **Haemolysis test**
  Take two test tubes with 2ml of 1.8% NaCl solution, 2ml of distilled water was added to one test tube and 2ml of 1% extract was added to another test tube. To each test tube, 5 drops of blood were added by pricking the thumb. The contents in each test tube were gently mixed and observed under the microscope if hemolysis occurred indicating the saponins.

2.12. Test for Tannins

2.12.1. The extracts were diluted with distilled water in small quantities and treated

- **Ferric chloride test –** The extracts were treated with $FeCl_3$ solution, the formation of blue colour indicates tannins.
- **Gelatin test –** The extracts were treated with gelatin solution, and the formation of a white precipitate indicates the presence of tannins.
- **Lead acetate test –** The formation of a yellow precipitate of treated extract with lead acetate solution indicates a positive result for tannins.

2.13. Test for proteins and amino acids

Small quantities of extracts were dissolved in distilled water separately and subjected to

- **Millon's test –** The extract was treated with Millon's reagent (Hg $(NO_3)_2$ in HNO_3), and a white precipitate appeared which turned red colour upon gentle heating.
- **Biuret test –** To the extract treated with NaOH solution, Copper sulphate was added dropwise and mixed, and the treated solution turned into pink.
- **Ninhydrin test –** The extract treated with Ninhydrin with α-amino acid and ammonium. The appearance of the violet colour on gentle heat indicates the presence of amino acids in the extract.

2.14. Test for Flavonoids

- **Zinc-hydrochloric acid test –** To the alcoholic extract adding a pinch of zinc dust and concentrated HCl along the sides of test tubes, formation of magenta colour confirms the presence of flavonoid.
- **Alkaline reagent test:** The extract was treated with NaOH with dilute HCl, solubility, and coloration were noted. A yellow-coloured precipitation appeared and it turned colourless with the addition of dilute HCl confirming the flavonoids.
- **Anthraquinones (Borntrager's test)**
- The crude extract was mixed with chloroform and filtered. Adding an equal volume of 10% ammonia solution to the filtered solution pink violet or red colour in the ammoniacal layer suggests positive results.

2.15. Test for Terpenoids

To the 2 ml of Chloroform, 5 ml of the extract was added and mixed, the addition of concentrated sulphuric acid formed a reddish-brown colour interface indicating the presence of terpenoids.

2.16. Test for Cardiac Glycosides (Keller-Killani test)

The extract was dissolved in 2 ml of chloroform and sulphuric acid was added, the formation of a brown ring at interphase confirms the presence of cardiac glycoside.

2.17. Test for Fixed oils and Fats

The few drops of concentrated crude extracts were pressed in between two filter papers and kept aside undisturbed. On the filter paper oil strains of extract indicate the presence of oils and fats in the material.

2.18. Test for Phenolic compounds

- **Ferric chloride test –**To the hydrolysable extract, a few drops of 5 % ferric chloride solution was added, and a dark green colour appeared indicating the presence of phenolic compounds.

2.19. Anti-bacterial Screening

The antibacterial activity of rice extracts was evaluated using the agar well diffusion method. Briefly, 500 µl of microbial suspension was added to a nutrient agar medium, and the mixture was poured into sterile Petri dishes. The bacterial strains, *Bacillus subtilis* (MTCC-1427) and *Bacillus cereus* (MTCC-430) (Gram-positive), and *Escherichia coli* (MTCC-294) and *Pseudomonas aeruginosa* (MTCC-1748) (Gram-negative), were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

2.19.1. Media preparation and inoculation

Nutrient Agar Media (NAM) was prepared by mixing 13g in sterile distilled water, followed by autoclaving at 121^0C/15lbs pressure for 20 minutes. The sterile liquid nutrient agar was cooled to below 50^0C, inoculated with 500µl of the selected microorganism, and poured into sterile Petri dishes. After solidification, 8 mm diameter wells were created in the nutrient agar plates using a sterile cork borer. The methanolic rice extracts (150 µl) were added to each well. The plates were incubated at 37^0C for 24 hours, and the zones of inhibition were measured using a Vernier Calliper. The diameter of the zones, including the well diameter, was recorded.

3. Results and discussion

3.1. Phytochemical analysis

The phytochemical analysis of the two brown rice extracts revealed the presence of various bioactive compounds. The results are presented in Table 1.

Table 1 Qualitative analysis of phytochemicals in two brown rice varieties CM (Chitti Muthyalu), KM (Khetri Maharaj)

S No.	Name of the Phytoconstituent	Results	
		CM	KM
1	Alkaloids	+	+
2	Amino acids	+	+
3	Anthraquinones	-	-
4	Carbohydrates	+	+
5	Cardiac Glycoside	+	+
6	Fixed oils and fats	-	-
7	Flavonoids	+	+
8	Phenolic Compounds	-	-
9	Proteins	+	+
10	Saponins	+	+
11	Terpenoids	+	+
12	Tannins	+	+

Where "+" indicates the presence of the phytoconstituent, and "-" indicates the absence of the phytoconstituent

The presence of alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, proteins, saponins, terpenoids, and tannins in both CM and KM extracts indicate a rich diversity of bioactive compounds. These compounds have been reported to possess various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. The absence of anthraquinones, fixed oils and fats, and phenolic compounds in both extracts suggests that these compounds may not be present in significant amounts or absent altogether. The presence of cardiac glycosides, which are known to possess cardioprotective properties, is noteworthy. Similarly, the presence of flavonoids, which are known to have antioxidant and anti-inflammatory properties, is also significant.

3.2. Anti-bacterial activity

The anti-bacterial activity of CM and KM extracts was evaluated against four microorganisms. The results show that both extracts exhibited antibacterial activity, with KM showing slightly higher activity against *E.coli* and *P.aeruginosa*. The zone of inhibition increased with increasing concentration of the extracts. These findings suggest that CM and KM extracts have potential antimicrobial properties.

Table 2 Antibacterial activity: zone of inhibition of methanolic extracts of two rice varieties against four bacterial strains

S No	Name of the bacterial strain	Sample	Zone of inhibition observed in mm		
			10 µg/ml	50 µg/ml	100 µg/ml
1	*Bacillus subtilis*	CM	-	-	3.3
		KM	-	-	3.0
2	*Bacillus cereus*	CM	-	-	2.4
		KM	-	-	2.8
3	*Escherichia coli*	CM	-	2.2	4.9
		KM	-	3.2	6.2
4	*Pseudomonas aeruginosa*	CM	-	3.2	6.4
		KM	-	3.1	6.9

CM (Chitti Muthyalu), KM (Khetri Maharaj)

Figure 1 Antibacterial activity of methanolic seed extract of A. Chitti Muthyalu and B. Khetri Maharaj against gram-positive bacteria 1. *Bacillus subtilis,* 2. *Bacillus cereus*

The minimum inhibitory concentration for *Bacillus subtilis* and *Bacillus cereus* is 100 µg of both rice varieties. Maximum 3.3 mm (1CM) and minimum 3.0 mm (2KM) zone of inhibitions were observed for *B. subtilis* and 2.8 mm (2KM), 2.4 mm (1CM) zone of inhibitions were observed for *B. cereus*.

For gram-negative bacteria the minimum inhibitory concentration is 50 µg and the zone of inhibitions observed were 2.2 mm (1CM), 3.2 mm (2KM) for *E. coli* and 3.2 mm (1CM), 3.1 mm (2KM) for *P. aeruginosa*. The maximum inhibitory concentration is 100 µg and zone of inhibitions observed were 4.9 mm (1CM), 6.2 mm (2KM) for *E. coli* and 6.4 mm (1CM), 6.9 mm (2KM) for *P. aeruginosa.*

The antibacterial activity of rice's methanolic seed extract depended on its phytochemical composition. Compared with standard control Gentamycin, the antibacterial activity of rice extracts was less. Although the bacterial activity is less with crude extract, it may show good activity with the pure form of extracts.

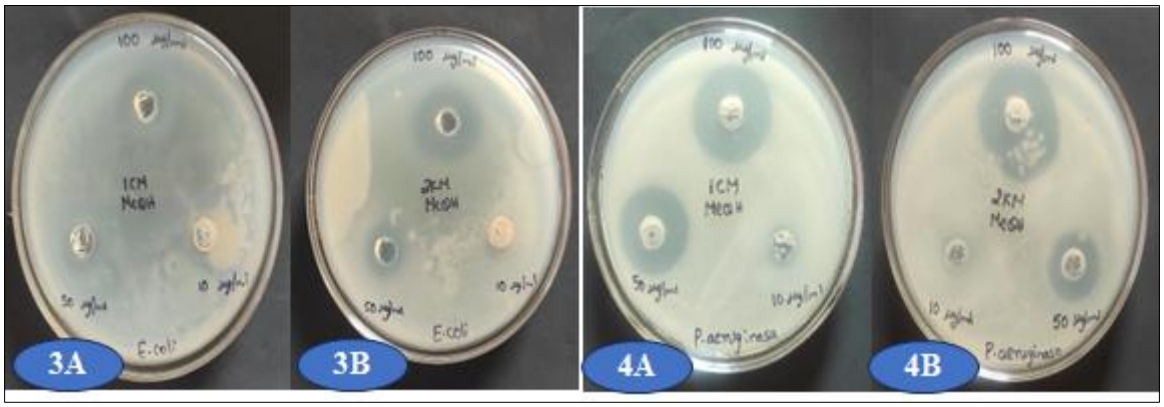

Figure 2 Antibacterial activity of methanolic seed extract of A. ChittiMuthyalu and B. Khetri Maharaj against gram-negative bacteria 3. *Escherichia coli,* 4. *Pseudomonas aeruginosa*

4. Conclusion

The phytochemical analysis of the CM and KM extracts reveals a rich diversity of bioactive compounds with potential therapeutic applications. Some non-nutritive phytochemicals may have disease-preventive properties. The preliminary phytochemical screening confirmed that the two rice varieties have alkaloids, amino acids, carbohydrates, cardiac glycosides, flavonoids, proteins, saponins, terpenoids, and tannins. The antimicrobial activity of CM and KM extracts against four microorganisms *Bacillus subtilis, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa* was demonstrated. Further studies are needed to isolate and characterize the pure compounds responsible for biological activities and to evaluate their potential applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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