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PHYTOCHEMICAL AND ANTIMICROBIAL INVESTIGATIONS OF METHANOLIC SEED EXTRACT OF BLACK RICE (*ORYZA SATIVA L.*) MENTIONED IN AN ANCIENT PALM LEAF MANUSCRIPT (TALAPATRA).

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ABSTRACT

Plants are being used as an alternative source for the development of safe, effective and inexpensive new drugs to treat and prevent bacterial infections. Recent studies have shown that black rice (*Oryza sativa* L.) is an important source of nutrients, consumed by most of the world's population, can suppress some bacterial infections. The use of black rice powder in various ayurvedic preparations was noticed in our ancient palm leaf manuscript. Our current study stems from this observation that black rice seed powder might be having lot of medicinal value. Keeping this in mind, preliminary phytochemical screening of methanolic seed extracts of black rice, *Oryza sativa* L. was carried out with an intention of finding the type and diversity of phytochemicals present in it. The phyto constituents in the plant were extracted using methanol as solvent. The extracts showed positive results for biologically active compounds like alkaloids, flavonoids, phenols and tannin compounds. The methanolic seed extract of black rice (*Oryza sativa* L.) was used to evaluate the antimicrobial activities. Antimicrobial activity of methanolic black rice seed extract was evaluated by determining the minimum inhibitory concentration and zone of inhibition. Different pathogenic organisms including 3 gram positive bacteria (*B. subtilis, S. aureus* and *P. pyogenes*), 4 gram negative bacteria (*P. aeruginosa, K. pneumoniae, Salmonella species* and *E.coli*) and two fungal species (*A.niger and C. albicans*) were evaluated for antimicrobial activity. The methanolic

extract of black rice seed showed high inhibition activity on all 3 gram positive bacteria and greater inhibition activity on *Salmonella species* among 4 gram negative bacteria. There was no antifungal activity by methanolic seed extract of black rice. This study indicates that the black rice seed contains a variety of chemical compounds that might be contributing to antimicrobial activity, which could be used for food additives and the development of useful natural antimicrobial compounds. As mentioned in our ancient talapatra, this study affirms that black rice grain powder can be used in the preparation of various antimicrobial agents.

KEYWORDS: Black rice, Methanolic extract, Phytochemicals, Flavonoids, Anti microbial activity.

1. INTRODUCTION

The black rice *(Oryza sativa L.*) is a super food, is an important source of nutrients consumed by most of the world population. It is a special cultivar of rice, rich in anthocyanins in the aleuron layer (Chaudhary, 2003) regarded as health promoting food and widely cultivated and consumed since ancient times in China, India, Thailand and other eastern Asian countries (Ling et al., 2002 and Kong et al., 2008). In ancient China, black rice is known as forbidden rice and it was considered as superior and rare, it was cultivated in very small amounts under protection, because it was reserved exclusively for the emperor's consumption. In China, the black rice is claimed to be good for the kidney, stomach and liver. Chinese black rice is rich in iron and is considered as a blood tonic. This rice is supposed to enhance longevity of life; hence it is also known as Long life rice. This is also called as Heaven rice, Imperial rice and King's rice.

Black rice has diversity in terms of colour due to anthocyanin content and other morphological characters. Looking black rice in the morning is an indication that the whole day will be successful. Based on historical record, black rice was only for the kings of China and Indonesia, so called forbidden rice (Rao et al., 2006). This is because black rice has a double function, namely as a source of staple food with good taste, fluffier and fragrance, as well as an efficacious medicine to cure various illnesses (Kristamtini, 2009). Thus the black rice is considered as a functional food in Indonesia (Pratiwi and Purwestri, 2017).

2. Black rice (*Oryza sativa L.*)

Kingdom : Plantae

Clade : Angiosperms

Clade : Monocots
Clade : Commelinids
Order : Poales
Family : Poaceae
Genus : *Oryza*
Species : *sativa*
Binomial name: *Oryza sativa Linn*.

Black rice is a herbaceous monocotyledon plant belonging to grass family Poaceae. It is distributed and cultivated in China, India, Thailand and other Eastern Asia countries (Ling et al., 2002; Kong et al., 2008). Only China is responsible for 62% of global production of black rice followed by Srilanka, Indonesia, India and Philippines. Thailand occupies the ninth position to black rice cultivation (Ichikawa et al., 2001; Sompong et al., 2011). Of all the coloured rice, especially black rice has long been consumed and is considered as a healthy food in Korea (Medhabhati et al., 2014).

In India, black rice is indigenous to north-east India and is extensively grown in Assam and Manipur states. Assam is rich in production of black rice. It is commonly eaten in Manipur because of its medicinal value called *chak-hao*. Chak means rice and ahaoba means delicious, therefore Chakhao means delicious rice. This black rice is eaten during traditional feasts (Devi et al., 2017) in Manipur and also served in standard hotels as a top rated rice. *Chak-haokheer* is a popular pudding in these regions and the water in which black rice is boiled is used in these parts to wash hair, in the belief that it makes hair strong. The ancient Indian ayurvedic practitioners noticed the importance and medicinal properties of black rice for treatment to Jaundice, fibroids and cancer wounds and conserved this information on in palm leaf manuscript (Talapatra) which are one of the ancient manuscripts of India (Mallikarjuna and Chandramouli, 2014).

Black rice is a whole grain is one of the potent functional food sources since it contains high amounts of phenolic compounds, especially anthocyanins in pericarp (Abdel et al., 2006; Ryu et al., 1998 and Yawadio et al., 2007). Black rice is getting popular in recent years because of its high nutritive value and anti oxidant properties. Consumption of black rice has resulted to atherosclerotic lesions (Ling et al., 2001), reduce oxidative stress and inflammatory (Xia et al., 2003) or cardiovascular protection (Xu et al., 2001). Anthocyanins are in the flavonoid pigments of black rice and are source of antioxidants which are able to prevent oxidative

stress. The pericarp of kernel of this rice colour is black due to a pigment known as anthocyanin, an antioxidant. Black rice special and medicinal values are truly stunning even today with all of our medical knowledge and tools. Thus black rice is a kind of food that can make us healthy and save our life.

Very recently rice researchers have begun to study the varieties of black rice to discover its medicinal and nutritional properties (Suzuki et al, 2004; Kristamtini, 2009). It has antimicrobial, antioxidant (Zhang et al., 2010; Chutipaijit et al., 2011), anti inflammatory, anti arthritic, hepato protective, anti-carcinogenic properties (Thomasset et al., 2009; Chen et al., 2006). This rice is becoming popular among rice consumers and dieticians day by day mainly because of its highly nutritive and medicinal value and also described as organic antibiotic.

However in India, the much research work has not been done on black rice. We came to know about black rice while documenting the ayurvedic knowledge present in an ancient palm leaf manuscript (talapatra) of our ancestors belonging to Rayalaseema region of Andhra Pradesh into digital form. Among all medicinal plants, the black rice has brought us a great attention owing vast literature citing its importance. In the present work, we are reporting the antimicrobial activity of black rice grain extract.

3. MATERIALS AND METHODS

Fig. 1: The ancient telugu palm leaf manuscript of Rayalaseema region of Andhra Pradesh.

3.1 Seeds collection and plant identification

The fresh black rice grains were collected from a farmer by name Yarakam Varaprasad Reddy, Nagula palli village, Kothapalli mandal, East Godavari district who has cultivated black rice in his fields. The plant was identified with the help of the Botanical survey of India, Hyderabad, Andhra Pradesh, India as Oryza sativa Linn. We tried to cultivate the black rice in earthen pots to study vegetative and floral characters. The seeds showed poor germination and fewer yields.

Fig. 2: The habit of black rice plants.

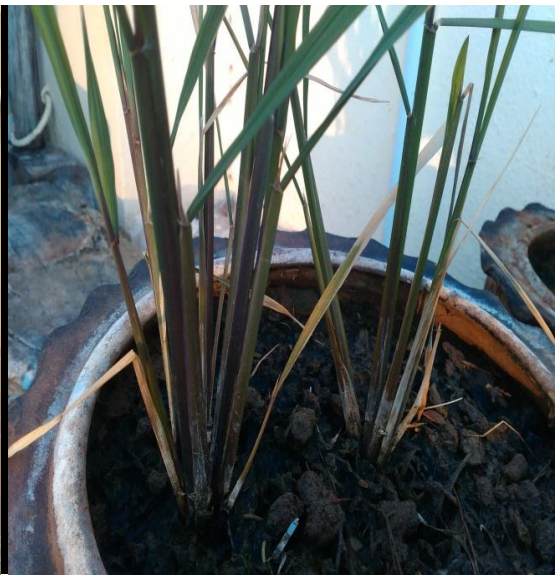


Fig. 3: Closure view of black rice.

Fig. 4: Vegetative stage of black rice.

Fig. 5: Flowering stage of black rice.

3.2 Extraction

The seeds were collected seeds from above plants and were de husked and made into course powder and the extraction process done with different solvents like Aqueous, Methanol, Chloroform and Hexane with occasional shaking for 72 hrs. But the black rice powder dissolved only in methanol but not in other solvents. The contents was filtered separately and collected each solvent and concentrated on rotary vapour using round bottom flask and the final crude extract was collected and preserved in refrigerator at 4^0C to avoid contamination for further study. The obtained extracts were used for preliminary phytochemical screening and antimicrobial activities.

4. Preliminary phytochemical screening

The methanolic crude extract of black rice was used for preliminary phytochemical screening by using standard procedures described by Gibbs (1974), Ayoola et al.,(2008), Harborne (1973), Sofowora (1993) and Trease & Evans (1989).

ALKALOIDS

Mayer's test

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

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

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.

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

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.

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

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 colour after few minutes indicates the presence of flavonoids.

Alkaline reagent test

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

GLYCOSIDES

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.

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 colour formation, suggests the presence of glycosides.

Baljet test

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

Legal test

The methanolic black rice crude extract is dissolved in pyridine, sodium nitro prusside solution is added to it and made alkaline, pink or red colour 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

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.

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

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.

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 colourless control, suggesting the presence of saponins.

STEROIDS

Lieberman-Burchard test

Few ml of the extract is treated with 0.5 ml of $CHCl_3$ followed by adding Conc. H_2SO_4 along the sides of the test tube. Formation of green colour indicates the presence of steroids.

Salkowski test

The methanolic crude extract of black rice was mixed with chloroform and a few drops of conc. H_2SO_4, shaked well and allowed to stand for some time. Formation of red colour shows the positive test for steroids.

TANNINS

Gelatin test

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

Ferric chloride test

To 5 ml of methanolic black rice crude extract, few drops of ferric chloride was added. Blue green colour 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 H_2S0_4 was added to form a layer. A reddish brown coloration of the interface was formed indicating the presence of terpenoids.

5. ANTI BACTERIAL ACTIVITY

5.1 Preparation of extract

Methanolic crude extract of black rice was collected and prepared different concentrations and then used to test the anti bacterial activity.

5.2 Test organisms

Gram positive strain: *S. aureus, B. subtilis, S. pyogenes.*

Gram negative strain: *E. coli, P. aeruginosa, K. pneumoniae, Salmonella species.*

Standard drug for gram positive: Norfloxacin.

Standard drug for gram negative: Ciprofloxacin.

5.3 Luria-Bertani broth medium preparation for antibacterial activity

Required amount of Luria-Bertani broth powder was weighed and added to a 1 litre Erlenmeyer flask containing 800 ml of distilled water. It is stirred to dissolve into solution and transferred the solution to a 1 litre volumetric flask and madeup into 1 litre. Then transferred the 1 L media solution to a 1 L media storage bottle and labelled with appropriate information. Then autoclaved the solution at 121°C for 15 min to ensure that, it has sterilized of all foreign matter and contaminants. After cooling of the broth, using a sterile pipette tip, a single colony was selected from a pure culture plate and dropped it into the liquid Luria-Bertani broth and swirled well and incubated at 37°C for 24 hrs in shaking incubator at 120 rpm.

5.4 Agar well diffusion assay

The anti bacterial activity of the methanolic crude extract of black rice was carried out using agar well diffusion method by Cheruiyot et al., 2009 with some modifications. Weighed Luria-Bertani Agar as per requirement and dissolved in distilled water as per the guidelines given by manufacturer. The medium was sterilized by an Autoclave at 121°C for 15 minutes. After autoclaving, the sterilized media poured into sterile petri plates and kept for solidification. A cork borer was taken and sterilized by autoclave and aseptically punched 4 holes on a LB agar plate. Marked the underside of the petri plate to label the wells using a marker. Aseptically spread 20µl of the indicator organism *Staphylococcus aureus, B. subtilis and S. pyogenes* (Gram positive) and *E. coli, P. aeruginosa, K. pneumonia and Salmonella species* (Gram negative) on to the LB agar plate. The petri plates kept aside for 5 minutes and poured 10µl, 25µl, 50µl, 75µl and 100µl of the methanolic crude extract of black rice and the standard drug Norfloxacin and Ciprofloxacin in the appropriate wells. All petri plates were Incubated at 37°C for 12-16hrs. The zone of inhibition was measured in millimetres, using a ruler on the underside of the plate. The relative antibacterial potency was calculated by comparing its zone of inhibition with that of the standard drug.

6. Anti fungal activity

The methanolic crude extract of black rice was used to test the anti fungal activity.

6.1 Test organisms

Standard inoculum of *Aspergillus niger* and *Candida albicans* were used.

6.2 Preparation of potato dextrose agar medium

Weighed out required amount of Potato Dextrose broth and added to a 1 L Erlenmeyer flask containing 800 ml of distilled water and stirred to dissolve into solution. transferred the solution to a 1 L Volumetric flask and madeup to 1 L. Transferred the 1 L media solution to a 1 L media storage bottle and labelled with appropriate formation. The prepared medium was sterilized by an autoclave at 121°C for 15 min to ensure that the Potato dextrose is sterilized of all foreign matter and contaminants.

6.3 Agar well diffusion assay

After autoclaving, the sterilized media is poured into sterile petri plates and kept for solidification. A sterilized cork borer was taken and punched 5 holes aseptically on a potato dextrose agar plates. All petri plates are marked underside to label the wells by using a marker. A standardized inoculum culture was spread evenly on the surface of gelled agar plates. All the petri plates were kept aside for 5 minutes and poured 10µl, 25µl, 50µl, 75µl and 100µl of the methanolic black rice crude extract in the appropriate wells. The petri plates were incubated at 25°C for 72 hrs. The zone of inhibition was measured in millimetres using a ruler on the underside of the plate.

7. RESULTS AND DISCUSSION

7.1 Preliminary phytochemical screening (Table. 1)

The preliminary phytochemical analysis of the methanolic crude extract from the seeds of black rice is shown in table 1. The curative properties of black rice are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, terpenoids, tannins etc.

Table 1: Phytochemical analysis of methanolic crude extract of black rice.

S. No	Name of the compounds	Result
1	Test for Alkaloids	+
2	Test for Carbohydrates	+
3	Tests for Fats	+
4	Test for Flavonoids	+
5	Test for Glycosides	+
6	Test for Phenols	+
7	Test for Proteins	+
8	Test for Saponins	-
9	Test for Steroids	-
10	Test for Tannins	+
11	Test for Terpenoids	+

Table 1 indicated the presence of different phytochemical components of black rice. The phytochemical studies of black rice has been carried out previously and showed the presence of secondary metabolites such as phenols, alkaloids, tannins, anthocyanins and flavonoids. Anthocyanins are flavonoid group of plant pigments which are potential antioxidants with many colours (Hansakul et al., 2011). Previous studies explained that the black rice contains high amounts of phenolic compounds, especially in pericarp (Abdel et al., 2006; Ryu et al., 1998 and Yawadio et al., 2007). Alkaloids, phenols and flavonoids are the main secondary metabolites reported in all most all black rice varities (Sompong et al., 2011; Hou et al., 2013).

In Andhra Pradesh, the phytochemical and anti microbial studies were carried out in wild rice *Oryza rufipogon* (Devi and Yasodamma, 2016). Our data is compared with the phytochemicals of *Oryza rufipogogon* from nallamalla forest area (Devi and Yasodamma, 2016). They reported the presence of alkaloids, flavonoids, phenols, terpenoids, steroids, saponins and glycosides from the husk, stem and root by alcohol and methanol extract. This indicates that almost similar phytochemicals reported but seeds exhibited poor number of alkaloids, phenols and flavonoids but with complete absence of terpenoids (Devi and Yasodamma, 2016). In our study, good amount of phytochemicals from seeds such as alkaloids, flavonoids, glycosides, phenols and tannins are found which might play a major role in pharmacological activities. Saponins and steroids are not reported from our study.

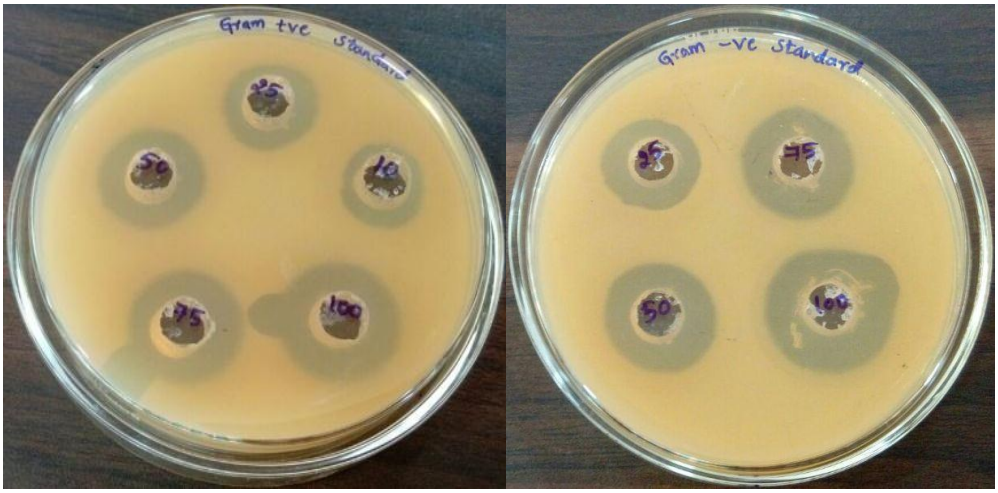
7.2 Anti bacterial activity

The anti bacterial activity of standard drugs (Norfloxacin for gram positive and Ciprofloxacin for gram negative bacteria) showed positive results as shown in table 2.

Table: 2. Anti bacterial activity of standard drugs (Norfloxacin and Ciprofloxacin).

Strain	Zone of inhibition in mm (10 µl)	Zone of inhibition in mm (25 µl)	Zone of inhibition in mm (50 µl)	Zone of inhibition in mm (75 µl)	Zone of inhibition in mm (100 µl)
S. aureus (Gram positive)	4	9	10	11	13
E. coli (Gram negative)	-	11	13	15	16

Zone of Inhibition in mm; A: Gram positive standard Norfloxacin; B: Gram negative standard Ciprofloxacin.

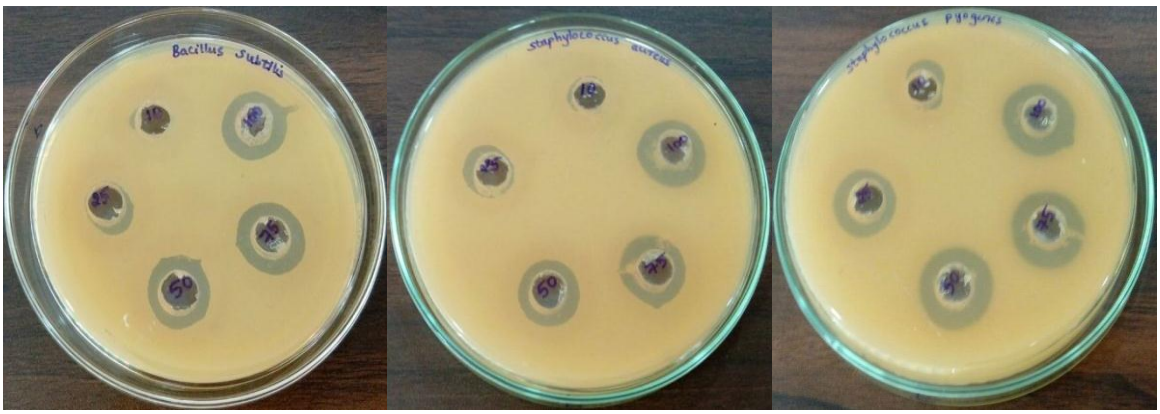


A **B**

Fig: 6. Anti bacterial activity of standard drugs
A: Norfloxacin for gram positive bacteria. B: Ciprofloxacin for gram negative bacteria.

Table: 3. Anti bacterial activity of methanolic extract of black rice showing zone of inhibition for gram positive bacteria.

Name	Zone of inhibition in mm (10µl)	Zone of inhibition in mm (25µl)	Zone of inhibition in mm (50µl)	Zone of inhibition in mm (75µl)	Zone of inhibition in mm (100µl)
B. subtilis	0	2	6	10	12
S. aureus	0	2	6	10	12
S. pyogenes	2	6	8	10	12

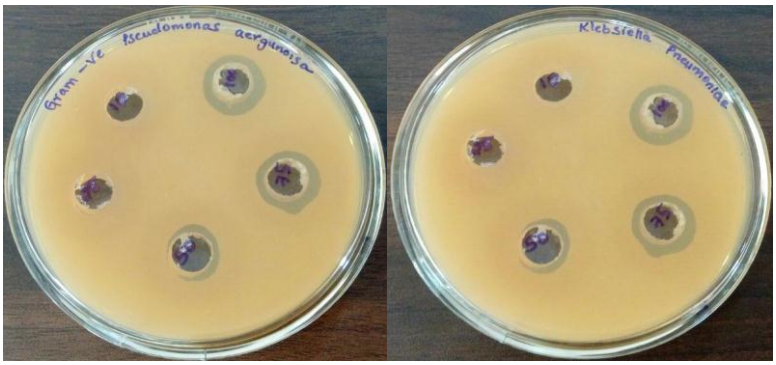


A **B** **C**

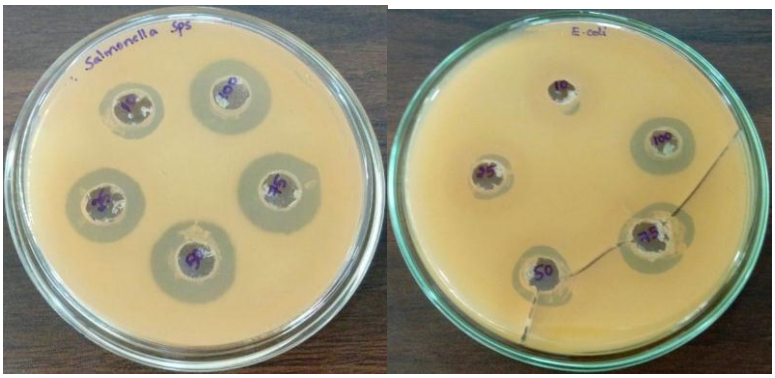
Fig: 7. Anti bacterial activity of methanolic extract of black rice against gram positive bacteria. A: *Bacillus subtilis*; B: *Staphylococcus aureus*; C: *Staphylococcus pyogenes.*

Table: 4. Anti bacterial activity of methanolic extract of black rice against gram negative bacteria with zone of inhibition. A: *Pseudomonas aeruginosa*; B: *Klebsiella pneumoniae*; C: *Salmonella sps*; D: *E. coli.*

Name	Zone of inhibition in mm (10µl)	Zone of inhibition in mm (25µl)	Zone of inhibition in mm (50µl)	Zone of inhibition in mm (75µl)	Zone of inhibition in mm (100µl)
P. aeruginosa	0	0	2	6	8
K. pneumonia	0	0	2	6	8
Salmonella	4	8	10	12	14
E. coli	0	1	2	4	8



A **B**



C **D**

Fig: 8. Anti bacterial activity of methanolic extract of black rice against gram negative bacteria. A: *Pseudomonas aeruginosa*; B: *Klebsiella pneumoniae*; C: *Salmonella Sps*; D: *E. coli.*

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because bacterial diseases are one of the major causes of mortality in these countries. Medicinal plants have been found to be useful in the cure of a number of diseases including bacterial diseases. Black rice is one among the medically important plants. Methanolic crude extracts of black rice was used to study anti

bacterial study. The antibacterial activity of the methanolic extract of black rice seeds was depended on its phyto chemical composition. The anti bacterial studies of methanolic seed extract of black rice showed an excellent antibacterial activity against gram positive and gram negative bacteria. These results indicated that the presence of broad spectrum of antibiotic compounds in black rice.

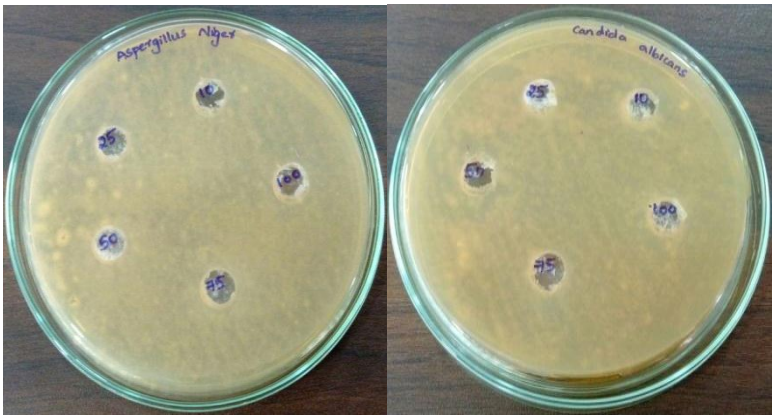
Antibacterial activity of methanolic extract of black rice were tested against *B. subtilis, S. aureus* and *S. pyogenes* from gram positive and *P. aeruginosa, K. pneumonia, Salmonella species* and *E. coli* from gram negative bacteria. The diameters of the inhibition zones were measured in millimetre.

The minimum inhibitory concentration for *B. subtilis* and *S. aureus* is 25 µl and maximum is 100 µl. 2mm of minimum and 12 mm of maximum zone of inhibitions were observed for both the bacteria. For *S. pyogenes* the minimum inhibitory concentration is 10 µl and zone of inhibition is 2 mm and maximum is 12mm at100 µl (Table. 3). In gram negative bacteria, the minimum inhibitory concentration for *P. aeruginosa* and *K. pneumonia* are 50µl with 2mm zone of inhibition and maximum is 8mm at 100 µl. In *E. coli* the minimum inhibitory concentration is 25 µl with 1mm zone of inhibition and maximum 8mm. Among all gram negative bacteria, the *Salmonella* species showed minimum of 4mm zone of inhibition at 10 µl and maximum of 14mm zone of inhibition at 100 µl (Table. 4). Methanolic and ethanolic extracts of Njavara rice (*Oryza sativa* L.) also showed anti bacterial activity against all above mentioned species (Paul and Moolan, 2014). The methanolic and alcoholic husk extracts of wild rice (*O. rufipogon*) showed more anti bacterial activity (Devi and Yasodamma, 2016) with good zone of inhibition. The basic understanding of the antibacterial properties of coloured rice against skin and wound pathogens were studied and the antibacterial activity of colored-rice crude extracts against common bacteria causing skin and soft-tissue infections were reported (Pumirat and Luplertlop, 2013). The crude methanolic extract of ordinary white rice husk (*Oryza sativa L.*) was investigated for possible phytochemical and antimicrobial activities (Saha et al., 2014).

7.3 Anti fungal activity

Table: 5. Anti fungal activity of methanolic extract of black rice against *A. niger* and *C. albicans.*

Name	Zone of Inhibition in mm (10µl)	Zone of Inhibition in mm (25 µl)	Zone of Inhibition in mm (50µl)	Zone of Inhibition in mm (75µl)	Zone of Inhibition in mm (100 µl)
Aspergillus niger	0	0	0	0	0
Candida albicans	0	0	0	0	0



A **B**

Fig: 9. Anti fungal activity of methanolic extract of black rice against A: *Aspergillus niger* and *B: Candida albicans* showing no zone of inhibition.

The anti fungal activity of methanolic extract of black rice against two fungal species, *A. niger* and *C. albicans* tested. But no significant anti fungal activity and zone of inhibitions were observed (Table. 5 and Fig. 9) in our study. In *Oryza rufipogon* species, there was a significant anti fungal activities on these two fungal species with good zone of inhibition (Devi and Yasodamma, 2016).

8. CONCLUSION

The preliminary phytochemical analysis showed the presence of many phyto constituents which would have played a role in the pharmacological activities. Secondary metabolites are the phytochemicals which are non-nutritive plant chemicals that have protective or disease preventive properties. In this study, the phytochemicals were isolated by using methanol as solvent from seeds of black rice. Preliminary phytochemical studies confirmed that the black rice is the rich source of alkaloids, phenols, flavonoids, tannins and carbohydrates. *Staphylococcus pyogenes, Salmonella* and *E.coli* bacterial strains are highly sensitive to methanolic seed extract of black rice. Incorporation of black rice crude extracts into

medicines to protect from the bacterial infections suggested. However, further intensive research in animals and humans is required, to verify the safety, efficacy and mechanism of action of these extracts. The biological activities of pure compounds present in black rice extract also need to be assessed in the above way.

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