Research Article

**In-Vitro Pharmacological Activity of Flavonoid Isolated from Njavara (Oryza sativa)**

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Received 7 April 2014; Accepted 16 April 2014; Published 21 May 2014

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**Abstract**

Rice is believed that some to have medicinal properties. In kerala, the variety navara is believed to have medicinal properties and is used to rejuvenate the nerves in properties and is used to rejuvenate the nerves in paralytic condition. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antioxidant activities. Our main objective in this investigation was to isolate the flavonoid compound from Njavara rice bran ethanolic extract and to analyze the *In vitro* Anti inflammatory Activity and Anti oxidant activity of Flavonoid and Njavara Rice. The sample possesses Alkaloid, Carbohydrate, Tannin, Flavonoid and Coumarine. The flavonol was isolated and characterized by UV and FTIR. The result indicted that the flavonols and paddy has significant anti-inflammatory property. In this present study, we have evaluated the free radical scavenger activity of flavonol and paddy which is possesses good antioxidant activity. Thus the selected sample is used as an analgesic to cure the skin inflammations and other related skin infections.

**Keywords:** Njavara, Flavonoids, Anti inflammatory and Anti-oxidant

**Introduction**

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses [1]. Cell injury may occur due to trauma, genetic defects, physical and chemical agents, tissue necrosis, foreign bodies, immune reactions and infections. Inflammation is a local reactive change that involves the release of antibacterial agents from nearby cells that defend the host against infection. In spite of our dependence on modern medicine and tremendous advances in synthetic drugs and a large number of populations (80% of the people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicine, which are derived from the plant material. The main action of anti-inflammatory agents is the inhibition of
Cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins.

**Antioxidant**

Reactive oxygen species (ROS) including super oxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors. A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases. Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer’s disease and cancer have appeared during last three decades [2]. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action. There is currently immense interest in natural antioxidants and their role in human health and nutrition. Considerable amount of data have been generated on antioxidant properties of food plants around the globe [3].

Rice is the most important cereal crop for human consumption. It is the staple food for over 3 billion people (most of them poor) constituting over half of the world's population. Rice is a high-carbohydrate food with 85 percent of the energy from carbohydrate, 7 percent from fat, and 8 percent from protein. However, rice also has a considerable amount of protein, with an excellent spectrum of amino acids. The protein quality of rice (66%) is higher than that of whole wheat (53%) or corn (49%). Of the small amount of fat in brown rice, much is polyunsaturated. White rice is extremely low in fat content.

**Medicinal Uses of Rice**

Rice is believed that some to have medicinal properties. Although, this is not scientifically proven effective, it has been used in many countries for medicinal purpose. Rice has the rare capability to enrich body elements, to exclude toxic metabolites, to strengthen, regenerate, energies body, to regulate blood pressure to prevent skin disease.

**Medicinal rice in Kerala:**

In kerala, the variety navara is believed to have medicinal properties and is used to rejuvenate the nerves in properties and is used to rejuvenate the nerves in paralytic condition. Oridine, an alkaloid present in rice, has some antineurotic properties. It is of two types

1. Black glumed
2. Golden-yellow glumed

Anthocyanin, pigment the active therapeutic components is blackrice, have been found to be most advantageous extracted from the separated outer layer of the rice grain. The composition of anthocyanin is cyaniding-3-0-glucoside and peoxidin-3-0-glucoside derived from black rice. Njavara, a medicinal rice, was assessed for its nutrient composition and physicochemical properties, in order to understand its therapeutic properties. Dehusked Njavara rice consisted of 73% carbohydrates, 9.5% fat, 1.4% ash and 1628 kJ per 100 g of energy.

**Medicinal uses:**

It is also believed that Njavara (Navara) rice increases Semen, and Fertility in male and is recommended for childless couple. It is recommended to pregnant woman as it increases the total weight of the Foetus , and also increases Mother milk.

Parassinikkara Hospital recommended Navara rice paste application in pustules formed due to the biting of Viper Snake reduce the pain and it is recommended as a safe food to Diabetic patients.

Njvarakizhi is rejuvenating and restoring therapy and an acclaimed remedy for Rheumatism and Neural disorders. Ayurvedic Doctors effectively uses Navara rice paste, Lepenam , to treat Psoriasis. The paste is also remedy for skin lesions.

**Flavonoid**

Flavonoids (or bioflavonoids) (from the Latin word flavus meaning yellow, their colour in nature) are a class of plant secondary metabolites. Flavonoids were referred to as Vitamin P [4] (probably because of the effect they had on the permeability of vascular capillaries) from the mid-1930s to early 50s, but the term has since fallen out of use [5].
Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health—they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities.

However, no scientific data are available on the isolation of flavonoids compound of the Njavara rice extract. Our main objective in this investigation was to isolate the flavonoid compound from Njavara rice bran ethanolic extract and to analyze the In vitro Anti inflammatory Activity and Anti oxidant activity of Flavonoid and Njavara Rice.

Materials and Methods

Sample Collection

Plant material Njavara was obtained from Musiri. Rice bran from the sample was obtained by milling rice grain in a local grinding mill, followed by sieving to separate grain from bran.

Preparation of rice bran extract

Rice bran and rice powder (5 g) was extracted thrice with 45 ml Ethanol for 3 h in an electrical shaker at 40°C. The extracts were filtered through Whatmann No.1 filter paper and evaporated. The extracted sample was used for the following analysis.

Preliminary Phytochemical Analysis

The extract of Rice extract was subjected to qualitative test for the identification of various plant constituents by Harborne method [6].

Determination of total flavonoid content

Total flavonoid content was determined using aluminium chloride (AlCl3) according to a known method using quercetin as a standard. The sample (0.1 ml) was added to 0.3 ml distilled water followed by 5% NaNO2 (0.03 ml). After 5 min at 25°C, AlCl3 (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg quercetin (QE)/g bran.

Column Chromatography

The sample extract (4g) was resolved by silica gel column chromatography (70- 230 mesh, Merck, petroleum ether, AcOEt, methanol gradients) so that main fractions were collected using as eluents a 9.5:0.5 CHCl3: MeOH mixture.

Quantitative Analysis

TLC

Thin layer chromatography is one of the valuable and versatile methods for analysis of wide range biomolecules. TLC is nothing but a modification of paper chromatography. Where the sheet of paper is replaced by thin layer of absorbent material. Therefore the separation in TLC is also due to the differential partition of solutes between the stationary and mobile phases. The flavonoids spots were separated using chloroform and methanol solvent mixture in the ratio of 19:1. The color and Rf value of these spots were recorded under ultraviolet (UV 254 nm) light [7].

Characterization of Flavonoids by UV and FT-IR

The presence of Flavonoid compound in the selected paddy was studied by UV and Fourier Transform Infrared (FT-IR) spectroscopy. A FT-IR spectrometer was used to record IR spectra.

Reducing Power Scavenging Activity

To determine the reducing power assay of isolated flavonol and paddy, by Yildrim et al., Method [8].

Procedure

1 ml of sample was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution
(2.5ml) was mixed with distilled water (2.5ml) and Ferric chloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

**Calculation**

\[
\text{Percentage scavenging activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100
\]

Where \(A_{\text{control}}\) is the absorbance of the control. \(A_{\text{test}}\) is the absorbance in the presence of the sample.

**Anti Inflammatory Activity by Human Red Blood Cell Membrane Stabilization Method (HRBC METHOD)**

To Determine The HRBC Assay of Sample by, Gandidasan [9].

**Procedure:**

HRBC method was used for the estimation of anti inflammatory activity in vitro. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsever’s solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were packed washed with isosaline solution and a 10% v/v suspension is made with isosaline. This HRBC suspension was used for the estimation of anti inflammatory property. The sample extract; reference sample and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. The reference used for this study is diclorofenac sodium. All the assay mixtures were incubated at 37ºC for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

**Calculation**

\[
\text{Percentage protection} = 100 - (\text{OD sample} / \text{OD control}) \times 100
\]

**Result and Discussion**

The result in phytochemical investigation of Selected Paddy Navara (Rice Bran, Husk and Rice) extract have been presented and discussed here. The present study of phytochemical investigation revealed that the presence of medicinally active constituents in the Paddy at various parts. There is a growing focus on the medicinal rice use as therapeutic agent because of their limited side effect and retention of appropriate period of activity.

**Qualitative phytochemical analysis**

The preliminary qualitative analysis of phytochemical investigation revealed the presence of alkaloids, flavonoids, tannins, and carbohydrates, were present in Rice with husk of the selected paddy as shown in Table-1 and figure 2. The rice also possess Alkaloid, Carbohydrate, Tannin, Flavonoid and Coumarine, where as the husk contains Alkaloid, Carbohydrate, Steroid, Tannin and Flavonoids. The flavonoids are present in all three selected parts of paddy hence, the flavonoids was isolated for the further study. Thus the preliminary screening test may be useful in the detection of the bioactive compounds.

**Quantitative phytochemical analysis by TLC**

The TLC profile of secondary metabolites (flavonoids) are tabulated in the table-2 and figure. The Rf Value of the flavonoids in selected sample was 0.97. The Chromatography showed that of Selected Paddy *Njavara* demonstrated the presence of alkaloids, flavonoids, and Tannin. The presence of some of these compounds had been demonstrated previously by other researchers. However some of the results obtained are not in agreement with the previous findings. This might be due to climatic and environmental factors.

The flavonoids content of the sample was estimated by spectrophotometer. The rice with husk sample contains 200.9mg of flavonoids, similarly the rice sample has 214.56mg of flavonoids respectively where as the husk possess 91.89mg of flavonoids which is the low amount compare with rice sample. Flavonoids have been reported to expert multiple biological effects such as, anti-inflammatory, anti-allergies, anti-viral and anti-cancer activities.
### Table 1: Preliminary phytochemical analysis of *Njavara*

<table>
<thead>
<tr>
<th>SI.No.</th>
<th>Name of the Test</th>
<th>Phytochemical constituents</th>
<th>Rice with Husk</th>
<th>Rice</th>
<th>Husk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wagner’s reagent</td>
<td>alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Dragondroff</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Molish Test</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Benedicts Test</td>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Salkowski</td>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Foam</td>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Ferric Chloride</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Hcl</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Shinoda’s Test</td>
<td>Flavones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Sodium Hydroxide</td>
<td>Anthocyanin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Sodium chloride</td>
<td>coumarin</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2: TLC Profile on Flavonoids in *Njavara*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical constituents</th>
<th>Rf Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rice with Husk</td>
</tr>
<tr>
<td>1.</td>
<td>Flavonoids</td>
<td>0.97</td>
</tr>
</tbody>
</table>

**TLC Profile For Flavonoids**

- Rice with Husk
- Rice
- Husk

Rf Values
Table 3: Estimation of Flavonoids

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>Flavonoids (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rice With Husk</td>
<td>200.9</td>
</tr>
<tr>
<td>2</td>
<td>Rice</td>
<td>214.56</td>
</tr>
<tr>
<td>3</td>
<td>Husk</td>
<td>91.89</td>
</tr>
</tbody>
</table>

FIG 1: UV ANALYSIS OF FLAVONOIDS

![UV Analysis of Flavonoids](image-url)
FIG 2: FTIR ANALYSIS OF FLAVONOID

Table 4: Invitro Anti inflammatory Activity by HRBC Method

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>% of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tablet Diclofenac</td>
<td>46.53</td>
</tr>
<tr>
<td>2</td>
<td>Njvara Paddy</td>
<td>46.55</td>
</tr>
<tr>
<td>3</td>
<td>Flavonol</td>
<td>46.94</td>
</tr>
</tbody>
</table>
Table 5: Invitro Anti Oxidant Activity by Power Reducing Assay

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>% of Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonol</td>
<td>44.3</td>
</tr>
<tr>
<td>2</td>
<td>Rice With Husk</td>
<td>12.6</td>
</tr>
<tr>
<td>3</td>
<td>Rice</td>
<td>16.1</td>
</tr>
<tr>
<td>4</td>
<td>Husk</td>
<td>14.0</td>
</tr>
</tbody>
</table>

Characterization of Flavonoid by UV and FTIR

The UV spectra of flavonoids are readily distinguished from those of other flavanols in that they exhibit a low intensity band absorption which often appears as a shoulder to band I. The UV spectrum of (Figure 3) was suggestive of a flavonoid with a flavonol skeleton from the high intensity band I peak (354nm).

The FT-IR result shows the structure of Flavonol compound from Paddy under Invitro method using different medium. Figure 4 shows the FT-IR spectrum of the isolated compound, which was acquired in the range of 400-4000cm⁻¹. The peaks in the range of 1576.39cm⁻¹ corresponds to the C=O bonds. The adsorbed band at 3408.53cm⁻¹ is assigned O-H bending vibrations respectively diminishes gradually for sample annealed at higher temperature.

There is an ever-increasing interest in the biological effects of the bioflavonoids, members of the large group of plant polyphenols. In addition, free radical products at sites of inflammatory processes react with bioflavonoids and their metabolites, generating important new compounds of as yet unknown properties [10].
Anti Inflammatory Activity

The flavonol shows its maximum anti-inflammatory (46.94%) activity with Human blood. The tablet shows its lesser activity than flavonol and paddy (46.55% and 46.53%) with respective to aqueous extract. The results are tabulated in Table 4.

The lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane [11]. HRBC method was selected for the in vitro evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indcoted that the flavonols and paddy has significant anti-inflammatory property.

Anti Oxidant Activity

The isolated flavonol shows the maximum anti oxidant activity (44.3%). Where as the Rice with Husk, Rice and Husk possess 12.64%, 16.1% and 14.0% of Antioxidant activity respectively. The table 5 shows the anti oxidant activity of flavonols and paddy. Natural antioxidants that are present in herbs, Rice and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices, rice and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In this present study, we have evaluated the free radical scavenger activity of flavonol and paddy which is possesses good antioxidant activity.

Conclusion

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role free radical. So it will be pertinent to examine the possible role of “free radical” in disease and “antioxidants” in its prevention. This indicates the isolated flavonol possess the strong antioxidant, anti inflammatory activity against skin problem. Thus sample is used as analgesic to cure the skin inflammations and other related skin infections. The further study will be carried out for the preparation and mechanism of wound healing and crack healing medicine using bioactive compound from this selected paddy.

References