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## Research Paper

# Evaluation of bioactive compounds of two locally cultivated pokkali rice (*Oryza sativa*. L) varieties

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## Abstract

The present study was carried out to evaluate the bioactive compounds present in the salt tolerant land races of rice varieties viz., pokkali (*pok*) and chettivirippu (*cv*). The phytochemical analyses indicated that the rice bran extracts have biologically active compounds such as alkaloids, phenols, tannins, quinones, flavonoids and anthocyanins. *pok* and *cv* rice varieties contains 29.2±1.43% and 32±1.12% carbohydrate respectively. Amylose is a non-nutritional polysaccharide and is of great importance in high starch rice varieties, the *pok* contains 23.2±1.31% and *cv* contains 6.4±0.03% amylose. The protein is another nutritional component and were 3.28±0.16g and 4.88±0.19g in *pok* and *cv* respectively. The total phenolic content of *pok* and *cv* (260 and 235 mg of gallic acid equivalent/ g of extract respectively) were high. In addition nutritionally important trace elements such as Copper, Zinc, Manganese, Calcium, Magnesium, Nitrogen, Boron, Potassium and Phosphorous concentration were significantly high in these two rice varieties. The active principles of *pok* and *cv* showed microbicidal property against *E. coli*. The results indicate that both *pok* and *cv* contains higher levels of nutritionally important compounds hence the varieties should be conserved for the cultivation even if the yield is less.

**Keywords:** Pokkali, rice, Chettivirippu, phenolics, salinity, trace elements

## Introduction

Rice predominates as staple food in majority of countries of Asia and the Pacific, nine countries in North and South America and eight countries in Africa and provides 20 percent of the world's dietary energy supply<sup>1</sup>. It is the main source of energy and protein. It provides instant energy as its important component is carbohydrate. Rice protein is biologically the richest by virtue of its high true digestibility (88%) among cereal proteins and also provides minerals and fibre<sup>2</sup>.

When the husk is removed, the rice is called brown rice. Brown rice contains the bran layer and the endosperm. The bran layer is made up of the pericarp and testa, the aleurone layer and the embryo. White rice is produced by removing the bran layer and the germ from the paddy and the bran layer is removed from the kernel through either abrasive or friction polishers<sup>3</sup>. Brown rice is more nutritious than white rice<sup>4</sup>.

Rice is a good source of thiamine, riboflavin and niacin. The nutrient content of rice varies depending on the variety of rice, soil and the conditions they grow. Protein content of brown rice is normally reported as twice that of white rice, i.e. 14.6 g/100 g (brown rice) vs 7.3 g/100 g (white rice). On the other hand, the fat content is so high, namely 24.8 g/100 g for pre-germinated brown rice and 1.5 g/100 g for white rice<sup>5</sup>. The amino acid profile of rice shows that glutamic and aspartic acids are the major amino acids present in rice, while lysine is the limiting amino acid<sup>1</sup>. Several types of rice had similar protein and crude fat contents, however, the ash contents among types of rice were slightly

different, mainly among milled samples. It is comprised of 77.5% carbohydrate. Amylose content varies from 2% to more than 25% and varieties with low (2-19%), intermediate (20- 25%) and high (>25%) amylose content are available in all grain types<sup>2</sup>. Rice bran is a good source of minerals and trace minerals. It is a good source Phosphorous, Iron and Calcium<sup>6</sup>. The other bioactive compounds present as minor constituents are lipoic acid, coenzyme Q etc. Except for dietary fibre, most of the other phytochemicals are present in the nonglyceride (unsaponifiable) fraction of the oil present in rice bran<sup>6</sup>.

Rice contains several phytochemicals such as carotenoids, phenolics, alkaloids, nitrogen and organosulfur containing compounds. Phenolic compounds are secondary metabolites of plants, with different activities such as protection against pathogens and predators, mechanical support, attraction of pollinating animals, and protection against ultraviolet radiation<sup>7,8</sup>. It has antioxidant activity. They may directly scavenge some reactive species, including hydroxyl, peroxy and superoxide radicals, acting as chain breaking antioxidants. They may suppress lipid peroxidation recycling other antioxidants, such as  $\alpha$ -tocopherol. Some phenolic compounds may bind pro-oxidant metals, such as iron and copper, preventing the formation of free radicals from these pro-oxidants while simultaneously maintaining their capacity to scavenge free radicals<sup>9-11</sup>. Besides, the effects of some phenolics are related to the increase in the activity of antioxidant enzymes<sup>12</sup> and induction of the synthesis of antioxidant protein<sup>13</sup>.

Soil salinity is a major environmental constraint to crop production with negative impacts on growth rates, tillering and seed production. Pokkali is a unique mode of saline rice production system in central Kerala using saline tolerant rice varieties that are cultivated exclusively in an organic way in the water logged acid sulphate soils of coastal regions of Ernakulam, Alappuzha and Thrissur districts of Kerala . In this method, a single crop of rice is grown in the low saline phase of the production cycle (June to mid-October/early November) followed by prawn farming during the high saline phase (mid November to mid April)<sup>14</sup>. Pokkali and Chettivirippu are salt tolerant rice varieties cultivated in Kerala. The present investigation was undertaken to determine the bioactive compounds present in the salt tolerant varieties, Pokkali and Chettivirippu. In addition assay of trace elements were also conducted in these two varieties.

## **Materials and Methods**

### **Collection of sample**

The seed samples viz. Pokkali (*pok*) and Chettivirippu (*cv*) were collected from Rice Research Station, Vyttila, Ernakulam, Kerala and authenticated by standard procedure<sup>15</sup>.

### **Separation of rice bran**

The separation of rice bran was performed according to the method of Luh (1991)<sup>16</sup>. The husk was separated and grains were winnowed to remove the husks. The rice was pounded again to separate the bran.

### **Extraction of rice bran**

The rice bran of two different cultivars was extracted by different solvents such as 80% ethanol, distilled water, ethanol and methanol<sup>17</sup>.

### **Determination of total carbohydrates**

Total carbohydrate of rice samples were determined according to the method of Hedge and Hofreiter(1962)<sup>18</sup>. Values are expressed in mean $\pm$ SD.

### **Determination of amylose**

The amylose content of the rice sample was determined according to the method of Williams *et al.*, (1970)<sup>19</sup>. Values are expressed in mean $\pm$ SD.

### **Determination of protein**

The protein content of the rice sample was determined according to Lowry *et al.*,(1951)<sup>20</sup>. Values are expressed in mean $\pm$ SD.

**Qualitative assay of phytochemicals**

Test for tannins, saponins, flavonoids, alkaloids, quinones, phenols, coumarins, sterols, phytosterols and anthocyanin were conducted as described elsewhere<sup>21</sup>.

**Determination of total phenolic contents (TPC)**

The total phenolic content of rice bran extracts were determined by spectrophotometric method using Folin –Ciocalteu's reagent<sup>22</sup>.

**Assay of mineral elements**

The analyses of elements were done with Atomic Absorption Spectroscope (Analytik-Jena– novAA-300) at Rice Research Station, Kerala Agricultural University.

**Digestion of plant samples using MARS**

Weighing 0.25 g sample + 10 ml concentrated nitric acid and was kept in rotating tube stand inside the digester for 45 minutes. After cooling, digested samples transferred to volumetric flasks and made up to the volume. A Blank without sample was also carried out for final calculation.

**Cu/Zn/Mn/Ca/Mg**

Calibrated atomic absorption spectrophotometer with known standards of Cu/Zn/Mn/Ca/Mg as detailed below,

S. No.	Element	Concentrations					
1	Cu	1ppm	2	4	5	6	7
2	Zn	0.5	1.0	1.5	2.0	2.25	2.50
3	Mn	1	2	3	4	5	6
4	Ca	0.5	1.0	2.0	3.5	4	5
5	Mg	0.2	0.3	0.4	0.5	0.6	0.7

**I. Phosphorous Vanadomolybdate method****Reagents**

- Bartons reagent (vanadomolybdate reagent)
- Standards of phosphorous (1, 1.5, 2, 2.5, 3, 3.5, 4ppm)

**Procedure**

5ml digested extract + 10ml vanadomolybdate reagent kept 10 min after thorough mixing and made up to the volume – colour developed is read in a spectrophotometer at 670nm.

**II. Boron**

Dried, ashed plant samples in muffle furnace at 500-600°C followed by extraction in 10 ml, 0.1 N HCl, and the suspension was filtered. 1ml extract + 2 ml buffer solution + 2ml azomethine-H reagent, mix well and read the absorbance after 30 min at 420nm on a spectrophotometer with known standards of B.

**III. Potassium**

10ml of the digested sample which is made up to 100 with distilled water is directly read in a flame photometer with a blank and known standards of potassium, % K was calculated.

**IV. Total Nitrogen**

Digestion: digested samples using Kjeldhal assembly (Kjelplus nitrogen estimation system). 0.5 g oven dried sample+10ml con.H<sub>2</sub>SO<sub>4</sub> +1 digestion catalyst. Digestion carried out in a digestion chamber; after cooling made volume of the digest to 100 ml in a volumetric flask, % Nitrogen in plant sample is noted by final titration.

**V. Distillation**

Pipette out 10ml of the digested extract and kept in the distillation rack followed by 4% boric acid and 40% sodium hydroxide. After distillation titrated against 0.1N H<sub>2</sub>SO<sub>4</sub>.

## Anti-Microbial Assay

### Preparation of bran extract

The rice bran was soaked in each of distilled water, ethanol and methanol (plant material to solvent ratio was 1:10, w/v) and extracted for 24 h at room temperature. Filtrates of the extracts were dried at 40°C<sup>23</sup>.

### Microbial strains

The bacterial strains used for antibacterial assay were pure cultures of *Escherichia coli*, *Pseudomonas aerogens*, *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumoniae*. All the strains were maintained in our laboratory.

### Preparation of bacterial culture

Nutrient broth medium: Nutrient broth – 1.3 g, Distilled water- 100ml

1.3 g of nutrient broth was emulsified in 100 ml of water. Dispensed 10 ml of broth to 10 test tubes. Plugged the mouth of the test tubes using cotton. Broth was then autoclaved and sterilized. Each tubes were inoculated with bacteria and was incubated. After 24 hours bacteria grew in broth medium.

Nutrient agar medium: Nutrient agar - 28 g, Distilled water – 1000 ml

Culture media was prepared by weighing 28 g of nutrient agar and was transferred in 1 L of distilled water taken in a beaker. Gently heated the contents. Petriplates and nutrient agar were subjected to sterilization in autoclave at 121°C for 15 minutes. Further operations were done in laminar air flow chamber. The sterilized nutrient agar medium was poured into petriplates and allowed to solidify at room temperature and kept in an inverted position. The bacterial culture in nutrient broth was swabbed using buds over the solidified agar medium from culture.

### Antibacterial assay by disc diffusion method

The assay was carried out according to CLSI<sup>24</sup>. Disc diffusion method was employed for antimicrobial activity. Filter paper discs were made of whatmann No 1 filter paper of 5mm. 10 µl of 1g/ml concentration of each extracts were impregnated to filter paper disc and allowed to dry. Using forceps impregnated filter paper discs were transferred to the media swabbed with test organism under a sterile condition. Negative control was prepared by dipping the sterilized disc into ethanol, methanol and distilled water extracts. After incubation of 24 hours at 37°C, zone of inhibition of growth was measured in millimeters.

## Results and Discussion

The total carbohydrate content of *pok* and *cv* were 29.2±1.43% and 32±1.12% respectively. A wide range of physiological functions, which are important to health were influenced by the total carbohydrate content. The major physiological activity, such as energy source, effects on satiety/gastric emptying, blood glucose and insulin metabolism, protein glycosylation, cholesterol and triglyceride metabolism, bile acid dehydroxylation, fermentation, colonic epithelial cell function, bowel habit / laxation/ motor activity, may be affected by the carbohydrate content<sup>25</sup>. Earlier workers reported<sup>26</sup> that the level of percentage of carbohydrate content in rice ranging from 73.63%- 85.18%. In the present study the level of carbohydrate content were low in both rice varieties (29.2±1.43% and 32±1.12%). This indicates the low glycaemic index.

In the present investigation *pok* had 23.2±1.31% and *cv* had 6.4±0.03% amylose. Amylose content is one of the important parameter which determines the cooking and eating characteristics of rice. High amylose content (25-30%) gives a firm and dry cooked product, while those with a intermediate amylose content (20-25%) gives much softer and stickier one and those with a low amylose content (<20%) gives quite soft and sticky products<sup>27</sup>. Amylose content varies from 2% to more than 25% and varieties with low (2-19%), intermediate (20- 25%) and high (>25%) amylose content were available in all grain types<sup>2</sup>. Among the different type of starch molecules, Amylose is an important form of resistant starch, which resistant to digestion than other types and hence found to be an effective prebiotic . Earlier workers reported that apparent amylose content in rice ranging from 1.5 to 26.4%<sup>28</sup>. Since the amylose content in *cv* was less than 20% the rice might be quite soft and sticky. The level of amylose content in *pok* was found to be 23.2±1.31% and hence the rice would be of better cooking quality.

In the present investigation *pok* contains  $3.28 \pm 0.16$  g/100g of protein and *cv* contains  $4.88 \pm 0.19$  g/100g of protein. Proteins are important for metabolism and other various activities, as hormones are proteins which control biochemical reactions and aid in the immune system. Cell division is also influenced by the protein contents. Earlier workers reported that rice contains 6.8 g/ 100 g of protein<sup>29</sup>. In the present study protein content of both rice varieties were found to be low.

**Table 1: Qualitative assay of various phytochemicals in two varieties of rice viz. *pok* and *cv***

Rice variety	<i>Pok</i>			<i>Cv</i>		
	Extract solvents	Ethanol	Methanol	Distilled water	Ethanol	Methanol
Tannins	-	+	-	+	+	-
Saponins	-	-	-	-	-	-
Flavonoids	+	+	-	+	+	-
Alkaloids	+	+	-	+	+	-
Phenols	+	+	-	+	+	-
Quinones	+	+	-	+	+	-
Coumarins	-	-	-	-	-	-
Steroids	-	-	-	-	-	-
Phytosterols	-	-	-	-	-	-
Anthocyanin	+	+	-	+	+	-

('+' - indicates presence and '-' indicates absence)

Secondary metabolites including alkaloids, flavonoids, terpenoids, and other phenolic compounds are important for the defence mechanisms of plants, mainly protection against biotic stresses, because of their repellent properties. Earlier worker reported that alkaloids, phenols, flavonoids, terpenoids were present in rice<sup>30</sup>. In the present study tannins, flavonoids, alkaloids, phenols, quinones, anthocyanin were assayed and showed its presence in both *pok* and *cv* (Figure 1). Alkaloids have been reported as one of the important group of phytoconstituents obtained from natural sources. Alkaloids belonging to beta-carboline group possess antimicrobial, anti-HIV and antiparasitic activities<sup>31</sup>. Both rice varieties *pok* and *cv* contains alkaloids. Phenolic compounds are important for their various activities such as protection against pathogens and predators, providing mechanical support, attraction of pollinating animals, and protection against ultraviolet radiation<sup>8</sup>. Several phenolic compounds have already been identified in rice. Earlier workers have reported that the concentration of total phenolics in the grain and antioxidant activity are positively associated<sup>32</sup>. In the present study both *pok* and *cv* contains phenols and hence might have antioxidant activity. Anthocyanin pigments are present in the bran layer. Anthocyanins are members of the flavonoid group of phytochemicals. Anthocyanin pigments are known to have free-radical-scavenging and antioxidant capacities. In the present study both *pok* and *cv* had anthocyanin and hence both varieties might have antioxidant activity. Flavonoids, including flavones, flavanols and tannins are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*<sup>33</sup>. In the present investigation both *pok* and *cv* had flavonoids. Hence both rice varieties might have antioxidant activity.

In the present investigation, *pok* had total phenolic content (TPC) of 260 mg of gallic acid equivalent/g of extract and *cv* had 235 mg of gallic acid equivalent/g of extract. Polyphenols are known for their defense mechanism mainly against ultraviolet radiation and aggression of pathogens<sup>34</sup>. The metabolites of polyphenols are rapidly eliminated from plasma, thus, daily consumption of plant products is essential in order to supply high metabolite concentrations in the blood. Phenolic compounds have redox properties, which allow them to act as antioxidants<sup>35</sup>. Earlier workers reported that the levels of TPC in three northern Thailand rice varieties were ranging from 36.14 - 305.30mg of gallic acid equivalent/g of extract<sup>17</sup>. Earlier studies reported that polyphenols displayed important functions, like inhibition of pathogens, anti-deposition of triglycerides, reduce the incidence of non-communicable diseases such as cardiovascular diseases, diabetes, cancer and stroke, anti-inflammation and anti-allergic effect through processes involving reactive oxygen species<sup>36</sup>. Both rice

varieties investigated contains significantly higher level of TPC. Hence it might have antioxidant, anti-inflammatory and antiallergic effect.

**Table 2: Level of various elements in two varieties of rice viz. *pok* and *cv***

S. No	Variety	Cu mg/kg	Zn mg/kg	Mn mg/kg	B mg/kg	P %	K%	N %	Ca%	Mg%
1	<i>Cv</i>	4.068	8.425	17.19	5.8	3.6	1.2	0.84	0.316	0.1001
2	<i>Pok</i>	5.42	8.994	20.41	6.2	2.1	0.9	1.07	0.282	0.0825

Presence of minerals is necessary for the maintenance of certain physicochemical processes which are essential to life, even though they yield no energy<sup>37,38</sup>. Every form of living matter requires these inorganic elements or minerals for their normal life processes<sup>39,40</sup>. The macro-minerals include Calcium, Phosphorus, Sodium and Chloride, while the micro-elements include Iron, Copper, Cobalt, Potassium, Magnesium, Iodine, Zinc, Manganese, Molybdenum, Fluoride, Chromium, Selenium and Sulfur<sup>38</sup>. When a trace element is deficient, a characteristic syndrome is produced which reflects the specific functions of the nutrient in the metabolism of the animal. Simple or conditioned deficiencies of mineral elements therefore have profound effects on metabolism and tissue structure. Iron deficiency anemia and goiter due to Iodine deficiency are reported to be problems of public health importance in some communities<sup>41</sup>.

Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin and also takes part in clotting. It plays a vital role in enzyme activation such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase etc. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability<sup>37</sup>. Earlier Studies have reported the level of Ca in 20 rice varieties ranged between 0.07 – 0.25%<sup>42</sup>. In the present study the level of Ca in both *pok* and *cv* found to be significantly high (Figure 2) and might useful to overcome Ca deficiencies.

Inorganic phosphate (Pi) plays a critical role in skeletal development, mineral metabolism, and diverse cellular functions involving intermediary metabolism and energy-transfer mechanisms. It is a vital component of bone mineralization, phospholipids in membranes, nucleotides that provide energy and serve as components of DNA and RNA, and phosphorylated intermediates in cellular signaling<sup>43</sup>. Earlier workers reported that the level of Phosphorous ranging from 0.50 – 0.55%<sup>42</sup>. In the present investigation the concentration of phosphorous is potentially high in both varieties. The percentage composition of phosphorous found to be higher in *cv* (3.6%) than *pok* (2.1%) (Figure 2).

Copper is an essential trace element<sup>44</sup>. It plays an important role in our metabolism because it allows many critical enzymes to function properly. It has selected biochemical function in hemoglobin synthesis, connective tissue metabolism, and bone development. In the present investigation level of Copper was significantly higher in *pok* as compared to *cv* (Figure 2). Phuong *et al.*, (1999)<sup>45</sup> has reported that the level of copper in 20 varieties of rice ranging from 1.1 – 7.9 mg/kg. Copper protects against free-radical damage to proteins, membrane lipids, and nucleic acids in a wide range of cells and organs<sup>46</sup>. In the present study *pok* contains 5.42 mg/kg and *cv* contains 4.06mg/kg of Copper, revealed that these varieties were of potential nutritional property.

Zinc is essential for a large number of enzymes particularly for the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as for the metabolism of other micronutrients. It stabilises the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Zinc is also important in polynucleotide transcription and thereby in genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms<sup>47</sup>. It plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity<sup>48</sup>. Earlier studies have reported the level of zinc ranging from 0.05-14.87 mg/kg<sup>49</sup>. In the present investigation level of zinc was significantly high in both *pok* and *cv* (Figure 2).

Manganese activates numerous enzymes such as hydrolases, transferases, kinases, and decarboxylases and is a constituent of some enzymes. One of the most well-known Manganese metalloenzyme is pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxalo-acetate.

Other enzymes include arginase, which is involved in the conversion of the amino acid arginine to urea, and mitochondrial superoxide dismutase (SOD)<sup>50</sup>. Earlier studies have reported the level of Manganese ranging between 5.9- 32.6 mg/kg in 20 rice varieties<sup>45</sup>. In the present study the level of Manganese was higher in *pok* (20.4 mg/kg) and *cv* (17.19 mg/kg) (Figure 2). This reveals the nutritional significance of *pok* and *cv*.

Potassium plays a major role being the principal cation in intracellular fluid. Potassium content affects activities like acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na<sup>+</sup> /K<sup>+</sup> -ATPase. Potassium is also required during glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid and probably has a role in many other basic cellular enzymatic reactions<sup>39</sup>. Studies have reported<sup>42</sup> that the level of Potassium in 20 rice varieties ranged between 0.15 – 0.23 %. In the present study the level Potassium in both *pok* and *cv* were found to be higher (Figure 2), hence are of nutritional significance.

Magnesium is very important for oxidative phosphorylation, which otherwise get greatly reduced in its absence. Mg is also an essential activator for the phosphate-transferring enzymes myokinase, diphosphopyridine nucleotide kinase, and creatine kinase. It also activates pyruvic acid carboxylase, pyruvic acid oxidase and the condensing enzyme for the reactions in the citric acid cycle. It is also a constituent of bones, teeth, enzyme cofactor, kinases, etc<sup>51</sup>. Earlier workers reported that the level of Mg in 20 rice varieties ranging between 0.07 – 0.25 %<sup>42</sup>. In the present study *pok* had 0.0825% Mg and *cv* had 0.1001% Mg. Acute Magnesium deficiency results in vasodilation, with erythema and hyperaemia appearing a few days on the deficient diet. In the present investigation the level of Mg in both varieties found to be higher. Hence consumption of both rice varieties *pok* and *cv* might overcome Mg deficiencies.

Boron is important for bone metabolism. It interacts with Calcium, vitamin D, and Magnesium<sup>52</sup>. It has also been found to increase steroid hormone concentrations in postmenopausal women and to have antioxidant properties, which could make it beneficial in preventing atherosclerosis<sup>53,54</sup>. In the present study the level of Boron was high in *pok* (6.2 mg/kg) and *cv*(5.8 mg/kg) (Figure 2). This indicates the nutritional superiority of these cultivars.

Nitrogen is a fundamental component of nucleic acids, proteins, hormones and coenzymes<sup>55</sup>. In the present study the percentage level of N was 0.84% in *cv* and 1.07% in *pok*.(Figure 2).

**Table 3: Microbicidal property of various fractions of *pok* and *cv* on different bacterial strains**

Sample	Extracts	<i>Staphylococcus</i>	<i>Klebsiella</i>	<i>Proteus</i>	<i>Pseudomonas</i>	<i>E. coli</i>
<i>Pok</i>	Ethanollic	x	x	x	x	✓ (1.5mm)
<i>Cv</i>		x	x	x	x	x
<i>Pok</i>	Methanollic	x	x	x	x	x
<i>Cv</i>		x	x	x	x	x
<i>Pok</i>	Distilled	x	x	x	x	x
<i>Cv</i>	water	x	x	x	x	✓ (1mm)

('X'- denotes negative; '✓' denotes positive); (Zone of inhibition in mm)

'pok' denotes Pokkali rice variety

'cv' denotes Chettivirippu rice variety

There is growing interest in plant-derived substances, secondary metabolites as alternative antimicrobial agents for the prophylaxis and treatment of diseases<sup>56,57</sup>. The advantage of using plant medicine is that it is a natural product, and normally safer than synthetic or chemical compounds. Earlier workers reported that brown rice had anti-microbicidal activity against *S. aureus* and *B. subtilis*<sup>58</sup>. In the earlier studies it had reported that aqueous leaf extract of *O. sativa* exhibited enormous activity against *E. coli*<sup>30</sup>. In the present investigation ethanolic extract of *pok* showed antibacterial activity against *E. coli* and distilled water fraction of *cv* also showed antibacterial activity against *E. coli* (Figure 3). *E. coli* is a major polluting organism of potable water. Enteroinvasive *E. coli* (EIEC) are capable of invading and multiplying in the intestinal epithelial cells of the distal large bowel

in humans. The illness is characterized by abdominal cramps, diarrhea, vomiting, fever, chills, a generalized malaise, and the appearance of blood and mucus in the stools of infected individuals. Enterotoxigenic *E. coli* (ETEC) serotypes can cause infantile gastroenteritis<sup>59</sup>. As both *cv* and *pok* fractions have principles that act against *E. coli* and consumption of these two varieties might decrease the pathogenicity due to *E. coli* contamination. However further investigations are warranted to identify the principle responsible for this microbicidal property. As both *pok* and *cv* contains nutritionally superior secondary metabolites and trace elements which are of nutraceutical value the variety should be conserved for the cultivation and consumption even if the yield is less.

## Conclusion

The bioactive compounds present in the salt tolerant land races of rice varieties viz., pokkali (*pok*) and chettivirippu (*cv*) were examined in detail in the present study. The carbohydrate content was found to be low in these varieties suggesting its low glycaemic index. Being a non nutritional polysaccharide, presence of intermediate amylose with low carbohydrate indicates its significance as diabetic rice. The total phenolic content and nutritionally important trace elements such as Copper, Zinc, Manganese, Calcium, Magnesium, Nitrogen, Boron, Potassium and Phosphorous concentration were significantly high in these two rice varieties. Apart from these, both *pok* and *cv* showed microbicidal property towards *E. coli*. These results indicate that both *pok* and *cv* contains higher levels of nutritionally important compounds.

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