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# Bioactivity and anthocyanin content of microwave-assisted subcritical water extracts of Manipur black rice (*Chakhao*) bran and straw

Kamaljit Moirangthem[a,b,1,*], Priya Ramakrishna[a,2], Mahetab H. Amer[c,3], Gregory A. Tucker[a]

[a] School of Biosciences, University of Nottingham, Loughborough LE12 5RD, UK
[b] Institute of Bioresources and Sustainable Development, An Autonomous Institute under the Department of Biotechnology, Government of India, Imphal 795001, India
[c] School of Pharmacy, Centre for Biomolecular Sciences, The University of Nottingham, University Park, Nottingham NG7 2RD, UK

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ABSTRACT

*Chakhao*, the Geographical Indication (GI) tagged aromatic black rice of Manipur, India, is a rich source of bioactive compounds such as anthocyanins. However, there is limited work available on *Chakhao* and research has focused on its grain. No reports on the anthocyanin, antioxidant properties or *in vitro* bioactivities of its straw are available. Anthocyanins content of its different fractions displayed a positive correlation with their antioxidant potentials ($r$ = 0.90). In this study, microwave-assisted sub-critical water extraction, at 90 °C for 5 min, from the straw gave an anthocyanins extraction efficiency of 85.8%. Furthermore, this microwave extract displayed higher antioxidant activity than an equivalent conventional methanol extract. *In vitro* studies on the microwave extracts of both straw and bran showed no apparent cytotoxicity on Jurkat cells and dose-dependent inhibition of colony growth in colorectal cancer cells. Black rice bran and straw are valuable by-products of the food industry that are rich in phytochemicals. This study reports the extraction of anthocyanins from black rice straw and presents evidence that the straw, in addition to the bran, contains important bioactive compounds; the extracts of which could further be explored as a natural antioxidant and/or functional ingredients.

## 1. Introduction

With a global production of over 758 million tons, rice provides more than 20% of calories for more than half of the human population (FAO, 2018). Rice can be differentiated based on its colours such as brown, white (polished brown rice), red, purple or black. Black rice are black due to the presence of anthocyanins. Anthocyanins are natural colorants that can impart vibrant red, purple, and blue colours to foods and are approved as clean label alternatives to synthetic colorants in the EU, Australia and New Zealand with the E number E163 (EFSA, 2013; Wallace and Giusti, 2015). They have been reported as chemo-preventative and anticancer agents (Hou, 2003; Li et al., 2015; Tsai et al., 2014), exhibiting a dose-dependent decrease in cell proliferation and growth of human oral, breast, colon, and prostate cancer cell lines (Kang et al., 2003) with different sensitivity between cell lines (Li et al., 2015). Hence, exploring potential non-food, natural plant sources rich in anthocyanins, which can be extracted with high efficiency at low cost would be of great interest.

Rice production results in three major forms of side streams - husk, bran and straw. Since the majority of the bioactive components in rice are present in the bran/germ fraction (Verma and Srivastav, 2020), less attention has been given to black rice side streams, such as straw and husk as a source of bioactive components (Murtey and Seeni, 2020). High-value utilization of rice side streams is still poorly developed (Wi et al., 2013). However, side streams such as rice straw are a useful bioresource with worldwide production of around 731 million tons (Bhattacharyya et al., 2020), of which India contributes no less than 126.6 million tons (Singh et al., 2016). Rice straw also contains a variety of bioactive compounds such as water-soluble phenolic acids (ferulic, p-coumaric and protocatechuic) and flavonoids (Karimi et al., 2014; Menzel et al., 2020). While, rice husk has been reported to contain bioactive compounds such as phenolic compounds, vitamin E and $\gamma$-oryzanol (Goufo and Trindade, 2014; Saikia et al., 2012). Murtey and Seeni (2020), provides a comprehensive review of the phytochemicals of rice husk and straw. They also report that only rice and its by-products (straw and husk) contain momilactone B (used to control ketosis), while p-hydroxybenzoic acid (used as a preservative) and lute-

* Corresponding author: Department of Engineering, Aarhus University, Aarhus N 8200, Denmark
*E-mail address:* kamaljit.moiranghtem@hotmail.com (K. Moirangthem).
[1] Present affiliation: Department of Engineering, Aarhus University, Aarhus N 8200, Denmark
[2] Present affiliation: Department of Botany and Plant Biology, University of Geneva, Geneva 1211, Switzerland
[3] Present affiliation: School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

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olin (an anti-cancer/apoptotic agent) are phytochemicals present in the straw but not in the husk. Additionally, straw extracts obtained using 70% ethanol have been reported to successfully reduce the viability of human cancer cells line such as liver HEPG2, breast MCF7 and prostate PC3 (Meselhy et al., 2020).

Although pigmented rice varieties, such as black rice, have a history of heritage and are admired all over the world, awareness concerning the benefits of consuming these types of rice is limited (Prasad et al., 2019). Owing to its limited consumption compared to normal rice, black rice can be categorised as an underutilized crop (Jha et al., 2017). Same is true for the black rice *Chakhao* of Manipur. However, with recent developments such as 100% *Chakhao* beer that showed enhanced oxidative stability (Moirangthem et al., 2020) and the awarding of the Geographical Indication (GI) status to *Chakhao* in April of 2020 (Intellectual Property India, 2020), there is a renewed interest in this aromatic black rice. The yield of anthocyanins from *Chakhao Poireiton* grain depends on the extraction method - 74 mg/100 g of powdered rice after extraction into acidified methanol (Asem et al., 2015) and 35.87 mg/100 g with acetone: water (75:25, v/v) (Saikia et al., 2012). Four main anthocyanins, *i.e.*, delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-galactoside and cyanidin 3-glucoside were identified in *Chakhao Poireiton* grain (Asem et al., 2015). Of the 27 anthocyanins identified, cyanidin-3-glucoside is the most predominant anthocyanins found in nature (Wallace and Giusti, 2015) and is also the main anthocyanins in black rice (Das et al., 2017).

Biorefining of waste rice side streams involves the breakdown of its structure to release its components (Goodman, 2020). Application of non-conventional methods such as ultrasonic, microwave and supercritical fluid in anthocyanins extractions are gaining interest in both industrial and the research arena (Roriz et al., 2017; Zhu et al., 2016). Peanparkdee and Iwamoto (2019) provide a comprehensive overview of different techniques for extracting bioactives from by-products of rice cultivation, including non-conventional methods. Like most bioactive compounds, the extraction method along with parameters that affect its stability such as temperature, pH and exposure time are crucial for efficient extraction of anthocyanins (López et al., 2018; Rodriguez-Amaya, 2016). Piyapanrungrueang et al. (2015) compared anthocyanin extraction methods such as microwave-assisted, ultrasound-assisted, ohmic heating and conventional heating. They showed that a low reaction time (up to 5 min) gave the highest yield irrespective of the extraction method. Additionally, Menzel et al. (2020) showed that aqueous extracts of rice straw had higher antioxidant activity compared to both 80% methanol and 80% ethanol extracts.

Microwave-assisted subcritical water extraction has been chosen for this study as it has high anthocyanins extraction efficiency (Piyapanrungrueang et al., 2015). It has also been shown to generate phenolic rich extracts from rice in a short time (minutes) compared to more conventional maceration (hours) (Setyaningsih et al., 2015). In addition, microwave-assisted extraction has also been used to extract protein and gamma oryzanol (rice bran oil) (Pandey and Shrivastava, 2018; Phongthai et al., 2016). When treatment is conducted in a pressurised vessel between 100 and 374 °C (Soto Ayala and Luque de Castro, 2001), it would create subcritical water with properties similar to less-polar organic solvents such as methanol (Teo et al., 2010). The method could overcome environmental concerns of utilizing organic solvents and potentially leaving chemical residues. Limited information is available about the use of microwave-assisted extraction of anthocyanins from Manipur black rice *Chakhao*. Although efficient saccharification of the *Chakhao* straw and recalcitrance of the husk (Moirangthem et al., 2017) and presence of 3.36 mg/100 g of anthocyanins in husk has been reported (Jha et al., 2017), there are no studies on the phytochemicals of black rice straw.

There have been no studies on the general phytochemical content of straw from black rice. However, it is known that normal white rice straw contains a number of phytochemicals (phenolics) (Karimi et al., 2014). It is thus probable that black rice straw may also have a similar phytochemical composition in addition to its more novel anthocyanin present in the black rice grain. Therefore, the present study aimed to investigate the effect of microwave-assisted subcritical water extraction (MA-SWE) on total anthocyanins and antioxidant activity of extracts compared to standard methanol extraction. Assessment of the effects of the straw microwave extract on Jurkat and colorectal cancer cells was also conducted and compared against the bran extract. Such works could promote a higher commercial value for this newly GI awarded crop.

## 2. Materials and methods

### 2.1. Plant material

The black rice *Chakhao Poireiton* straw, along with the industrially milled fractions of the paddy were imported from Manipur (India). These were categorised and labelled into different fractions. Paddy rice was categorised into 4 fractions: paddy rice (de-husked, unpolished grain), polished rice (brown rice with some bran removed), husk and bran. While the straw was air-dried and categorised into 5 fractions: straw (whole straw containing both stem and leaf), leaf (whole leaf containing leaf sheath and leaf blade); stem (whole stem containing nodes and internodes), stem top (consisting of only the first internode connected to the rachis); and the stem lower (consisting of the remaining stem internodes and nodes). Straw fractions were milled separately to 2 mm mesh size using a FRITSCH Pulverisette 19 knife mill and stored along with the other fractions, at 4 °C in separate bags with airtight seals. Rice grain and husk were ground using a coffee grinder. Three replicates of each fraction were carried out for the extraction.

### 2.2. Conventional solvent extraction using methanol

Anthocyanins extraction was carried out with a slight modification to that described by Sutharut and Sudarat (2012). To 10 mL of methanol, 500 mg plant material was added and vortexed for 30 s followed by incubation at 60 °C for 20 min with samples being vortexed twice during the incubation. The samples were centrifuged at 4472 × *g* for 10 min and the supernatant collected. The pellet was mixed with another 5 mL of methanol, vortexed, centrifuged and the supernatant collected. The pooled supernatants were centrifuged again, filtered through a 0.22 μm syringe filter and stored at −20 °C for further analysis. All extractions were done in triplicate.

### 2.3. Microwave-assisted subcritical water extraction (MA-SWE)

MA-SWE was conducted in a pressurised Monowave 300 microwave synthesis reactor (Anton Paar, Germany) with 850 W maximum magnetron output power. Accurate temperature control was maintained using both external infrared and internal ruby based fibre optic thermometers. Output power and pressure were not controllable, but controlled heating to a given set temperature in a given time was achieved by choosing the inbuilt “as-fast-as-possible” mode followed by rapid cooling with a flow of nitrogen. To 10 mL of water, 500 mg of plant material was added, steeped at room temperature for 2 min and then heated at temperatures between 25–200 °C for 5 min. The samples were then centrifuged at 4472 × *g* for 10 min, the supernatant filtered through 0.22 μm syringe filters and stored at −20 °C for further analysis. All extractions were done in triplicates.

### 2.4. Measurement of anthocyanins

The total anthocyanins content (TAC) was determined using the pH-differential method (Piyapanrungrueang et al., 2015). The absorbance of the samples was measured at 515 and 700 nm in pH 1.0 and 4.5 using a spectrophotometer-7315 (Jenway, UK). The TAC was calculated using Eq. (1) and expressed as milligrams of cyanidin 3-glucoside (cyn 3-glu)

equivalent per 100 g dry sample (mg cyn 3-glu/100 g):

$$TAC(mg/100g)=(A \times MW \times DF \times V \times 100)/(\varepsilon \times L \times m) \quad (1)$$

Where, $A$= (A510–A700) pH 1.0 – (A510–A700) pH 4.5; MW is the molecular weight of anthocyanins (449.2 g/mol), DF is the dilution factor, V is the extract volume (mL), $\varepsilon$ is molar absorbance of cyn 3-glu (26,900 L/mol/cm), L is the cell path length (1 cm), and m the sample weight (g).

### 2.5. Antioxidant Assay- 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH)

The DPPH free radical scavenging method was adapted from Wong et al. (2006). Briefly, 0.1 mL of extract was pipetted into the test tube followed by 3 mL of 0.1 mM DPPH (Sigma Aldrich). For blank, the sample was replaced with the extraction solvent. The mixture was then vortexed thoroughly and kept for 30 min in the dark. The absorbance was measured at a wavelength of 515 nm with spectrophotometer 7315 (Jenway, UK). Standard Trolox solutions of 20, 40, 80 and 160 μg/mL was used and results expressed as mg of Trolox equivalent (TE) per 100 gm of sample (mg TE/100 g).

### 2.6. Drying the straw and bran extracts

Methanol extracts were concentrated using a rotary drum evaporator (Heidolph Rotary Evaporator, Germany) connected to a chiller unit (Julabo F250). The evaporator was set to 20 ˚C and 30 rpm and the chiller unit was maintained at −9 ˚C under vacuum. The methanol extracts were then subjected to drying over a flow of nitrogen until all the methanol had evaporated and a constant weight was achieved. Microwaved extracts were initially frozen in a glass flask by placing in a bath containing dry ice and acetone to form a thin film. They were then freeze-dried under vacuum overnight (lyophilised) (Telstar Cryodos, Spain).

### 2.7. In vitro *studies*

#### 2.7.1. Cell culture

Biocompatibility of bran and straw extracts was investigated using two different human cell lines: Jurkat and Colorectal cancer cells. Jurkat (J.RT3-T3.5) cells (ATCC TIB-153; human T lymphocyte cell line) were grown in Roswell Park Memorial Institute (RPMI)−1640 medium containing 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, 1500 mg/L sodium bicarbonate and 10% Foetal calf serum (FCS). Human colorectal cancer cells (HCT-116) were cultured in RPMI 1640 medium with 10% foetal bovine serum (FBS), and 1% penicillin/streptomycin. Cells were cultured at 37 ˚C in 5% $CO_2$.

#### 2.7.2. Cytotoxicity on Jurkat cell lines using flow cytometry

Jurkat cells (100,000 cells per well) were incubated with extracts at 100, 200, 400, 800 μg/mL in 100 μl total volume for 24 h at 37 ˚C with 5% $CO_2$. Cells incubated without the extract were used as negative controls and cells incubated with 3 $\mu$g/mL of cytotoxic calcium ionophore (A23187, Sigma) in absence of extract were used as positive controls. After incubation, cells were washed with Annexin-V binding buffer (MACS, UK), and stained with Annexin-V and propidium iodide (Sigma P4864) both at 5 μg/mL and incubated at 4 ˚C for 30 min. Cells were then washed and re-suspended in 500 μl of binding buffer and analysed using an FC500 flow cytometer (Beckman Coulter, UK). Ten thousand events gated by forward/side scatter analysis were acquired for each sample and data analysed using WEASEL version 3.0 (Walter and Eliza Hall Institute of Medical Research). Cells were defined as: live Annexin-V -ve/PI -ve; apoptotic Annexin-*V* +ve/PI -ve or dead Annexin-*V* +ve/PI +ve.

#### 2.7.3. Colony formation assay of extracts on colorectal cell lines

Colorectal cancer cells (HCT-116) were seeded at a density of 250 cells/well and incubated for 7 days with the bran and straw extracts at concentrations of 50, 100 and 200 μg/mL. Cells were stained with methylene blue and colonies counted on ImageJ software using a colony counter plugin.

### 2.8. Statistics

Statistical analysis of variance (ANOVA), standard deviations and Pearson correlations were calculated using Prism 8.4.2 (GraphPad Software, Inc). All experiments were carried out in triplicates and results are mean ± standard deviation (SD). Differences were considered significant at $p < 0.05$.

## 3. Results and discussion

### 3.1. Anthocyanins content of black rice fractions

Anthocyanins were extracted from the paddy and straw fractions using methanol and quantified using the pH differential method (Fig. 1). As expected, the bran had the highest anthocyanins content. This could make bran an attractive candidate for anthocyanins extraction. However, to maintain the colour and quality of black rice, only a small fraction of bran is generally removed during processing (Kraithong et al., 2018). Removal of the bran in the present study resulted in a ~40% reduction of anthocyanins. Additionally, most of the ways in which *Chakhao* grain is consumed involves cooking/heat, which drastically reduces its phytochemicals and antioxidant capacities (Saikia et al., 2012). Similarly, thermal processing of food containing anthocyanins can strongly impact the final content (Giusti and Wrolstad, 2003). Hence, mild extraction from non-edible fractions such as the straw are important.

The anthocyanins content of the unfractionated straw was 62.8 ± 4.2 mg/100 g and this was present mainly in the stem lower fraction (175.3 ± 12.8 mg/100 g), while the stem top fraction and the leaf had negligible anthocyanins. Commercial sources of anthocyanins include grape marc which contains ~100 mg/100 g (Liang et al., 2008), while whole grape has 27 mg/100 g (Wu et al., 2006). This would suggest the straw is a promising source of anthocyanins especially, from a by-product, with most countries having an anthocyanins intake of less than 50% below the recommended minimum intake of 80 mg/day (Finland highest with 47 mg/day) (Igwe et al., 2019; Zhang et al., 2020).

### 3.2. Microwave-assisted subcritical water extraction (MA-SWE) of anthocyanins

MA-SWE was explored as an alternative to organic solvent for the extraction of the anthocyanins from the straw. The anthocyanins content determined using methanol extraction was used as an indication of the theoretical maximum yield and the efficiency of the MA-SWE at temperatures ranging from 25 to 200 ˚C for 5 min was assessed (Fig. 2).

The maximum efficiency of MA-SWE was at 90 ˚C and this extracted 85.8% of the theoretical maximum anthocyanins. However, the difference in the anthocyanins content between the methanol and 90 ˚C MA-SWE extracts was not significant ($p = 0.095$). Beyond this temperature, anthocyanins content of the extract declined and was undetectable from 160 ˚C onwards possibly due to the heat-labile nature of anthocyanins resulting in their degradation. The differences between the extraction efficiencies at 90 and 100 ˚C were statistically insignificant ($p = 0.454$). Hence, an extraction at 100 ˚C may be more commercially suitable as it is easier to maintain water at 100 ˚C. However, the difference in anthocyanins content between the methanol and 100 ˚C MA-SWE extracts was significant ($p = 0.016$). For both, the methanol and MA-SWE, solid loading of 5% (w/v) was chosen as high loading has been reported to have a negligible impact on anthocyanins extraction (Bridgers et al., 2010).

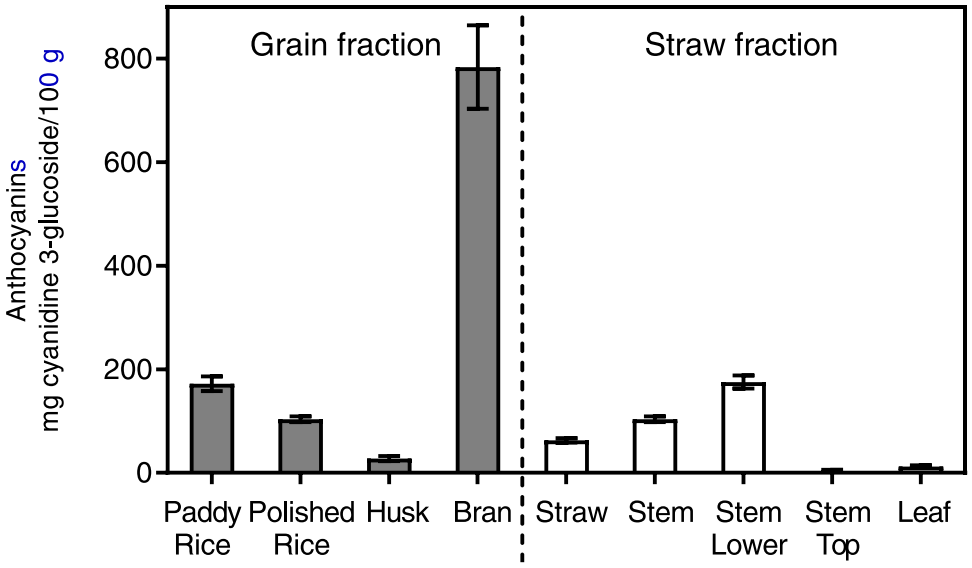


**Fig. 1.** Anthocyanins content of various rice fractions extracted by methanol and expressed as mg of cyanidin 3-glucoside/100 g of dry biomass (mean ± SD, $n = 3$).

**Table 1**
Anthocyanins content and antioxidant activity of methanol and microwave extracts. Values are mean ± SD, $n = 3$.

| Fractions | Anthocyaninsmg cyanidin-3 glucoside equivalent per 100 g dry biomass | DPPH activitymg Trolox equivalent per 100 g dry biomass |
|---|---|---|
| **Methanol Extracts** | | |
| Grain | 172.0 ± 14.1 | 96.67 ± 6.67 |
| Bran | 783.7 ± 80.5 | 456.30 ± 10.37 |
| Stem | 101.1 ± 5.4 | 203.89 ± 3.89 |
| Stem lower | 175.3 ± 12.8 | 256.3 ± 10.15 |
| Stem top | 4.0 ± 2.8 | 0 |
| Leaf | 12.0 ± 2.7 | 0 |
| Straw | 62.8 ± 4.2 | 73.06 ± 2.0 |
| **Microwaved extracts** | | |
| Straw 100 °C –5 min | 50.8 ± 3.1 | 98.12 ± 4.07 |
| Straw 90 °C –5 min | 53.9 ± 5.8 | 100.74 ± 7.26 |

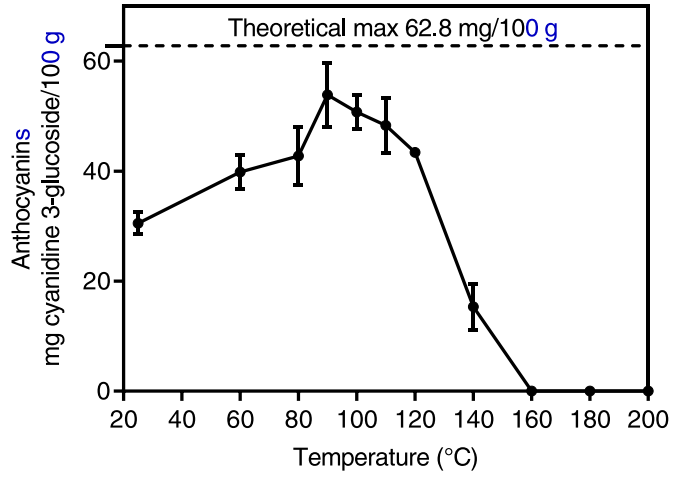


**Fig. 2.** Microwave-assisted subcritical water extraction (MA-SWE) of anthocyanins from straw. Straw was heated in a pressurised microwave reactor for 5 min at different temperatures and extracts assessed for anthocyanins content expressed as mg cyanidin 3-glucoside/100 g of dry biomass (mean ± SD, $n = 3$).

### 3.3. Antioxidant activity of extracts

Anthocyanins content and antioxidant activities, measured via DPPH assay, of the microwave and methanol extracts, are shown in Table 1. There was a positive correlation between anthocyanins content and antioxidant activity ($r = 0.90$), possibly indicating that anthocyanins could be a major contributor in the observed antioxidant effect.

As shown previously the microwave extracts of straw, at both 90 and 100 °C, contained similar amounts of anthocyanin but this was significantly lower than the equivalent methanol extract in the case of 100 °C extract. The 90 and 100 °C microwave extracts also showed similar antioxidant activity, and more importantly, both were significantly higher than the methanol extract ($p = 0.030$ and 0.026 for microwave extracts at 90 and 100 °C, respectively). Microwave-assisted extracts have been reported to display much higher antioxidant activity than those obtained by traditional solvent extraction (Dorta et al., 2013). This could be due to easier release of phytochemical compounds from the disruption of plant matrix by microwave (Hemwimon et al., 2007), and low thermal degradation, decomposition or oxidation of bioactive owing to its quick heating property (Liazid et al., 2007). Menzel et al. (2020) also reported an aqueous extract of rice straw displayed higher antioxidant activity compared to 80% methanol and 80% ethanol extracts, which correlated with higher phenolic compounds in the water extracts. The microwave and higher temperature extraction could have also released additional antioxidant compounds such as bound phenolic acids from cell wall matrices (Samyor et al., 2017). Additionally, methanol extracts were dried at 20 °C compared to freeze-drying of the MA-SWE extracts, which could have influenced the activity of the methanol extract. The measurement of antioxidant activity was crucial to demonstrate that the anthocyanins were not denatured during MA-SWE and extracts were comparable to methanol extracts. This antioxidant property of anthocyanins may contribute to its role in the prevention of neuronal and cardiovascular illness, cancer and diabetes to name a few (Konczak and Zhang, 2004). The antioxidant activity of anthocyanins

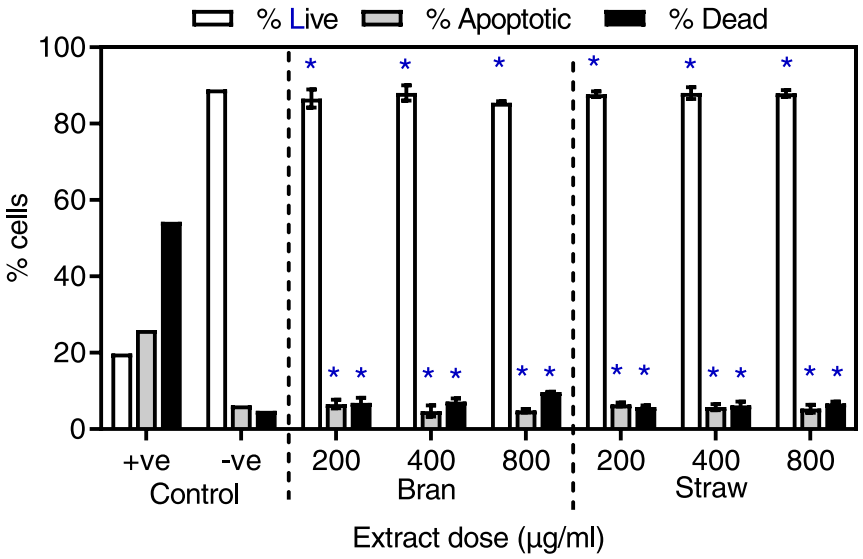


**Fig. 3.** Cytotoxicity and apoptosis cell responses to straw and bran microwave extracts at 200–800 µg/mL on Jurkat cell lines after 24 h of incubation measured with flow cytometry following staining with propidium iodide and annexin V. Control +ve is cells incubated with 3 µg/mL of ionophore in absence of extract. Control -ve is cells incubated without the extract and ionophore (mean ± SD, $n = 3$). * represents values that are not significantly different to their respective -ve control ($p > 0.05$).

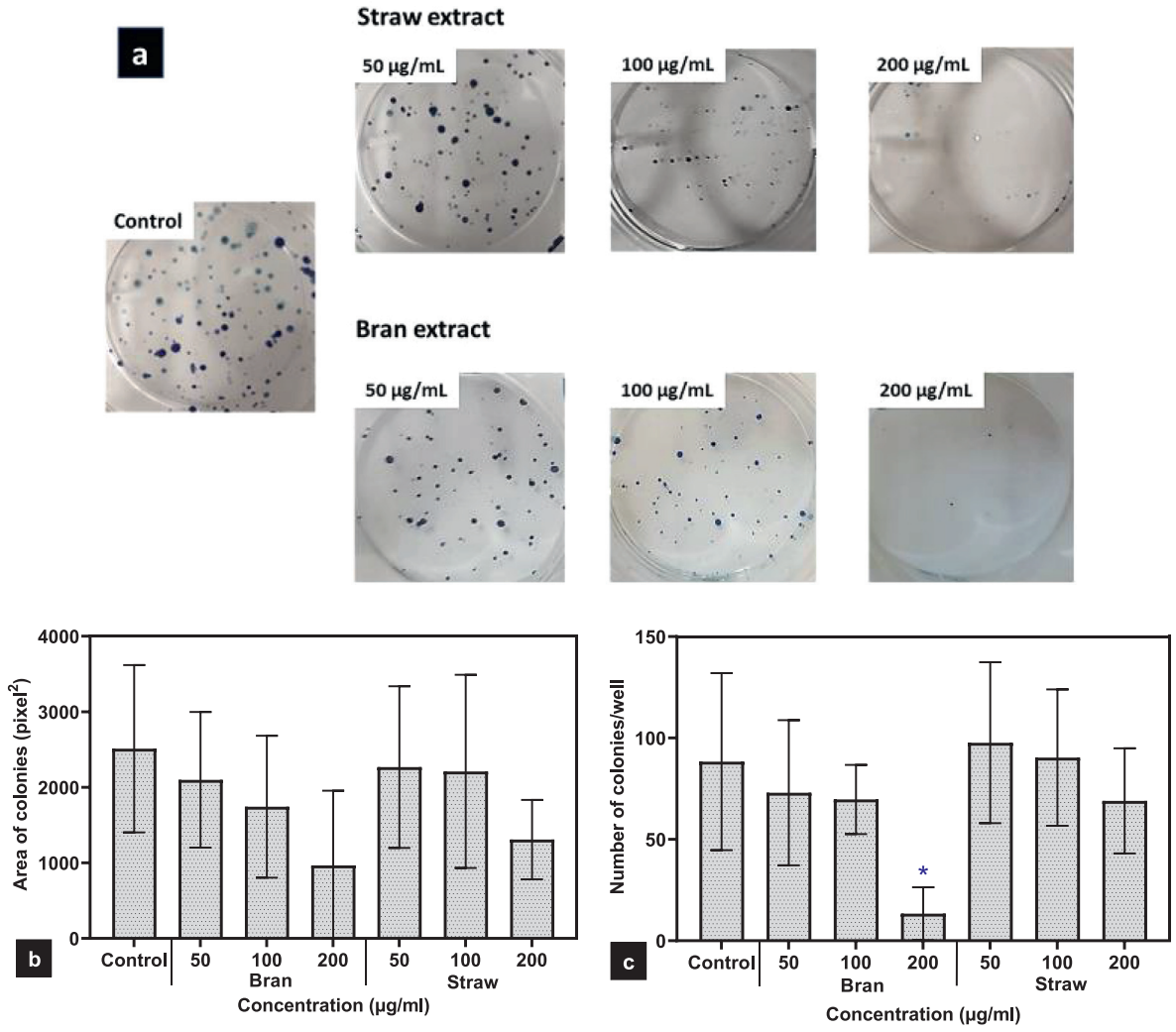


**Fig. 4.** Extract effects on colony-forming ability of colorectal cells (HCT-116). Colorectal cell lines were incubated for 7 days with extracts at the indicated concentrations. (a) Representative images showing colony formation in the presence of various concentrations of straw and bran extracts, stained using methylene blue. (b) The mean area of colonies per well and (c) the total number of colonies formed per well under each condition are presented. The results are representative of three independent experiments ± SD. * represent a significant difference ($p < 0.05$) compared to control.

has been reported to be much higher than that of other natural antioxidants such as vitamin C (Ascorbic acid) and vitamin E (alpha-tocopherol) (Motohashi and Sakagami, 2008), and artificial antioxidants such as butylated hydroxytoluene (BHT) (Fukumoto and Mazza, 2000). Phenolic compounds extracted via alkaline pretreatments of rice straw also exhibited high antioxidant activity. However, being obtained via chemical methods they would have fewer applications in the food and pharmaceutical industries than those obtained via biological methods or cleaner extraction methods (Chen et al., 2018).

### 3.4. In vitro *cytotoxicity study on Jurkat cell lines*

An *in vitro* assessment of the extracts was carried out by measuring the viability of Jurkat cells following exposure to 200 to 800 μg/mL microwave extract from straw. Microwave bran extract was included to assess if the two extracts induce similar responses. Apoptosis and cytotoxicity of Jurkat cells were detected by double staining using Annexin-V and propidium iodide (PI), respectively, followed by analysis with fluorescence-activated cell sorting (FACS) - Fig. 3. The respective representative dot plots are available in Supplementary Figure S1.

Cell viability was not affected by either extract, with results showing a decrease of no more than 10% in cell viability after 24 h of incubation (relative to -ve control). There are no significant effects of either extract at any concentration compared to the -ve control ($p > 0.05$). Increasing extract concentration from 200 to 800 μg/mL does not result in any further death of cells nor induction of apoptosis. This could mean that the extract may be potentially suitable to fortify foods, boosting the nutraceutical value of the food while also acting as a natural antioxidant colorant.

### 3.5. In vitro *colony formation assay on colorectal cells*

Human colorectal cancer cells (HCT-116) were treated with different concentrations of microwaved black rice straw and bran extracts (50 to 200 μg/mL), and the colony-forming ability of the cells were determined by incubation with the extract for 7 days followed by staining with methylene blue (Fig. 4, a–c).

The treatment revealed a dose-dependent reduction in colony formation. However, a significant reduction of over 50% compared to the control was only observed when treated with 200 μg/mL of the bran extract ($p = 0.04$). Hence, bran extract was more potent in inhibiting cell growth in comparison with the straw extract. This could be because the majority of the bioactive components in rice are present in the bran/germ fraction (Verma and Srivastav, 2020). Table 1 also shows that the bran had the highest anthocyanins content amongst the different fractions under study. This dose-dependent decrease in colony formation ability suggests potential anticancer activity of the extract. This is in line with previous studies, where it has been reported that anthocyanins induced cell cycle arrest and apoptosis in several human cell lines, including colon adenocarcinoma and HCT-116 (Lazzè et al., 2004; Yun et al., 2009). Since the crude extract obtained through MA-SWE showed bioactivity in cell lines, it warrants bioactivity guided fractionation to narrow down on the active ingredient(s).

## 4. Conclusions

The present study provides strong evidence that through microwave-assisted subcritical water extraction (MA-SWE), efficient anthocyanins extraction with an antioxidant capacity comparable or even better than conventional solvent extraction can be achieved. Currently, costly feedstocks and production technology make natural anthocyanins production relatively expensive (Jezek et al., 2018). Hence, agricultural by-products such as black rice straw and bran are an attractive alternative. Both straw and bran crude extracts were able to inhibit colony formation of colorectal cells in a dose-dependent manner and were non-cytotoxic to Jurkat cell lines. This study presents evidence that straw of *Chakhao*, in addition to the bran, has important bioactive compounds and that MA-SWE is an efficient technology for the valorisation of the newly GI awarded Manipur Black rice *Chakhao*.

These results indicate that the black rice straw could be exploited as a natural and safe source of functional antioxidants, natural colourants or functional ingredients for dietary/pharmaceutical applications. The bioactive recovered from food processing by-products can be put back into the food industry value chain for the development of functional foods. Although there was a strong correlation between the antioxidant activity and anthocyanin content, the antioxidant activity could also have been influenced by the presence of other phytochemicals in the black rice straw extract. Some of the reported phytochemicals from normal/white rice straw include phenolics which have antioxidant potential. Further extensive investigation on the phytochemical composition of the black rice straw microwave-assisted extracts and its bioactivity are needed. Additionally, validations at a small-scale level are needed to assess its economic feasibility of MA-SWE. Such work could promote a higher commercial value of this newly GI awarded crop.

## Declaration of Competing Interest

The authors declare that they have no conflict of interest with respect to the work described in the manuscript.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2021.100030.

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