Antioxidant and antibacterial activity of *Rhoeo spathacea* (Swartz) Stearn leaves

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Abstract The decoction and infusion of *Rhoeo spathacea* (Swartz) Stearn leaves have been recognized as a functional food particularly in South America, but has not yet gained international popularity as a beverage. The primary aim of this study was to establish the viability of *R. spathacea* aqueous leaf extracts as a beverage, in terms of its antioxidant activity and antibacterial activity. The antioxidant contents of aqueous and methanol leaf extracts were evaluated by the total phenolic content (TPC) and total flavonoid content (TFC) assays. The antioxidant activities measured were DPPH radical scavenging activity (FRS), ferric reducing power (FRP) and ferrous ion chelating (FIC) activity. The aqueous leaf extracts in the forms of decoction and infusion, were found to have comparable TPC and antioxidant activity with other herbal teas previously reported by our research group. Both decoction and infusion also exhibited antibacterial activity against six species of Gram positive and four species of Gram negative bacteria, notably methicillin-resistant *Staphylococcus aureus* and *Neisseria gonorrhoeae*. A total of four different known phenolic compounds were identified by HPLC and MS, three of which have not been previously reported to be found in this plant. Both the decoction and infusion of the leaves *R. spathacea* have potential to be popularized into a common beverage.

Keywords *Rhoeo spathacea* · Antioxidant · Antibacterial · Decoction · Infusion · Aqueous extracts

Introduction

There are two main types of water-based preparations that involve boiling water - decoctions and infusions. Decoctions require a prolonged application of heat, often used in preparation of many traditional remedies, while infusions involve the steeping of plant material in boiling water for a short period, such as in the preparation of teas. The herbal plant *Rhoeo spathacea* (Sw.) Stearn, also known as *Tradescantia spathacea*, has been recognized as a functional food particularly in South America with the dried leaves having the potential to be developed into a tea-like beverage (Rosales-Reyes et al. 2008). The decoction is taken orally on a daily basis as a treatment for cancer (Rosales-Reyes et al. 2008), as an anti-inflammatory agent (Longuefosse and Nossin 1996), and is also purported to be capable of treating *Neisseria gonorrhoeae* (Halberstein 2005). Despite these traditional applications, *R. spathacea* remains uncommonly used outside of South America, but shows promise to be established as a beverage internationally.

The consumption of antioxidants, which are ubiquitously present in many medicinal and herbal plants, has been associated with a reduced risk of the incidence of oxidative diseases ranging from cancer, cardiovascular disorders, diabetes mellitus, rheumatic arthritis to aging (Halliwell 1996). In recent years, a number of antibiotics have lost their effectiveness due to the development of resistant strains. In addition, antibiotics are sometimes linked to adverse effects such as hypersensitivity, immune-suppression and allergic reactions (Berahou et al. 2007). There is therefore a need to develop new antibiotics from natural sources such as plants.

Thus, the primary aim of this study was to establish the viability of *R. spathacea* aqueous leaf extracts as a beverage, in terms of its antioxidant activity in comparison with other tropical and herbal teas previously reported by our research group (Chan et al. 2010), as well as its antibacterial activity

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against several species, including *N. gonorrhoeae*. The decoction and infusion methods were compared, as both techniques were traditionally used in the preparation of this plant for oral consumption (Reyes-Munguía et al. 2009; Rosales-Reyes et al. 2008). In addition, an optimized methanolic extract and aqueous extracts using water at room temperature (as opposed to boiling water) were used as controls to respectively determine the extraction efficiency compared to an organic solvent, and to demonstrate the effect of heat on the extraction efficiency. Lastly, qualitative phytochemical screening, reverse phase high pressure liquid chromatography (RP-HPLC) and mass spectrometry (LC-MS) were used to determine the classes of compounds present, and to identify compounds present in extracts of *R. spathacea*.

Materials and methods

Samples

Fresh *R. spathacea* leaf samples were obtained from Petaling Jaya, Malaysia. The identity of the plant was confirmed by Dr Wong Khoon Meng, former professor of Botany, Institute of Biological Sciences, University of Malaya. They were freeze-dried in a freeze dryer (Christ Alpha 1–4 unit) for 48 hours, and weighed before and after drying to determine the moisture content. Bacterial isolates were obtained from American Type Culture Collection (ATCC), with the exception of *Serratia marcescens* and *Proteus vulgaris*, which were obtained from Institute of Medical Research (IMR), Malaysia. All bacteria were grown on nutrient agar, with the exception of *Neisseria gonorrhoeae*, which was grown on chocolate II agar with haemoglobin.

Chemicals and reagents

The various reagents used throughout this project were purchased from suppliers as follows. TPC analysis: Folin-Ciocalteu's phenol reagent (2 N, R and M Chemicals, Essex, U.K.), gallic acid (98 %, Fluka, Steinheim, France), anhydrous sodium carbonate (99 %, J. Kollin, U.K.); diphenyl-2-picrylhydrazyl (DPPH·) assay: 1,1-diphenyl-2-picrylhydrazyl (90 %, Sigma, St. Louis, MO); ferric reducing power (FRP) assay: ferric chloride hexa-hydrate (100 %, Fisher Scientific, Loughborough, UK), potassium ferricyanide (99 %, Unilab, Auburn, Australia), trichloroacetic acid (99.8 %, HmbG Chemicals, Barcelona, Spain), potassium dihydrogen orthophosphate (99.5 %, Fisher Scientific), dipotassium hydrogen phosphate (99 %, Merck, Darmstadt, Germany), iron chloride (99 %, RandM Chemicals); ferrous ion chelating (FIC) assay: ferrozine (98 %, Acros Organics, Morris Plains, NJ), ferrous sulphate hepta-hydrate (HmbG Chemicals), ethylenediamine-tetraacetic acid (EDTA) (98 %, Sigma); total flavonoid

content (TFC) analysis: aluminium chloride (99.5 %, Bendosen Laboratory Chemicals, Bendosen, Norway), potassium acetate (99 %, R and M chemicals), quercetin (98 %, Sigma); phytochemical screening: sulfuric acid (95–97 %, HmbG Chemicals), hydrochloric acid (37 %, Merck, Darmstadt, Germany), Dragendorff reagent (Fluka), α -naphthol (99 %, Sigma); antimicrobial activity: nutrient broth (Oxoid, Hampshire, England), carbon dioxide pack (Oxoid), nutrient agar (Oxoid), vancomycin (Sigma), chocolate II agar with haemoglobin (Fisher Scientific, Selangor, Malaysia).

Extraction of samples

The fresh leaf was split down the middle (along the vertical axis) and each half was weighed and extracted at a ratio of 50 mL water to 1 g of leaf material after liquid nitrogen-aided crushing. The first half was kept boiling under constant heat for 60 min, while the other half of the leaf was suspended in water at room temperature for 60 min to function as the control. The same methodology was used for the preparation of the infusion, but instead of boiling under constant heat for 60 min, boiling water was added instead and the sample was left to stand for 15 min without any additional heating. The control was the leaves suspended for 15 min in water at room temperature. Methanolic extracts were prepared with 70 % methanol, which was previously found to have the highest extraction efficiency for phenolic compounds in a preliminary study in our laboratory.

Determination of total phenolic content (TPC)

The determination of the total phenolic content of the samples was done using a procedure modified from Kähkönen et al. (1999) utilizing the Folin-Ciocalteu reagent. Samples (300 μ L, in triplicate) were mixed with 1.5 mL of the 10 % Folin-Ciocalteu reagent, followed by an addition of 1.2 mL of 7.5 % (w/v) sodium carbonate (Na_2CO_3) solution. The test tubes were then left to stand for 30 min in the dark at room temperature before the absorbance values were measured at 765 nm. The total phenolic content was expressed as mg gallic acid equivalent per 100 g of sample (mg GAE/100 g).

DPPH radical scavenging assay (FRS)

The DPPH· assay was based on the procedures described in Leong and Shui (2002) and Miliauskas et al. (2004) where the reduction of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured spectrometrically to determine the radical scavenging activity of the extract. Two mL of DPPH· solution (5.9 mg in 100 mL methanol) was added to 1 mL of three different concentrations of the sample extract. The absorbance of the solution was measured at 517 nm after a 30 min incubation time. The free radical scavenging activity (FRS)

was expressed as ascorbic acid (AA) equivalent antioxidant capacity, in mg AA/100 g using the equation: $FRS = IC_{50(AA)} / IC_{50(sample)} \times 10^5$. IC_{50} of AA used for calculation of FRS was 0.00387 mg/mL (Chan et al. 2007).

Ferric reducing power (FRP) assay

The reducing power of the extracts was determined using potassium hexacyanoferrate(III) as described in the procedure described by Juntachote and Berghofer (2005). The FRP assay was used to assess the ability of any antioxidants present in the extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). One mL of sample extract of different concentration was added with 2.5 mL of 0.2 M phosphate buffer (pH 6.7) and the same volume of 1 % (w/v) potassium ferricyanide. The solutions were mixed and incubated in 50 °C water bath for 20 min. Subsequently, 2.5 mL of 10 % trichloroacetic acid was added to stop the reaction. Then, the solution in each test tube was separated into aliquots of 2.5 mL, added with 2.5 mL of miliQ water and 0.5 mL of 0.1 % $FeCl_3$. The solutions were mixed and left on bench for 30 min before the absorbance was measured at 700 nm. FRP was expressed as mg gallic acid equivalent per gram of sample, mg GAE/g.

Ferrous ion chelating (FIC) assay

The determination of ferrous ion chelating strength of the extract was based on the procedures described in Mau et al. (2003), and Singh and Rajini (2004). One mL of 0.25 mM ferrozine was added to 1 mL of sample of different concentrations (0.2, 0.5 and 1 mL of extract), followed by 1 mL of 0.1 mM $FeSO_4$. The mixtures were incubated at room temperature for 10 min before the absorbance was measured at 562 nm. It was expressed as the percentage of iron chelating

activity. EDTA (0.017 - 0.067 mg/mL) was used as a positive control.

Determination of total flavonoid content (TFC)

Flavonoid content in the extract was determined with the aluminium chloride colorimetric method as described in Chang et al. (2002). Equal volumes of 10 % aluminium chloride and 1.0 M potassium acetate (0.1 mL each) were added to 0.5 mL of extract, followed by 2.8 mL of distilled water. The solutions were mixed well and incubated at room temperature for 30 min before the absorbance was taken at 435 nm. The flavonoid concentration was expressed as mg quercetin equivalent per 100 g sample, mg QE/100 g.

Phytochemical screening

The extracts and fractions were tested for the following classes of compounds - terpenoids/sterols, alkaloids, saponins and glycosides - by employing methods described by Nayak et al. (2009) and Silva et al. (1998). Presence of anthocyanins was confirmed by employing the method described by Nielson and Harley (2004).

Identification of phenolic compounds

RP-HPLC analysis was performed using Agilent Technologies 1200 Series with quaternary pump (model G1311A) and a G1315B diode array detector. The stationary phase used was an Agilent Eclipse XDB C18 column (4.6 × 250 mm, 5 µm), with the two mobile phases being water + 0.1 % trifluoroacetic acid (Solvent A) and methanol + 0.1 % trifluoroacetic acid (Solvent B), with a linear gradient of 95 % Solvent A to 100 % Solvent B in 15 min, and maintained at 100 % Solvent B for an additional 5 min. The mobile phase

Table 1 Antioxidant properties of methanolic extract, decoction and infusion of *R. spathacea* leaves

Treatment	TPC (mg GAE/100 g)	FRS (mg AA/100 g)	FRP (mg GAE/g)	TFC (QE mg/100 g)
Decoction	379.3 ± 47.3 ^a (4712 ± 588 ^{a1})	309.0 ± 46.9 ^a (3839 ± 583 ^{a1})	2.2 ± 0.1 ^a (27.3 ± 1.2 ^{a1})	2.1 ± 0.8 ^a (26.1 ± 9.9 ^{a1})
Decoction Control	179.1 ± 65.9 ^b (2225 ± 819 ^{b1})	60.8 ± 16.9 ^b (755.3 ± 209.9 ^{b1})	0.9 ± 0.1 ^b (11.2 ± 1.2 ^{b1})	2.1 ± 0.6 ^a (26.1 ± 7.5 ^{a1})
Infusion	463.6 ± 60.9 ^a (5759 ± 757 ^{a1})	377.8 ± 90.8 ^a (4963 ± 1128 ^{a1})	2.2 ± 1.0 ^a (27.3 ± 12.4 ^{a1})	2.9 ± 0.4 ^a (36.0 ± 5.0 ^{a1})
Infusion Control	178.6 ± 67.9 ^b (2219 ± 844 ^{b1})	117.1 ± 33.4 ^b (1455 ± 415 ^{b1})	1.0 ± 0.1 ^b (12.4 ± 1.2 ^{b1})	2.8 ± 1.0 ^a (34.8 ± 12.4 ^{a1})
Methanolic Extract	432.6 ± 100.0 ^a (5374 ± 1242 ^{a1})	486.4 ± 115.6 ^a (6042 ± 1436 ^{a1})	4.2 ± 1.0 ^c (52.1 ± 12.4 ^{c1})	2.3 ± 1.4 ^a (28.9 ± 17.4 ^{a1})

¹ Values in parenthesis are expressed in terms of 100 g dry weight of leaf samples

² No FIC was observed in any of the samples

³ Results are expressed as mean ± S.D. (n=6). For each column, values followed by the same letter are not significantly different at $p < 0.05$ as measured by the Tukey HSD test.

Table 2 Phytochemical analysis of methanolic extract, decoction and infusion of *R. spathacea* leaves

Class	Decoction	Decoction Control	Infusion	Infusion Control	Methanolic Extract
Tannins	✓	✓	✓	✓	✓
Terpenoids/sterols	✓	✓	✓	✓	✓
Alkaloids	✓	—	✓	—	✓
Glycosides	✓	✓	✓	✓	✓
Saponins	✓	✓	✓	✓	✓
Anthocyanins	✓	✓	✓	✓	✓

was delivered through the column with the flow rate of 1.0 mL/min during the running of the samples. The samples were analyzed at four wavelengths: 210 nm, 254 nm, 280 nm and 365 nm.

LC-MS spectra were recorded on a ThermoFinnigan model LCQ Deca coupled mass spectrometer fitted with an ESI source. Electrospray ionization was used under the following conditions for both positive and negative ion modes: capillary voltage, 2.7 kV; source temperature, 100 °C; desolvation temperature, 350 °C; cone gas flow, 30 L/h; desolvation gas flow, 700 L/h. The column used was ACQUITY UPLC BEH C18 1.7 μ m, 2.1 $\times$ 50 mm column, with an ACQUITY PDA Detector (ACQ-PDA) Version 1.40.1932 detector. The mobile phase consisted of water+0.1 % formic acid (solvent A) and acetonitrile+0.1 % formic acid (solvent B). Gradient elution was performed as follows: from 0 to 5 min, linear gradient of 5 % solvent B to 30 % solvent B; from 5 to 6 min, linear gradient of 30 % solvent B to 100 % solvent B; from 6 to 8 min, isocratic at 8 min. The software used was Waters MassLynx 4.1.

Determination of antimicrobial activity

The minimum inhibitory concentration (MIC) of the samples was determined using the broth microdilution technique in 96-well flat bottom microtiter plates as described by the Clinical and Laboratory Standards Institute (2009) with a few modifications. One hundred and eighty microlitres of nutrient broth was loaded into all the wells of the first column of the 96-well plate, followed by 100 μ L of nutrient broth in all the other wells. Twenty μ L of each sample type (200 mg/mL stock concentration) was loaded into the first column in triplicate,

enabling two samples to be run concurrently on a single plate. Serial doubling dilution was then performed nine times, keeping the volume of each well at 100 μ L. One hundred μ L of nutrient broth inoculated with bacteria the day prior was standardized with the Mcfarland standard and then loaded into these wells for a final working concentration of sample ranging from 10 mg/mL till 0.02 mg/mL. The plates were then incubated overnight. The lowest concentration where complete inhibition was observed with the unaided eye was noted as the MIC. Vancomycin (10 mg/mL – 0.02 mg/mL) was used as a positive control. For *N. gonorrhoeae*, the bacteria was incubated for 48 hours in an anaerobic jar containing a carbon dioxide pack, both during the initial inoculation of the broth that was to be loaded into the 96-well plate, as well as the 96-well plate itself.

Statistical analysis

One-way analysis of variance (ANOVA) with post-hoc Tukey was performed to determine significance. A probability value of $p < 0.05$ was considered significant. Analysis was done using SPSS16.

Results and discussion

There was no significant difference in TPC, TFC, FRS and FRP between the decoction and infusion, thus indicating that prolonged exposure to boiling temperatures did not negatively impact the overall antioxidant properties. The data in Table 1 is expressed in terms of both 100 g fresh weight and 100 g dry weight in parentheses, with a 92 % water content in the leaves of *R. spathacea* which was determined by freeze drying.

Fig 1 HPLC chromatogram at 254 nm of decoction of *R. spathacea* leaves, labeled with peaks corresponding to compounds 1 to 4 in Table 4

Table 3 Minimum inhibitory concentration of decoction and infusion of *R. spathacea* leaves for sixteen species of bacteria

Gram Positive						
Sample	<i>Bacillus cereus</i> (ATCC 14579)	<i>Bacillus subtilis</i> (ATCC 8188)	<i>Micrococcus luteus</i> (ATCC 4698)	MRSA (ATCC 33591)	<i>Staphylococcus epidermidis</i> (ATCC 12226)	<i>Staphylococcus saprophyticus</i> (ATCC 15305)
Decoction	10	10	10	2.5	10	10
Infusion	10	5	10	2.5	10	5
Methanolic extract	10	10	10	10	10	10
Gram Negative						
	<i>Aeromonas hydrophila</i> (ATCC 49140)		<i>Klebsiella pneumonia</i> (ATCC 10031)	<i>Neisseria gonorrhoeae</i> (ATCC 49226)		<i>Proteus vulgaris</i> (Clinical)
Decoction	5		10	10		10
Infusion	5		10	10		10
Methanolic extract	10					

^a MIC expressed as mg/mL^b No activity was observed for *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 10145), *Proteus mirabilis* (ATCC 12453), *Serratia marcescens* (Clinical) and *Salmonella typhimurium* (ATCC 14028)^c Decoction control and infusion control showed no activity^d Vancomycin MIC < 0.02 mg/mL

Compared to a study published by our research group on the eighteen tropical and temperate herbal teas (Chan et al. 2010), the TPC of the decoction and infusion of *R. spathacea* was only lower than lemon myrtle (*Backhousia citrifolia*) which has the highest TPC of 7,560 mg GAE/ 100 g dry weight and it is similar to guava (*Psidium guajava*), legundi (*Vitex negundo*), oregano (*Origanum vulgare*) and mint (*Mentha spicata*) (4,350 to 5,930 mg GAE/100 g dry weight). Similarly, the FRS activity of the decoction and infusion of *R. spathacea* ranked fourth-highest, tied with Misai Kucing (*Orthosiphon aristatus*), mint (*M. spicata*) and legundi (*V. negundo*). The FRP values were also comparable with that of tropical herbal teas (3–61 mg GAE/g dry weight) and temperate herbal teas (8–49 mg GAE/g dry weight), with the decoction and infusion ranking third-highest out of the eighteen teas. However, both species showed no chelating activity at concentrations as high as 0.17 mg/mL. This indicates that the antioxidants present in both species lack the ability to chelate ferrous ions (compared to ferrozine) and thus have low or no secondary antioxidant activity. It is to be noted that although the antioxidant property of ethanolic extract of *R. discolor* in terms of FRS activity has been reported (González-Avila et al. 2003),

no comparison can be made as the extract TPC and DPPH[•] IC₅₀ values were not reported in that paper.

Overall, the antioxidant activity of *R. spathacea* consistently ranked high compared to the herbal teas, further supporting the hypothesis that this plant has potential to be promoted as a beverage internationally.

Qualitative phytochemical tests (Table 2) revealed for the first time the presence of tannins and alkaloids in *R. spathacea*, while terpenoids/sterols, alkaloids, saponins and anthocyanins, were previously known to exist in *R. spathacea* (Rosales-Reyes et al. 2008). Similarly, glycosides were present in all treatments, as certain glycosides are heat stable (Ohta et al. 1991). This is expected, due to the known presence of flavonoids, anthocyanins and saponins, which may have attached sugar moiety in the structures. Thus far no specific compounds have ever been reported to be identified in leaves of *R. spathacea* with the exception of rheonin (cyanidin 3-O-[6-O-(2-O-(feruloyl)-arabinosyl)-glucoside]-7,3'-di-O-[6-O-(feruloyl)-glucoside]), the primary anthocyanin present in the species (Idaka et al. 1987; Tatsuzawa et al. 2010). Rheonin was confirmed also to be present in our sample, as evidenced by its HPLC-DAD and MS data (Table 4

Table 4 Phenolic compounds identified in decoction of *R. spathacea* leaves

Compound	R _t (min)	Putative identity	HPLC-DAD λ _{max} (nm)	m/z, [M+H] ⁺	m/z, [M-H] ⁻	Mode of identification
1	7.3	Epigallocatechin	279	307	305	UV/MS+standard
2	11.6	Rheonin	285, 327, 538	1433	1431	UV/MS
3	11.9	Peltatoside	267, 341	619 [M+Na], 303	595	UV/MS
4	12.0	Rutin	270, 350	633[M+Na], 303	609	UV/MS + standard

and Fig. 1). HPLC-DAD analysis revealed that rheonin was the only anthocyanin compound with an absorbance at ~530 nm in the infusion and decoction, indicating that the reddish color of the decoction was primarily due to rheonin.

Out of the six different species of Gram-positive bacteria and ten different species of Gram-negative bacteria screened (Table 3), six Gram-positive species were inhibited by both the decoction and infusion, while only four of the ten Gram-negative bacteria tested were susceptible. The lower susceptibility of Gram-negative bacteria can be attributed to the naturally higher impermeability of the bacterial cell wall (Russell 1999). The MIC values range from 2.5 to 10 mg/mL. Of particular note is the inhibition of *N. gonorrhoeae*, which the plant had been purported to work against in traditional applications (Halberstein 2005), and methicillin-resistant *Staphylococcus aureus* (MRSA) that was the most susceptible out of all the sixteen species of bacteria screened, despite its resistance to β -lactam antibiotics. Two of the phenolic compounds identified in *R. spathacea* (Table 4 and Fig. 1): epigallocatechin (Akiyama et al. 2001) and rutin (Cushnie and Lamb 2005) are known to exhibit antibacterial activity. It is therefore very likely that these phenolic compounds are contributors to the antibacterial activity observed.

In addition to their antibacterial activity, the compounds identified in Table 4 are all antioxidants, and have also been purported to be beneficial to human health. Peltatoside is an anti-fungal agent, and may also have potential in treating cataract (Vit and Jacob 2008). Rutin exhibits antiangiogenic activity, and has been reported to reduce risk of atherosclerosis in humans (Fabjan et al. 2003), in addition to exhibiting antiviral activity (Akiyama et al. 2001). The four phenolic compounds identified in this project are highlighted in Fig. 1. Both the decoction and infusion showed matching HPLC profiles, lending extra credence to the comparable antioxidant and antibacterial activity observed in both the decoction and infusion.

Both the decoction and infusion were found to have significantly higher TPC, FRS and FRP than their respective controls which were extracted at room temperature. The presence of heat thus vastly improved the TPC, FRS and FRP, but extraction time did not significantly impact the overall TPC. Heat was crucial in the extraction of alkaloids, which were only present in the decoction and infusion but not the controls (Table 2). This is in agreement with literature where an increase in heat significantly improved the extraction efficiency of alkaloids (Zhang et al. 2005). While alkaloids are often toxic, a separate study conducted in our laboratory using a method as described by Ribeiro et al. (2008) determined the alkaloid concentration to be very low in *R. spathacea* leaves (under 1 % w/w dry weight). This low concentration may have contributed to the observed negative result in the controls. Both controls also exhibited no antibacterial activity at the concentrations tested, further reinforcing the aforementioned

antibacterial role of the polyphenols present, given that both controls had significantly lower TPC compared to the boiling treatments. Thus, the application of boiling heat is crucial in improving the extraction efficiency of *R. spathacea* leaves.

The boiling water treatments also showed comparable TPC, FRS with the methanolic extract (Table 1). Methanol was chosen due to its ability to extract most compounds of both hydrophilic and hydrophobic nature, and is commonly used in phytochemical studies due to this property. The concentration of methanol reported in Table 1 was 70 % as it had the highest extraction efficiency based on TPC (84.0 % $\pm$ 0.1 %), compared to 50 % and 100 % methanol (81.6 % $\pm$ 0.3 % and 82.6 % $\pm$ 0.3 % respectively). The methanolic extract did however exhibit inferior antibacterial activity compared to the boiling treatments (Table 3). This is likely due to the presence of other less hydrophilic compounds that were extracted by methanol along with the hydrophilic antibacterial compounds, resulting in the dilution of these antibacterial compounds. Overall, this shows that boiling water has comparable extraction efficiency with a commonly-used organic solvent, and is superior for the extraction of bioactive hydrophilic compounds.

Conclusion

Both the decoction and infusion methods are viable ways to extract the leaves *R. spathacea*, with TPC and antioxidant activity comparable to many tropical and herbal teas. This, compounded by the antibacterial activity against a range of different bacteria including *N. gonorrhoeae*, MRSA, and several other Gram-negative species indicates that this plant lives up to its reputation in South America as a functional food, and has potential to be popularized into a common beverage.

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