AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2020; 8(3): 01-08

Received: 01-04-2020
Accepted: 02-06-2020

Theresa F Rambaran
a) Department of Chemistry, Faculty of Science and Technology, the University of the West Indies, Mona Campus, Kingston 7, Jamaica.
b) Institute of Applied Sciences, Faculty of Science, Technology and Environment, University of the South Pacific, Suva, Fiji.

Joape Ginigini
Institute of Applied Sciences, Faculty of Science, Technology and Environment, University of the South Pacific, Suva, Fiji.

Corresponding Author:
Theresa F Rambaran
a) Department of Chemistry, Faculty of Science and Technology, the University of the West Indies, Mona Campus, Kingston 7, Jamaica.
b) Institute of Applied Sciences, Faculty of Science, Technology and Environment, University of the South Pacific, Suva, Fiji.

Essential oil profiles of two *Rubus* varieties and the antimicrobial activities and lethality of their extracts

Theresa F Rambaran and Joape Ginigini

Abstract
The essential oil composition of two varieties of the red raspberry, *Rubus rosifolius* (Red, R and Wine red, WR) was examined and the antimicrobial activity and brine shrimp lethality of their extracts determined. Plant samples were obtained and their essential oil profiles assessed using GC-MS analysis. Antimicrobial activity of the crude extracts of both plant varieties was assessed against drug-resistant and foodborne pathogens and the brine shrimp ethality assay was used to assess the potential lethality of the extracts. The quantity and identified essential oil components of the fruits varied between the varieties with the R variety having in greatest quantity the monoterpene, linalool, while that of the WR variety was found to be the sesquiterpene, α-cadinol. Analysis of the antimicrobial activity of the extracts against the growth of drug resistant pathogens showed the methanol extracts having greatest activity with zones of inhibition of 11 and 13 mm (for the R and WR varieties, respectively) against methicillin-resistant *Staphylococcus aureus* (MRSA). The methanol extracts also exhibited minimal activity against several food-borne pathogens including two strains of *Escherichia coli*, *Listeriamono cytogenes* and *Enterobacter aerogenes*. Brine shrimp lethality of the various extracts revealed that the essential oils of both plant varieties had the greatest activity with a LC_{50} value of 63 and 48 ppm for R_{Hex} and WR_{Hex}, respectively. The biological activities of the fruit extracts indicate that their utilization in the production of functional foods, nutraceuticals and pharmaceuticals should be further explored.

Keywords: *Rubus rosifolius*, essential oils, brine shrimp lethality, antimicrobial, MRSA

1. Introduction
Essential oils can be extracted from various parts of aromatic plants and can be utilised in the production of perfumes, cosmetics, and spices, and applied in areas such as aromatherapy, phytotherapy, and nutrition. The chemical composition of essential oilscan be impacted by exogenous and endogenous factors where examples of the former include environmental conditions, the drying procedure, the storage conditions, the method of isolation, and the analytical conditions; while, factors included in the latter are related to anatomical and physiological characteristics of the plants [1]. The yield, taste, and flavour of the oils depend on the extraction protocol used [2]. Terpenes arethe main phytochemicals present in these oils, along with a few other non-terpenoid components [3].
Antimicrobial activity of essential oils has been reported from as early as the 1950s, when the vapour activity of essential oils was evaluated [4]. Consumers have become particularly interested in green consumerism and, as a result of this, there has been a renewal of interest in essential oils [5]. Essential oils from *Rubus* species have been reported to demonstrate antibacterial activity against a wide spectrum of pathogenic bacterial strains [6, 7]. A study by Rauha *et al.* [8] revealed that the most widespread bactericidal activity of berries was obtained from those belonging to the *Rubus* genus. Different classes of compounds demonstrate varied activity against different microbes and, in some cases, activity also varied among strains. The use of phytochemicals as antibacterial agents can vastly impact the health sector. According to the World Health Organization (WHO), unsafe food causes 600 million cases of food borne diseases and 420,000 deaths [9]. In Jamaica, the occurrence of food borne illnesses is of particular concern due to possible ill-health and fatalities that may result, and also due to the movement of people and goods across borders. While the prevalence of such diseases in Jamaica is important to nationals, it is also of great concern to tourists. Jamaica is a popular tourist destination and travelers'diarrhea is one of the most common travel-related illnesses [10]. Given the significance of tourism in the region (contributing 27.2% to GDP in Jamaica in 2014), it is critical that measures be implemented to address the prevalence of foodborne illnesses [11].

Microorganisms can undergo certain changes and develop resistance to antimicrobial agents. There has been a rapid emergence of multiple drug-resistant human pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), rifamycin resistant *S. aureus* (RRSA), wild-type *S. aureus* (WTSA) and vancomycin-resistant *Enterococcus faecium* (VREF). *S. aureus* is a common cause of healthcare-associated infections (HAI) and was outlined as the second most common overall cause of HAIs reported to the National Healthcare Safety Network (NHSN) at the Centres for Disease Control and Prevention (second to the less virulent coagulase-negative staphylococci) [12]. There always exists, therefore, a thrust towards finding new antimicrobial agents.

The brine shrimp lethality of an agent refers to its ability to kill a laboratory cultured larvae (nauplii) and as such, brine shrimp lethality assays can be employed as a preliminary method for the assessment of plant extracts for potential cytotoxic effects [13, 14]. Additionally, it has been reported that the assay shows good correlation with the detection of anticancer compounds in plant extracts [15-17].

In this study, the essential oil composition of two *Rubus rosifolius* varieties were assessed. Brine shrimp lethality and antimicrobial activity of various plant extracts against food-borne and drug-resistant pathogens were also examined.

2. Materials and methods

2.1 Plant material

The berries of two *Rubus rosifolius* varieties identified as red (R) and wine red (WR) were collected from the Holywell Community which is located over 900 m above sea level in the Blue and John Crow Mountains in St Andrew, Jamaica. The plant varieties were collected between April 2011 and January 2012 and were identified by comparison with authentic samples at the herbarium in the Department of Botany, The University of the West Indies, Mona campus, by the herbarium curator. The samples were assigned voucher specimen numbers 35595 and 35596 for the red and wine red varieties, respectively, and were stored at 4 °C.

2.2 Preparation of extracts

The mature berries of the red (R) and wine red (WR) *R. rosifolius* plants were lyophilised (using a Labconco Freezone Freeze Dryer system, model 7522800, Kansas City, MO, USA) and extracted successively with n-hexane, ethyl acetate, and methanol, with a solvent to plant ratio of 4:1 (160g, 11.8% FW, R) and (202g, 14.8% FW, WR). The extraction protocol in all cases included an initial blending at high speed for 3mins, followed by sonication (using a Fisher Scientific Sonicator, model FS110D, MA, USA) for 1hr after which the mixture was filtered. The resulting filter cake was resubmerged in solvent in a similar ratio and left overnight to percolate after which it was filtered. The filter cake was finally washed with the extracting solvent (150 mL) and the resulting filtrates pooled and concentrated *in vacuo* using a BüchiiRotavaporR-215 (Switzerland) to yield viscous yellow oils for the hexane extracts (4.68 g and 7.60 g for the R and WR fruits, respectively) and reddish-brown and bright red gums for the ethyl acetate (5.66 g R and 6.09 g WR) and methanol extracts (79.50 g R and 98.66 g WR).

2.3 Extraction of essential oils

The fresh fruits of the two *Rubus* varieties (1 kg) were blended at low speed for 30 seconds and subjected to steam-distillation for 3 hrs using a Clevenger-type apparatus. The resulting yellow essential oils (with those from the Wine Red fruits having a brighter hue) were then collected and stored at -10 °C for further analysis.

2.3.1 Analytical conditions and component identification of essential oils

The essential oils of the red (R) and wine red (WR) fruits were investigated by GC-MSan HP6890 gas chromatograph equipped with a HP5973 mass spectrometer and an MSD Chem Station software. The carrier gas was helium with a flow rate of 1 ml/min. The injector and detector temperatures were 250 and 280 ºC, respectively and the injection volume was 1 µL. A splitless injector was used along with an ion-source heating of 280 ºC. The temperature program was 80 ºC for 2 min to 280 ºC for 6 min with a heating rate of 10 ºC min^{-1}. The column was a HP-5MS with dimensions of 60 m x 0.25 mm and a film thickness of 0.25 µm. The El-mode was 70 eV and the scan range was 50-500 amu. Identification of the essential oil components was done by employing mass spectral correlations using the NIST ’98 library (NIST-EPA-NIH Mass Spectral Library) as well as comparison of their Kovat’s retention indices (KI) and mass spectral databases with data published elsewhere [18-20].

2.4 Antimicrobial activity

2.4.1 Microorganisms

All microorganisms were obtained from the New Zealand Reference Culture Collection and supplied by the Microbiology section of the Analytical Unit at the Institute of Applied Sciences, The University of the South Pacific, Fiji. The strains of microorganisms were stored at -80 °C and included *Escherichia coli* (416 and 4355), *Bacillus cereus, Listeria monocytogenes, Pseudomonas aeruginosa, Streptococcus pyogenes, Klebsiellapneumoniae, Enterococcus faecalis, Enterobacteraerogenes,* Vancomycin-resistant *Enterococcus faecium* (VREF), methicillin-resistant *Staphylococcus aureus* (MRSA), wild-type *Staphylococcus aureus* (WTSA), rifamycin-resistant *Staphylococcus aureus* (RRSA), amphotericin B resistant *Candida albicans* (ARCA) and wild-type *Candida albicans* (WTCA).

2.4.2 Antimicrobial activity assay

The antimicrobial activities of the extracts and essential oils from two varieties of *R. rosifolius* were assessed. Two loopfuls of each microbe were added to the sterile culturing media (10 mL) and incubated at 37 °C for 48 hours (for foodborne pathogens) and 24 hrs (for drug-resistant pathogens). The concentration of the bacterial seed broth used was gauged by the resulting optical density (under UV at 600 to 800 nm) where the optimum growth range was 0.1-0.3. Tryptic soy broth (TSB) was used for the culturing medium except for the fungal pathogens for which potato dextrose broth (PDB) was used. Each pathogen was pour plated (200 µL per mL of media). The medium used for the food borne pathogens (FBP) was Tryptic soy agar (TSA), while nutrient agar (NA) was used for the drug resistant *S. aureus* species, luriabertani agar (LBA) for the *Enterococcus faecium* species and potato dextrose agar (PDA) for the drug resistant *Candida albicans* species. Susceptibility discs (6 mm in diameter) were impregnated with 15µL (0.75 mg) of the essential oils/extracts dissolved in their respective solvents at 50 mg/mL (750 µg/disc). The disks were allowed to dry (that is, the solvent evaporated) before the disks were positioned on the plates. The plates were wrapped with para film and observed for zones of inhibition after 24 hrs of incubation at 37 °C. Zones of inhibition were calculated by measuring the diameter in

mm (including disc). The foodborne pathogens used were *E. coli* (416 and 4355), *B. cereus, L. monocytogenes, P. aeruginosa, S. pyogenes, K. pneumoniae, Enterococcus faecalis*, and *Enterobacter aerogenes*. The drug-resistant bacterial pathogens used were Vancomycin-resistant *Enterococcus faecium* (VREF), methicillin-resistant *S. aureus* (MRSA), wild-type *S. aureus* (WTSA) and rifamycin-resistant *S. aureus* (RRSA). The drug resistant fungal pathogens were amphotericin B resistant *Candida albicans* (ARCA) and wild-type *Candida albicans* (WTCA). The positive controls employed were Vancomycin (for all FBPs, MRSA and RRSA) and rifamycin (for VREF), which were used at concentrations of 10 mg/mL (150 µg/disc).

The minimum inhibitory concentrations (MIC) needed to inhibit growth of the drug-resistant pathogens (MRSA, RRSA, WTSA and VREF) were determined. The analysis was conducted using only the methanol extracts (since these were the only extracts to produce zones of inhibition), of which solutions of 25 mg/ml were prepared. Microbial culture (20uL) was added to 10 mL of sterile nutrient broth and incubated at 37 °C for 24 hrs. The respective cell suspension (190uL) was then added to the first well of each column after which the methanol extract (10uL) was added. Cell suspension (100uL) was then added to the proceeding wells and serial dilutions done by adding 100uL of the previous well to the subsequent wells until the 8th row was filled. The concentration range for the samples was therefore 1250-9.8 µg/mL.

2.5 Brine shrimp lethality assay

The brine shrimp (*Artemia salina*) eggs were purchased from Sep-Art Technology (INVE Aquaculture Ltd., Thailand). The method of Subramania *et al.* [21] was used to determine the lethality of the fruit extracts. Brine shrimp eggs were hatched in filtered sea water (150 mL) under constant aeration for 48 hrs at 26 ± 2 ºC. After hatching, active nauplii, free from eggshells, were collected and used for the assay. Sea water (100 µL) containing 7-14 nauplii were added to each well of a 96-well plate. The hexane, ethyl acetate, and essential oil extracts of the fruits were dissolved in 10% v/v Tween 80 and the methanol extracts dissolved in methanol. A sample of the extracts (100 µL) from the two varieties of *R. rosifolius* was then added to the respective wells at 500, 250, 125, 62.5, and 31.3 ppm in triplicates, and maintained at room temperature (26±2 ºC) for 24 hrs. Filtered sea water and the various solvents used to dissolve the extracts were used as solvent controls, while potassium dichromate with a concentration range of 1-500 ppm was used as a positive control. The number of dead nauplii present in each well was counted using a compound light microscope (10X) (Nikon, NZ). The LD_{50} values were then determined statistically using the Reed-Muench method, as outlined by Sam [22] where the percentage mortality was plotted against the logarithm of the concentrations of the extracts. The LD_{50} was then calculated from the linear equation by taking the antilogarithm.

3. Results and Discussion

3.1 Essential oil constituents

The chemical compositions of the essential oils resulting from the R and WR fruits were examined and the data presented in Table 1. While one of the major compounds in the oils from both varieties was the same (the sesquiterpene, α-cadinol), their chemical compositions generally revealed variability. The monoterpenes linalool,α-terpineol and geraniol, and the sesquiterpene δ-cadinene were present in greater quantities in the red variety, while in the wine red variety the sesquiterpenes α-copaene and α-cadinol were in greater abundance. Of all the compounds identified, 9 were common to both varieties. A total of 13 compounds were identified from the red variety, representing 94% of the essential oil content. While a greater number of compounds were identified in the wine red variety (17), only 71% of the essential oils were positively identified. The major compound present (22%) remained unidentified (mass spectrum shown in Figure 1) and this along with the remaining unidentified components were below the desired 80% mark of certainty provided by the NIST library, which was the benchmark for acceptability. The sesquiterpene, (+)-α-copaene, is believed to be rareand is usually present in small quantities in the essential oils of various plants including oranges and mangoes. The compound has been reported as a potent attractant for the agricultural pest, the Mediterranean fruit fly, *Ceratitis capitata*, (while showing selectivity for the males) and is therefore of economic importance [23]. The compounds found in the two *Rubus* varieties, linalool, α-terpineol, and geraniol were also reported in five raspberry cultivars (‘Chilliwack’, ‘Tulameen’, ‘Willamette’, ‘Yellow Meeker’ and ‘Meeker’) grown in the Pacific Northwest [24]. The essential oil profiles of the fruits of the two raspberry varieties, however, differ from those reported by Southwell [25] and Southwell and Tucker [26], who examined the essential oil composition of the aerial parts of *R. rosifolius*. The compounds reported were cyclic sesquiterpenes and include discussion, β-kessane, pregeijerene, β-caryophyllene, humulene, dihydro agar furan, hedycaryol, Bicyclogermacrene, an unidentified sesquiterpene ether, and rosifoliol which is said to be a biogenetically interesting molecule.

The wine red fruits produced a notably larger yield of essential oils than its red counterpart (34 vs. 6 ppm). Different essential oil components are capable of exhibiting varying biological properties. The variation seen in the essential oil composition of the two red raspberry varieties therefore indicates the potential for variation in biological activity of the essential oils. The extraction protocol utilised involved the steam distillation of the fresh fruits. This protocol was selected because it is known to result in a higher yield of oil in a shorter time than other methods such as water distillation. According to Handa [27], the oil quality produced using this method is also more reproducible. One of the main drawbacks to this method of essential oil extraction is, however, the low pressure of rising steam, which causes oils of a high boiling point to require a longer distillation period in order to vaporise.

Table 1: Essential oil composition of the R and WR varieties of *Rubus rosifolius* fruits

Component	KI*	KI* (Literature)	Red (R) Variety/%	Wine Red (WR) Variety %
Monoterpenoids				
α-Pinene	943	939	-	0.5
Linalool	1104	1098	21.0	4.9
Hotrienol	1109	1101	-	1.6
α-Terpineol	1203	1198	13.1	3.4

Geraniol	1256	1249	3.5	0.6
Sesquiterpenoids				
α-Cubebene	1358	1351	3.0	2.3
α-Copaene	1378	1374	6.6	9.2
Ylangene	1393	1382	-	1.0
β-Cubebene	1401	1388	-	0.4
β-Gurjunene	1427	1431	5.8	-
γ-Elemene	1430	1436	-	6.8
α-Muurolene	1495	1500	-	1.1
δ-Cadinene	1526	1523	7.1	6.6
Calamenene	1531	1528	-	3.6
β-Calacorene	1572	1564	9.8	8.9
α-Cadinol	1660	1652	10.6	17.0
14-Hydroxy-4,5-dihydro- β-caryophyllene	1706	1706	2.4	2.4
Others				
Thymol methyl ether	1240	1232	-	0.2
2-Undecanone	1293	1294	5.0	-
2-[(2-butylcyclopropyl) methyl]- methyl ester, cyclopropanenonanoic acid	2202	2203	4.3	-
2-(9-octadecenyloxy)ethanol (Z)	2329	2336	1.6	-
Unidentified‡	1674	-	-	22.0
Components, %			94	93
Total Monoterpenoids (%)			37.6	11.0
Total Sesquiterpenoids (%)			45.3	59.3
Oil yield (mg/kg FW)			6.0 ± 0.00	34.0 ± 0.01

*KI: Kovats Retention Index
(-): not detected
‡: Mass spectrum shown in Figure 1

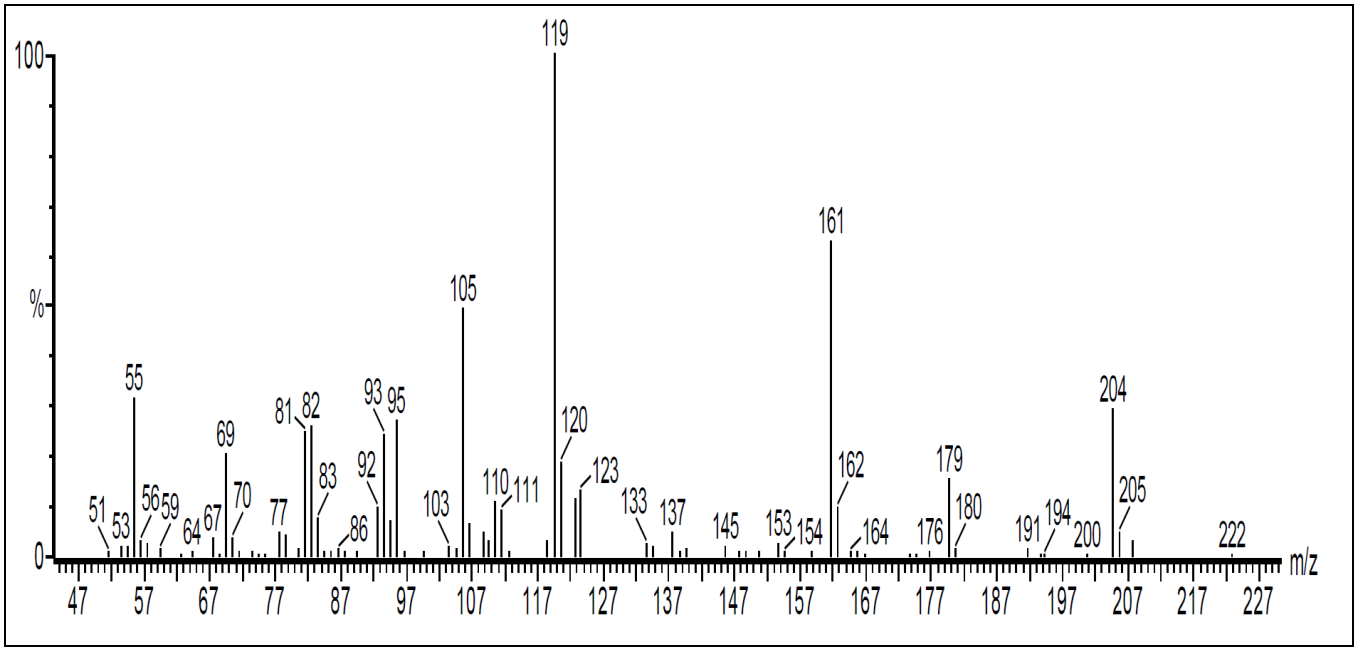

Fig 1: Mass spectrum of an unidentified compound in the essential oil of the Wine Red *Rubus rosifolius* plant variety

3.2 Antimicrobial Activity

Extracts from various *Rubus* species have exhibited potent antimicrobial activity against both food borne and drug-resistant pathogens [28, 29]. Lower respiratory infections and diarrhoeal diseases, which are among the 10 main causes of death worldwide, contribute respectively to 5.5% and 2.7% of total deaths [9]. Given the role microbial agents play in these diseased states, research focused on developing antimicrobial agents is critical. The solvent and essential oil extracts of the red and wine red fruits were therefore examined for their antimicrobial properties against foodborne and drug-resistant bacteria, and the results are outlined in Table 2. The methanol extracts, which were the only ones that demonstrated antimicrobial activity, were effective only against MRSA (with R_{MeOH} displaying greater activity) and RRSA (with R_{MeOH} demonstrating weak activity). The activities of these extracts against WTSA are lower than those reported by Bobinaitė *et al.* [30] where a 1% solution of an 80% methanol extract of three raspberry cultivars yielded zones of inhibition of 9, 10.5, and 14.5 mm for the black raspberry cultivar 'Bristol', and the two red raspberry cultivars 'Benefis' and 'Novokitaevskaja', respectively. The zone of inhibition of 9 mm was comparable to our findings for WR_{MeOH} against WTSA.

The minimal inhibitory concentration of the methanol extracts against the MRSA pathogen was found to be 1.25 mg/ml for both plant varieties. This high minimum concentration however renders the inhibitory action of the extract weak

since potent activity of an extract is usually ≤100 µg/mL, with that of a pure compound being < 10 µg/mL [31].

Consistent with the results observed in our analysis of antimicrobial activity against drug-resistant pathogens, only the methanol extracts demonstrated activity against the microbial pathogens tested (Table 2). Also, although essential oils have been reported to possess antibacterial properties [3], no activity was exhibited by the oils from the *Rubus rosifolius* fruit varieties against the organisms tested. It is important to note that since the diffusivity of the compounds is able to impact the antimicrobial effect they confer using this assay, a difference in the rate of diffusion of the compounds in the different extracts could be an influencing factor for the different activities noted.

The activities against food-borne pathogens were very similar for the methanol extracts from the two varieties; however, in almost every instance, the wine red (WR) variety exhibited slightly higher activities. The greatest activity was observed against the *E coli* and *E aerogenes* strains (both Gram-negative). Both extracts were more effective against *E. coli* 416 compared to *E. coli 4355*. This difference in activity of the extracts against the two strains of *E. coli* is not surprising, since it has been reported that the activity of extracts varies among strains [32]. The activities of R_{MeOH} and WR_{MeOH} against *E faecalis*was lower than the range reported by Bobinaitė *et al.* [30]; that is, 9.0-11.5 mm using a 1% solution under similar experimental conditions. No activity was observed for the fruit extracts against *P aeruginosa* or *K pneumonia*. It has been reported that the aqueous extract of the leaves of *R rosifolius* inhibited the growth of *S. aureus* and that the ethanol extract was effective against *P. aeruginosa* and *E. coli*, where a lethal effect was observed 2 days after inoculation [33, 34]. While these results support our findings with respect to *E. coli*, the same is not true for *P. aeruginosa.*

The antimicrobial activity of the methanol extracts may be due to the presence of tannins. The presence of these compounds in the methanol extracts was previously reported in our research group by Campbell *et al.* [35]. Two mechanisms through which tannins are believed to impart their antimicrobial effects are deprivation of substrates needed to sustain microbial growth and inhibition of extracellular microbial enzymes. These effects can be achieved through its astringent character, which can induce complexation of substrates and enzymes [36].

It has been postulated that there is a correlation between Gram reactivity and antimicrobial activity. According to Puupponen-Pimiä *et al.* [32], berry extracts are more effective against Gram-positive than Gram-negative bacteria. In this case however, no Gram selectivity was observed. Instead, slightly higher values were obtained against the Gram-negative microbes. This finding has been supported by Cavanagh *et al.* [28].

The assessed foodborne pathogens are of great concern within the food industry. Due to the degree of harm that can result from the ingestion of food contaminated with these species, the need for safe and effective preservation systems in formulations is of paramount importance to manufacturers. On the other hand, the MRSA pathogen is quite problematic and is considered the most prevalent and important antimicrobial-resistant pathogen, causing serious nosocomial and community-acquired infections. A limitation of treatment options for infections resulting from this pathogen results in an increase in morbidity and mortality [37]. Research on the effect of natural products against MRSA could possibly lead to a breakthrough in the discovery of potential treatment options.

Table 2: Antimicrobial activity for R_{MeOH} and WR_{MeOH} extracts against drug-resistant and foodborne pathogens

Pathogens		**Zone of Inhibition (mm)**	
Drug-resistant		**Red (R)**	**Wine Red (WR)**
MRSA		13.0	11.0
RRSA		8.0	10.0
WTSA		8.0	9.0
VREF		8.0	9.0
WTCA		-	-
ARCA		-	-
Foodborne	**Gram Reaction**		
E. coli 4355	-	9.0	10.0
E. coli 416	-	10.0	13.0
E. aerogenes	-	10.0	11.0
P. aeruginosa	-	-	-
K. pneumoniae	-	-	-
B. cereus	+	-	8.0
L. monocytogenes	+	8.0	9.0
S. pyogenes	+	7.0	8.0
E. faecalis	+	7.0	7.0
Positive Controls			
Vancomycin		20	
Rifamycin		15	

Extracts analysed at 750 µg/disc and controls at 150 µg/disc
(-): No activity observed

Zones of inhibition include the diameter of the disc
Strong activity (Susceptible organisms): zone > 14 mm
Intermediate: zone = 12-14 mm
Weakly active: zone = 10-12 mm
No activity (Resistant organisms): zone < 10 mm [38]

3.3 Lethality assay

The brine shrimp lethality assay examines the ability of a test sample to kill laboratory-cultured *Artemianauplii* from the brine shrimp (*Artemiasalina*). It is a useful tool which assesses the preliminary toxicity of the tested materials. Along with being rapid and inexpensive, another critical criterion satisfied by the assay is the small amount of test sample (2-20 mg or less) required to conduct the assay. The concentration of a substance needed to kill 50% of the population of brine shrimp (LD_{50}) was determined for the plant extracts and essential oils from *R. rosifolius*. The results are outlined in Table 3.

For the fruit extracts, the percentage of solvents used in the assay was of paramount importance. Since the sole basis on which the activity of the extracts was determined was the number of dead or live nauplii present at the end of the test period, it was critical that the solvent did not impart any toxicity to the cells. The brine shrimp larvae are tolerant of up to 11% of dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethanol, and methanol. Surfactants can also be used as solubilising aids for plant materials that are difficult to dissolve. It was reported that *Artemia* larvae can survive for at least 24 hrs in media containing Tween 80 (polyoxyethylene sorbitan) at concentrations of 50,000 ppm [22]. The solvents used for the analysis were methanol (for the methanol extract) and a 10% v/v Tween 80 solution for the ethyl acetate, hexane, and essential oil samples. Initially high concentrations of the extracts were prepared so that only an extremely small quantity had to be delivered to the wells to achieve the desired

concentrations. This way, the volume of solvent transferred was minimal, not exceeding 2% in the total well content. The Reed-Muench method was used to determine the lethality of the test samples. The method is convenient for this assay since the assumption is made that an animal that survived a given dose of a sample would also survive any lower dose; and conversely, an animal that died after being exposed to a certain dose would also die at any other higher dose [22]. All nauplii were alive at the end of the assay for the solvent controls. The extracts were considered to be of minimal lethality if the LD_{50} was greater than 500 ppm. This was the case for the R_{MeOH}, WR $_{MeOH}$, and WR $_{EtOAc}$, with R_{EtOAc} having only weak activity.

Crude extracts resulting in LC_{50} values less than 250 µg/ml were considered significantly active and therefore the hexane extracts demonstrated significant lethality towards the brine shrimp larvae (LD_{50} 177 and 250 µg/mL), with WR $_{Hex}$ producing the greater activity [39]. The hexane, ethyl acetate, and methanol extracts of the fruits of *R. rosifolius* were analysed against several cancer cell lines by our research group (not reported here) and it was found that the hexane and ethyl acetate extracts exhibited minimal activity (against the gastric cancer cell line) while the methanol extract was found to be inactive at 250 µg/mL [40]. Additionally, the three mentioned solvent extracts of the two *R. rosifolius* varieties were also assessed previously for their chemo preventive properties against cytochromes P450 (CYP) enzymes and it was found that the methanol extract from the R variety (99.2% inhibition) and the hexane and ethyl acetate extracts from the WR variety (81.1 and 82.0% inhibition, respectively) were most effective [35]. These findings do not support claims of any strong correlation between the brine shrimp lethality of the extracts and their chemo preventive effects; however, some similarity exists between the former and the anticancer effects reported.

In this study, the essential oils produced even greater lethality than the hexane extracts, with LD_{50} values of 63 and 48 µg/mL for R_{Hex} and WR $_{Hex}$, respectively. This activity, compared to the activity of the positive control (potassium dichromate), with an LD_{50} value of 23, is promising. The components of the essential oils are outlined in Table 1. Some of the major components of the oils were α-cadinol, linalool and α-terpineol which have also been reported as major compounds in plant extracts for which similar brine shrimp lethality has been reported [41-43]. It is important to note that although the findings of the brine shrimp lethality assay are reproducible, a probable source of error is in the decision whether a nau plus is actually dead or is simply immobilised at the time the results are gathered [22].

Table 3: LD_{50} of extracts of the R and WR *Rubus* fruits

Sample/Extracts	LD_{50} (µg/mL)	
	Red (R)	Wine Red (WR)
Hexane	250	177
Ethyl acetate	498	>500
Methanol	>500	>500
Essential Oil	63	48
Positive control		
Potassium dichromate	23	

4. Conclusion

The biological activity of phytochemicals determines their possible uses which can include their incorporation into food productsand/or pharmaceuticals. The two varieties of *R. rosifolius* explored differed on several bases in their essential oil compositions but displayed similar antimicrobial activity against drug resistant and selected food-borne pathogens as it relates to their methanol extracts. Greatest inhibition resulted against MRSA and *E. coli* 416. The hexane extracts and essential oils reported the most potent lethality towards the brine shrimp nauplii while the methanol extract was inactive. Comparison of these results to previously reported chemo preventive effects of the *R. rosifolius* solvent extracts are inconclusive in terms of whether an association exists between the aforementioned and brine shrimp lethality. Studies have however showed that common cancer therapies, when combined with dietary compounds, may exert enhanced anti-tumour activity which can occur through synergic action or by decreasing the systemic toxicity, thereby allowing lower doses of cancer treatments to be used [44-45]. This is therefore another promising area of research that can be explored using the active plant extracts.

Acknowledgements

The authors are grateful for the research guidance provided by the late Prof. William Aalsbersberg of the University of the South Pacific, Fiji. We also express gratitude to Dr. Camille Bowen-Forbes from The University of the West Indies, Mona Campus, for her comments on the manuscript. Special thanks to the Education, Audio visual and Culture Executive Agency (EACEA) of the European Union for offering a Caribbean-Pacific Island Mobility Scheme (CARPIMS) scholarship to Dr. T Rambaran which enabled her to conduct aspects of this research at the University of the South Pacific, Fiji.

5. References

1. Barra A. Factors affecting chemical variability of essential oils: a review of recent developments, Natural product communications. 2009; 4(8):1147-54.
2. Stanojević L, Stankovic M, Cakic M, Nikolic V, Nikolic L, Ilic D *et al.* The effect of hydrodisitillation techniques on yield, kinetics, composition and antimicrobial activity of essential oils from flowers of *Lavandula officinalis* L Hemijska Industrija. 2011; 65:455-463.
3. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review Phytotherapy Research. 2007; 21(4):308-23.
4. Maruzzella JC, Balter J, Katz A. The action of perfume-oil flavours on fungi, American perfumer and aromatics. 1957; 74:21-22
5. Nychas GJE. Natural antimicrobials from plants, In New Methods of Food Preservation, 1st ed; Gould, G.W, Ed. London: Blackie academic & professional, 1995, 58-89.
6. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils, International journal of food microbiology. 2002; 74(1-2):101-9.
7. Dorman HJ, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils, Journal of applied microbiology. 2000; 88(2):308-16.
8. Rauha JP, Remes S, Heinonen M, Hopia A, Kahkonen M, Kujala T *et al.* Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds, International journal of food microbiology. 2000; 56(1):3-12.
9. WHO. World Health Organization: Estimating the burden of foodborne diseases. https://www.who.int/activities/estimating-the-burden-of-foodborne-diseases, (Accessed Nov. 2019).

10. Brown CM. Select destinations: The Americas and the Caribbean. In Yellow Book, Centres for Disease Control and Prevention, Atlanta, GA, 2013, 75.
11. WTTC. World Travel & Tourism Council. Travel & tourism economic impact 2015 Jamaica. http://www.caribbeanhotelandtourism.com/wp-content/uploads/data_center/destinations/Jamaica-WTTC-EconomicImpact2015.pdf, (Accessed 2019).
12. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA *et al.* NHSN Annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007, Infection control and hospital epidemiology. 2008; 29(11):996-1011.
13. Cardellina JH FR, Gamble WR, Westergaard C, Boswell J, Munro MHG, Currens M *et al.* Evolving strategies for the selection, DE replication and prioritization of antitumour and HIV-Inhibitory natural products extracts. In Bioassaay methods in natural product research and development, Bohlin L, Bruhn J, Ed. Dordrecht: Kluwer Academic Publishers., 1999, 25-36.
14. Sarah QS, Anny FC, Mir M. Brine shrimp lethality assay, Bangladesh Journal of pharmacology. 2017; 12(2):186-189.
15. Ogugu SE KA, James BI, Paul DK. Assessment of cytotoxic effects of methanol extract of *Calliandra portoricensis* using brine shrimp (*Artemia salina*) lethality bioassay Glob, Journal of bioscience and biotechnology. 2012; 2:257-260.
16. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals, drug information journal. 1998; 32(2):513-524.
17. Hamidi M, Jovanova B, Panovska T. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L) model.Makedonsko Farmacevtski Bilte. 2014; 60(1):9-18.
18. Adams R. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy Carol Stream. 2005; 16:65-120.
19. Said-Al Ahl HH, Hussein MS, Tkachenko KG, Nkomo M, Mudau F. Essential oil composition of *Artemisia vulgaris* grown in Egypt, International journal of pharmacy and pharmaceutical sciences. 2016; 1302(2.07):1.7030.
20. Pirzadah TB, Malik B, Tahir I, Rehman R. Metabolite profiling of tartary buckwheat-An underutilized neutraceutical crop of Kashmir Himalaya, Journal of Phytology. 2016, 49-54
21. Subramani R, Kumar R, Prasad P, Aalbersberg W, Retheesh ST. Cytotoxic and antibacterial substances against multi-drug resistant pathogens from marine sponge symbiotic: Citrinin, a secondary metabolite of *Penicillium* sp, Asian pacific journal of tropical biomedicine. 2013; 3(4):291-6.
22. Sam TW. Toxicity testing using the brine shrimp: *Artemia salina*, In Bioactive natural products: detection, isolation and structural determination, Colegate S, Molyneux RJ, Ed. Boca Raton FL: CRC Press, 1993, 442-452.
23. Nishida R, Shelly T, Whittier T, Kaneshiro K. α-Copaene a potential rendezvous cue for the Mediterranean fruit fly, *Ceratitis Capitata*? Journal of chemical ecology. 2000; 26:87-100.
24. Malowicki SM, Martin R, Qian MC. Volatile composition in raspberry cultivars grown in the Pacific Northwest determined by stir bar sorptive extraction-gas chromatography-mass spectrometry, Journal of agricultural and food chemistry. 2008; 56(11):4128-33.
25. Southwell I. The constituents of *Rubus rosifolius*. The structure of rosifoliol, a biogenetically significant sesquiterpenoid, Australian journal of chemistry. 1978; 31:2527-2538.
26. Southwell I, Tucker D. Isokessane and β-kessane from *Rubus rosifolius* Sm, Journal of essential oil research. 1996; 8:143-148.
27. Handa SS. An overview of extraction technologies for medicinal and aromatic plants, In extraction technologies for medicinal and aromatic plants, Handa S.S.K, S Longo G, Rakesh D, Eds; Ed. Italy: United Nations industrial development organization and the international centre for science and high technology, 2008, 36-42.
28. Cavanagh HM, Hipwell M, Wilkinson JM. Antibacterial activity of berry fruits used for culinary purposes, Journal of medicinal food. 2003; 6(1):57-61.
29. Miyasaki Y, Rabenstein JD, Rhea J, Crouch ML, Mocek UM, Kittell PE *et al.* Isolation and characterization of antimicrobial compounds in plant extracts against multidrug-resistant *Acinetobacter baumannii* PloS One. 2013; 8(4):e61594
30. Bobinaitė R, Viskelis P, Sarkinas A, Venskutonis R. Phytochemical composition antioxidant and antimicrobial properties of raspberry fruit, pulp, and marc extracts, CyTA - Journal of food. 2013, 11.
31. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review Planta Medica. 2010; 76(14):1479-1491.
32. Puupponen-Pimia R, Nohynek L, Meier C, Kahkonen M, Heinonen M, Hopia A *et al.* Antimicrobial properties of phenolic compounds from berries, Journal of applied microbiology. 2001; 90(4):494-507.
33. Mauro C, Cardoso CMZ, Shultze C, Yamamichi E, Lopes P, Marcondes EMC. Estudo botânico, fitoquímico e avaliação da atividade antimicrobiana de *Rubus rosaefolius* Rev Bras Farmacogn. 2002; 12:23-25.
34. Ostrosky EA, Marcondes EM, Nishikawa Sde O, Lopes PS, Varca GH, Pinto Tde J *et al. Rubus rosaefolius* extract as a natural preservative candidate in topical formulations AAPS Pharm Sci Tech. 2011; 12(2):732-7.
35. Campbell TF, McKenzie J, Murray J, Delgoda R, Bowen-Forbes CS. *Rubus rosifolius* varieties as antioxidant and potential chemo preventive agents, Journal of functional foods. 2017; 37:49-57.
36. Scalbert A. Antimicrobial properties of tannins Phytochemistry 1991; 30(12):3875-3883.
37. Jernigan J, Kallen A. HAI elimination Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections activity C: ELC Prevention Collaboratives Centres for Disease Control and Prevention. http://www.cdc.gov/hai/pdfs/toolkits/MRSA_toolkit_white_020910_v2.pdf.
38. Facey PC, Pascoe KO, Porter RB, Jones AD. Investigation of plants used in Jamaican folk medicine for anti-bacterial activity, The Journal of pharmacy and pharmacology. 1999; 51(12):1455-60.
39. Rieser MJ, Gu ZM, Fang XP, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*, Journal of natural products. 1996; 59(2):100-8

40. Bowen-Forbes CS, Zhang Y, Nair MG. Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits, Journal of food composition and analysis. 2010; 23(6):554-560.
41. Soares BV, Morais SM, dos Santos Fontenelle RO, Queiroz VA, Vila-Nova NS, Pereira C *et al.* Antifungal activity, toxicity and chemical composition of the essential oil of *Coriandrum sativum* L fruits Molecules (Basel, Switzerland). 2012; 17(7):8439-8448.
42. Satyal P, Paudel P, Raut J, Deo A, Dosoky NS, Setzer WN. Volatile constituents of *Pinus roxburghii* from Nepal, Pharmacognosy Research. 2013; 5(1):43.
43. Tawaha KA, Alali FQ, Hudaib MM. Chemical composition and general cytotoxicity evaluation of essential oil from the flowers of *Anthemis palestina* Reut, ex Boiss, growing in Jordan, Journal of essential oil bearing plants. 2015; 18(5):1070-1077.
44. Hussain A, Brahmbhatt K, Priyani A, Ahmed M, Rizvi TA, Sharma C. Eugenol enhances the chemotherapeutic potential of gemcitabine and induces anticarcinogenic and anti-inflammatory activity in human cervical cancer cells, Cancer Biotherapy & Radiopharmaceuticals 2011; 26(5):519-27.
45. Ho JW, Cheung MW. Combination of phytochemicals as adjuvants for cancer therapy. Recent patents on anti-cancer drug discovery. 2014; 9(3):297-302.
46. HA Ibekwe. In vitro anthelmintic activities of aqueous crude extract of Azadirachta indica on Paramphistomum cervi and Fasciola hepatica. Int J Vet Sci Anim Husbandry 2019;4(1):14-18.