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Antidandruff property of *Psidium guajava* leaf extracts

Cheetra Bhajan [1,*], Joyce Soulange Govinden [1], Vijayanti Mala Ranghoo Sanmukhiya [1]

[1]Department of Agricultural and Food Science, University of Mauritius, Mauritius

ABSTRACT: *Psidium guajava* leaf paste and decoction have long been used in Mauritius for their efficacy in dandruff management. The study aimed to test *P. guajava* leaf extracts against fungi collected from dandruff patients' scalps, as previous research has shown a strong link between dandruff and dermatophytes. Fungi were isolated and cultured on Sabouraud dextrose agar (SDA), which contained 2% olive oil, 1% Tween 80, and chloramphenicol. The isolates were identified using physiological, macroscopic, microscopic, biochemical, and molecular techniques. Hydrodistillation and Soxhlet extraction of Mauritian *P. guajava* leaves were used to obtain essential oil and different solvent fractions, which were then screened for the presence of phytochemicals. The microdilution assay was used to test the antifungal activity of harvested extracts against isolates. *Neurospora intermedia*, *Trichoderma atroviride*, and *Trichosporon asahii* were identified as fungi. The solvents chloroform, methanol, and hexane produced higher extract yields (16.2%, 15.2% and 14.7%). The phytochemicals were most abundant in the chloroform methanol (4:1 v/v) fraction. The methanol fraction had the highest total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), with concentrations of 298.27 19.66 g GAE/g, 132.1 6.15 g QUE/g, and 118.53 1.60 g GAE/g, respectively. Isolates were most sensitive to chloroform-methanol (4:1 v/v) and methanol fractions, with a MIC of 1.56 mg/mL. From the study, it can be deduced that *P. guajava* leaf extracts are effective antifungal agents and potential ingredients for natural antidandruff hair products, which are in high demand in the cosmetic industry.

1. INTRODUCTION

Dandruff, a condition in which patients experience excessive shedding of dead skin cells from their scalps, is a major concern worldwide, particularly from a cosmetic standpoint (Keragala et al., 2020; M, 2000). Cellular debris usually clumps together, forming white visible flakes on the hair that can land on the face, ears, brows, and shoulders, causing social embarrassment and lowering self-esteem. Affected individuals also experience discomforts such as itching and hair loss (Borda & Wikramanayake, 2015). Almost half of the population is affected by the condition, with teenagers and adults bearing the brunt of the burden. In fact, it is believed that almost every human being will experience dandruff at some point in their lives (Pramodani & Wickramarachchi, 2017) .

Several studies on scalp microbiomes from different populations have demonstrated a link between dandruff and bacterial and fungal dysbiosis. *Propionibacterium acnes* is commonly found on healthy scalps, whereas *Staphylococcus epidermidis* predominates on dandruff patient's scalps. *Malassezia restricta* and *Malassezia globosa* are the most common fungi found on the scalps of dandruff patients. There is still a strong link between dandruff and unidentified fungal species (Saxena et al., 2018). The presence of fungi is highly dependent on the scalp's sebum content. Sebum production is known to be influenced by genes, hormones, humidity, and food. During pregnancy, menopause, or puberty, hormonal imbalances can cause an oily scalp (Riffat, 2018). Lipophilic yeasts that live on the mammalian scalp multiply in the presence of sebum. Because the scalp is densely packed with sebaceous glands, it provides an ideal environment for the fungi. To multiply, the microorganisms use the enzyme lipase to digest oleic acid found in sebum. Eradicating or controlling the yeast content on patients' scalps may provide potential dandruff treatment solutions (Martinsdos et al., 2017) .

Various fungicidal products, such as shampoos, conditioners, scalp lotions, and creams, are already commercially available; however, the majority of them are synthetic in nature. Chemical products may appear to be the best solution, but they eventually damage the hair roots, resulting in premature hair aging, scalp dryness, split ends, and additional hair loss. The NHS (2019). claims that the main active ingredients in these products are zinc pyrithione and ketoconazole, which act as fungicides, coal tar and selenium sulfide, which slow down the death of scalp

* Corresponding author.
E-mail address: cheetrabhajan@gmail.com (Cheetra Bhajan)

cells, and salicylic acid, which detaches flakes so they can be washed away. Although these ingredients effectively combat dandruff, skin and eye irritations and rashes are common side effects. Despite its medicinal properties, coal tar is also a known carcinogen and may be harmful to the skin's health. Even shampoos that claim to reduce flakes can cause more dandruff over time. Furthermore, little is known about their biodegradability, which could have a negative impact on the environment (Kröhnert & Stucki, 2021). To combat the issues associated with chemical dandruff products, it is best to switch to natural ingredients such as essential oils and scalp masks, which have been shown to have antimicrobial activity as well as conditioning properties, benefiting both the hair and scalp. Because they are high in vitamins, glycosides, terpenoids, flavonoids, alkaloids, coumarins, and tannins, leaves are the most commonly used plant part in natural shampoos (Bakr et al., 2019). These secondary metabolites are useful in cosmetic formulations because they are easily degradable. Furthermore, when compared to chemicals, the availability of plants is simple and inexpensive (Altemimi et al., 2017).

The guava tree (*Psidium guajava* Linn.), a member of the Myrtaceae family, is a prolific tropical plant, and Mauritius, being a tropical island, provides ideal growing conditions. Different parts of the plant have been used in folk medicine, and as a result, modern day researchers have conducted extensive research to test the pharmacological effects of extracts obtained from its fruits, leaves, barks, and roots (Mohiuddin, 2019). For millennia, guava leaf paste and decoction have been used as a home remedy for dandruff. Guava leaf extracts contain over 50 bioactive compounds and have shown a few notable antifungal properties against a variety of fungi (Bezerra et al., 2018). Despite the plant's widespread biological uses, there is a paucity of data on its therapeutic effect against scalp fungi, which warrants further investigation. As a result, the study aimed to identify the fungal agents of dandruff in a Mauritian population as well as assess the phytochemical composition and efficacy of Mauritian *P. guajava* leaf extracts against those fungi.

2. MATERIALS AND METHOD

2.1. Fungi Isolation

Patients of either sex with relatively visible flakes aged 20-25 years old at the University of Mauritius who were willing to participate were the study group's inclusion criteria. Patients who were already receiving dandruff treatment and who had other known skin conditions were excluded. After partitioning the hair with a sterile comb to select an appropriate affected area, a moist rayon swab was rubbed and rolled back and forth firmly several times across the sampling area (Bhattacharyya et al., 2017). Using a swab, samples were plated on Petri dishes containing SDA incorporated with chloramphenicol and supplemented with 2% olive oil and 1% tween 80 to create a lawn culture in a laminar flow. The Petri dishes were parafilm-sealed, labelled, and incubated at room temperature. Every day for 7-21 days, the inoculated plates were observed (Kindo et al., 2004).

2.2. Identification of fungal isolates

All of the isolates were identified using physiological, macroscopic, microscopic, biochemical, and molecular characteristics. One drop of 10% potassium hydroxide (KOH) and two drops of methylene blue were used separately for direct microscopy. Gram's staining was used to detect any gram-positive budding yeast cells using a few drops of crystal violet dye, iodine alcohol, and safranin (M, 2000). Following the morphology of potential dermatophyte colonies, biochemical tests were used to further identify them. In a test tube for each isolate, a catalase test using 3ml of 3 percent hydrogen peroxide solution was performed, followed by an esculin hydrolysis test using bile esculin agar and a tween assimilation test using 30 μl of tween 20 and 80 (Khosravi et al., 2009). The CTAB (hexadecetyltrimethylammonium bromide) protocol using calculated volumes of 1M Tris-HCl (pH 8), 0.5M EDTA, 5M NaCl, hexadecyltrimethyl ammonium bromide, and water was followed to extract DNA. In an Applied Biosystems Thermal Cycler 2720, a 250 μl Mastermix was used for forward primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and reverse primer ITS4 (5′-TCC TCC GCT TGA TAT GC-3′). The QIAquick® PCR Purification Kit was used to purify PCR products which were sent for sequencing at Inqaba Biotec in South Africa. The Bioedit Sequence Alignment Editor Version 7.0.9.0 was used to assemble the DNA sequences while the alignment was done using MAFFT Version 5.66 (Katoh et al., 2005). Blast searches were carried out to identify the most similar sequences.

2.3. Secondary Metabolites Extraction

The *P. guajava* leaves were collected from Curepipe, which is situated in the central region of Mauritius and is 151 m above sea level, benefitting from a mild tropical maritime climate throughout the year (Aumeeruddy, 2017). The plant's accession number was MAU 0024165. Fresh leaves were gently plucked, separated from twigs, thoroughly washed with tap water, air dried at room temperature, and finely cut into pieces. Around 300-600g of the cut leaves were weighed on an electronic balance and placed into a steam distiller of a Clevenger type apparatus. In a 500ml separating funnel, 50ml of hexane was added two times to 150ml of the condensed mixture of oil and water. The extract was dried over powdered anhydrous magnesium sulfate and filtered using a number 1 Whatmann filter paper (Kulisic et al., 2004; Sokovic & Van Griensven, 2006). 5g of *P. guajava* leaf was ground to a fine powder, wrapped in number 1 Whatmann filter paper, and extracted in 250ml of solvent using a Soxhlet apparatus partitioned by five solvents in increasing polarity: hexane, chloroform, chloroform methanol (4:1 v/v), chloroform methanol (1:1 v/v), and methanol. Each of the resulting fractions was dried by evaporating under reduced pressure at a temperature not exceeding 45°C in a rotary evaporator (Hussain et al., 2008). All extracts were stored at 4°C in dark vials until further use. Extract yields were calculated for all fractions and expressed as a

percentage using the following formula:

$$Percentage\ Yeild = \frac{Net\ Weight\ of\ Extract}{Total\ Weight\ of\ Leaves} \times 100$$

2.4. Qualitative phytochemical Analysis

2.4.1 Coumarins

To the plant extracts, a few drops of concentrated ammonia solution were added. A smear of the solution was placed on a microscope slide and viewed under long-wave (366 nm) UV light (Crowden et al., 1969).

2.4.2 Steroid / Terpenes

The plant extracts were separated on thin layer chromatography (TLC) in solvent 9:1 chloroform/methanol. The plate was developed by spraying with Liebermann - Burchard's reagent (Schoonhoven et al., 2005).

2.4.3 Tannins

The plant extracts were washed with petroleum ether and filtered. To a portion of the filtrate, an equal amount of freshly prepared ferric chloride and potassium hexacyanoferrate (III) were added dropwise (Hampton-Hoch, 1912).

2.4.4 Phenols

The plant extracts were spotted on the TLC plates by spraying with Follin reagent. Phenols were sprayed on the plate (M, 2000).

2.4.5 Alkaloids

To a few drops of extracts, a few drops of Wagner's reagent were added (Hultin & Torsell, 1965).

2.4.6 Flavonols

The extracts were washed with petroleum ether until all pigments were extracted. Ethanol was added to the mixtures and filtered through. Concentrated hydrochloric acid was added to the filtrates and the tubes were placed in a hot water bath and allowed to stand for thirty minutes (Harbourne, 1973).

2.4.7 Anthraquinones

The plant extracts were dissolved in warm distilled water. The solution was filtered and the filtrate was extracted with benzene. A few drops of ammonia solution were added and the mixture shaken (Harbourne, 1973).

2.4.8 Leucoanthocyanins and Flavonols

The methanol extract was washed with petroleum ether until all pigments were extracted. Ethanol was then added, the solution filtered and aliquots of the filtrate were placed in separate test tubes. Concentrated hydrochloric acid was added to both tubes. In one of the tubes, magnesium turnings were added and allowed to stand for 10 minutes. The second tube was placed in a hot water bath and allowed to stand for 30 minutes (Harbourne, 1973).

2.4.9 Saponins

0.5g of dried crushed guava leaves were treated with boiling water for 5 minutes and allowed to cool (Hostettmann & Marston, 1995).

2.5. Quantitative phytochemical test of extracts

2.5.1 Total Phenolic Content (TPC)

The TPC of the essential oil and each solvent fraction of *P. guajava* were determined using the Folin–Ciocalteu method. 1 ml of water and 200 μl of freshly prepared Folin–Ciocalteu reagent were added to 200 μl of crude sample. Then, 800 μl of sodium carbonate solution (2%) was added and the mixture was placed in a water bath at 40°C for 1 minute. After cooling, the absorbance of the solution was measured spectrophotometrically at 650 nm in triplicates. TPC was expressed as μg of gallic acid in crude extract (Kaur & Kapoor, 2002).

2.5.2 Total Flavonoid Content (TFC)

To 2.5 ml of crude extract, 150 μl of sodium nitrite (5%) was added. After 5 minutes, 150 μl of aluminium chloride (10%) was added. After 1 minute, 1 mL of sodium hydroxide (1 M) was added. The absorbance of the solution was measured spectrophotometrically at 510 nm in triplicates. TFC was expressed as μg of quercetin of crude extract (Chang et al., 2002).

2.5.3 Total Tannin Content (TTC

TTC was determined by the Folin-Ciocalteu method. 100μl of the sample extract was added to a volumetric flask containing 7.5 ml of distilled water, 500 μl of Folin-Ciocalteuphenol reagent, and 1ml of sodium carbonate (35%) solution and adjusted to 10 ml with distilled water. The flask was swirled to allow proper mixing, and the mixture was kept at room temperature for 30 min. The absorbance of the solution was measured spectrophotometrically at 725 nm in triplicates. TTC was expressed as μg of gallic acid of crude extract (Afifyael et al., 2012).

2.6. Antimicrobial assay

The well microdilution technique (Eloff, 1998) was used to study the antimicrobial properties of the plant extracts. In a 96-well microplate, 100μL of sterile water was dispensed in each well using a micropipette. 100μL of extract was then dispensed in well A and mixed. 100μL of mixture was pipetted from well A to well B and mixed again. This procedure of 2-fold dilution was continued up to well H which was the final well and 100μL of the last mixture discarded. 100μL of all six solvents was used as the negative control and 100μL of a commercial antidandruff shampoo as the positive control. To each well, 100μL of dilute fungal culture with absorbance 0.400 - 0.600nm at a wavelength of 600 nm was dispensed and the

plate incubated at 37°C overnight. 40μl of 0.2 mg/mL p-iodonitrotetrazolium violet (INT) dye was added to each well to test for fungal growth whereby a red coloration indicated growth while no colour change indicated no growth. The microplate containing the dye was then incubated for another 30 minutes at 37°C and then observed for colour changes to determine the minimum inhibitory concentration (MIC). The MIC was corelated with the TPC, TFC and TTC.

2.7. Statistical Analysis

All experimental data were processed and analyzed in Microsoft Excel 2013, and the results were expressed as mean standard deviation. Standard curves were plotted using linear regression to obtain a line of best fit. Frequency counts, Pearson's correlation coefficient, and pairwise comparison using Tukey's test were obtained. Significant differences between the variables were obtained through a completely randomized design (one-way ANOVA). A *p-value* of 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Fungi Identification

The colonies of the cultured fungi were observed and observations recorded in Table 1 . The colony morphology of the isolates was white to cream in color, the texture was fluffy to smooth and the diameter varied from 7–10.1 mm, while the hyphae type varied. The features of the fungal cells as viewed under the microscope using different staining techniques are recorded in Table 2 . All the tested samples showed positive results when tested with KOH. Gram-positive budding yeast cells upon gram staining were seen in isolate 4. The presence of clusters of cells with circular disarculated hyphae was observed when stained with methylene blue. Observations for the biochemical tests were recorded in Table 3 . The tested samples showed positive biochemical tests except isolate 3, which was negative for the catalase test and isolate 4, which was negative for the bile esculin test. Single PCR products of 650 bp were obtained and submitted to GenBank. Blast searches revealed *Neurospora intermedia*, *Trichoderma atroviride*, and *Trichosporon asahii* with a percent similarity of 100.00% each.

Table 1
Colony morphology of fungi isolated in the study

Isolate	Colony Morphology	Colony diameter (mm)	Mycelia	Spores	Conidiophores
1	White, fluffy	10.1 ± 2.09^{a}	Thick, circular	Feather-like	Powdery
2	White, fluffy	8.92 ± 1.75^{ab}	Thick, circular	Feather-like	Powdery
3	White, fluffy	7.0 ± 1.41^{ab}	Thick, circular	Rough	Effused
4	Cream, smooth	8.5 ± 0.71^{b}	Disarticulated	Bud-like	Elliptical

Different letter superscript in lowercase represents significant difference between samples (p<0.05).

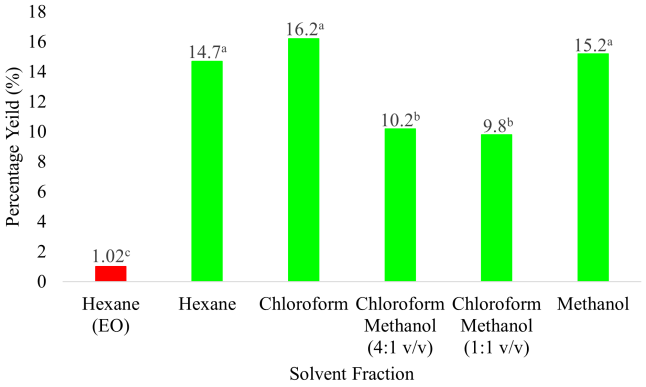

Figure 1. Comparativeanalysis of the percentage yield of extracts. Different lettersuperscript in lowercase between bars represent significant difference betweensamples ($p<0.05$).

Table 2
Microscopical features of the pure cultures of fungi isolated in the study

Isolate	KOH mount	Gram staining	Methylene Blue staining
1	+	-	-
2	+	-	-
3	+	-	+
4	+	+	+

+: Positive, -: Negative

The identification test results are in line with the work of Begum et al. (2019) who used a similar approach to identify dandruff causing fungi. In a study conducted by Cernansky et al. (2020), the authors revealed that *Neurospora* species are often found on humans as a result of exposure to contaminated food and plants, especially sugar cane. The fungi were not thought to be pathogenic until Kochar et al. (2004) reported that participants in his study showed positive skin reaction to it. It can be deduced that the presence of *N. intermedia* on the scalps of dandruff patients makes it an emergent fungal pathogen. *T. atroviride*. On the other hand, is a known fungal pathogen which is rare but becoming more frequent nowadays (Hatvani et al., 2013). Sandoval-Denis et al. 2014 highlighted that *Trichoderma* species have been the causative agents of skin and subcutaneous infections in several cases. *T. asahii* is another opportunistic pathogen residing in human skin commensals. It is usually harmless, but its virulence is activated in immunosuppressed individuals and upon exposure to brisk environmental changes. It is especially frequent in moist and tropical regions. Zhang et al. (2011) associated it with atopic dermatitis and white piedra, conditions which cause similar symptoms to dandruff. The fungal isolates obtained in this study have often been difficult to identify due to their inconsistent morphology (Dolenc-Voljč, 2017). The identification data indicates that various fungi can be responsible for dandruff. Since Mauritius is plentiful in sugar cane fields it is possible that Mauritians are more exposed to *N. intermedia* and that it may be causing dandruff on the scalps of the inhabitants. Being a tropical country with

Table 3
Observations for biochemical tests performed on the isolated fungi of the study.

Isolate	Catalase Test	Bile Esculin Test	Tween Assimilation Test
Observation for positive result	Formation of bubbles	Browning of agar	Growth on agar
1	+	+	+
2	+	+	+
3	-	+	+
4	+	+	-

+: Positive, -: Negative

Table 4
Qualitative phytochemical composition of different solvent extracts used for biomolecules identification of guava leaves.

Phytochemicals	Essential Oil	Hexane	Chloroform	Chloroform Methanol (4:1 v/v)	Chloroform Methanol (1:1 v/v)	Methanol
Chlorophyll	+	++	++	+	++	++
Steroids	-	+	+	+	-	-
Coumarins	-	+	-	+	+	++
Terpenes	-	+	+	+	-	-
Tannins	++	+	+	+	+	+
Phenols	+	+	+	++	++	++
Saponins	-	-	-	-	-	-
Quinones	-	-	-	+	+	++
Alkaloids	-	++	++	++	++	++
Flavonoids	++	+	+	+	+	++

++: Highly Present, +: Low, -: Absent

a considerable amount of humidity, *T. asahii*, can also be associated with dandruff in Mauritians since the climate is ideal for its growth. Finally, allergies, skin diseases such as eczema and white piedra may often be mistaken for dandruff due to overlapping symptoms.

3.2. Plant Extraction

A comparison between the two methods of extraction used for *P. guajava* leaf is illustrated in Figure 1. Higher yields of extract were obtained from Soxhlet extraction method than from hydrodistillation. Among the five Soxhlet fractions, chloroform, methanol, and hexane yielded significantly higher amounts of extract, followed by chloroform methanol (4:1 v/v) and chloroform methanol (1:1 v/v). The yield of essential oil was lowest. Porwal et al. (2012) explain that distillation methods usually result in low yields of oil and this can be attributed to the maturity of the leaves. Young leaves tend to contain less oil since the production of monoterpene is restricted, hampering oil gland development and leading to evaporative losses of oil components (Dhifi et al., 2016). Other factors, such as the plant's chemical composition, geographical location and genetic variation may also contribute to the low oil yield (Nouman et al., 2015; Omoboyowa et al., 2016). Soxhlet extraction gave a higher yield as compared with hydrodistillation. Chloroform and methanol gave yields of 16.2% and 15.2%, respectively. Chloroform contains an electronegative Cl-ion in its molecule, while methanol contains an electronegative OH-ion, which makes them polar. Many organic molecules present in *P. guajava* leaves were thus capable of binding to the ions and dissolving in the solvents (Sophie, 2017). Hexane, which yielded 14.7% of the extract, is a molecule that has a low electronegativity difference between its hydrogen and carbon atoms and hence is a non-polar solvent. It extracts mainly non-polar, hydrophobic and lipophilic compounds, as suggested by the simple rule "like dissolves like". This implies that the *P. guajava* leaves used in this study contained a considerable number of non-polar compounds as well. A mixture of chloroform and methanol can extract hydrophobic substances and allow them to remain soluble. Joyard et al. (1981) revealed that this mixture extracts trapped molecules from the plasma membrane, chloroplast envelope, thylakoid membranes, mitochondria, and tonoplast rather than from the cytosol. The lower yields of the chloroform methanol (4:1 v/v) and chloroform methanol (1:1 v/v) fractions, being 10.2% and 9.8%, could imply that after most of the cytosol was extracted by chloroform, methanol, and hexane, the remaining trapped molecules were then extracted by the solvent mixture. The study shows that Soxhlet extraction using different solvent systems is a superior method of extraction since it can extract both soluble and non-soluble bioactive compounds with the advantage of low solvent requirements and solvent recycling.

3.3. Phytochemical Analysis

Table 4 shows the qualitative phytochemical compositions of the tested samples. Preliminary phytochemical screening revealed the presence of chlorophyll in all solvent fractions, including essential oil. Steroids and terpenes were present in the hexane, chloroform, and chloroform methanol (4:1 v/v) fractions. All fractions contained coumarins except for

Table 5
TPC, TFC and TTC of the essential oil and different solvent extracts of guava leaves

Fraction	Total phenolic (μg GAE/g)	Total flavonoid (μg QUE/g)	Total tannin (μg GAE/g)
Essential Oil	31.20 ± 4.72^{c}	12.97 ± 2.32^{c}	11.63 ± 1.16^{bc}
hexane	54.33 ± 2.50^{c}	14.33 ± 1.66^{c}	10.77 ± 1.19^{bc}
chloroform	26.30 ± 1.87^{c}	10.87 ± 3.33^{c}	12.53 ± 0.67^{b}
Chloroform Methanol (4:1 v/v)	$153.53\ 3.00^{b}$	136.67 ± 3.37^{a}	7.97 ± 2.06^{c}
Chloroform Methanol (1:1 v/v)	282.53 ± 31.76^{a}	90.83 ± 7.16^{b}	13.77 ± 2.36^{b}
Methanol	298.27 ± 19.66^{a}	132.1 ± 6.15^{a}	118.53 ± 1.60^{a}

Different letter superscript in lowercase between bars represent significant difference between samples ($p<0.05$).

Table 6
Minimum inhibitory concentrations of the essential oil and extracts from different solvent fractions against the 4 fungal isolates

Extract Fraction	Minimum Inhibitory Concentration (MIC) (mg/mL)			
	1	2	4	4
Hexane (Essential Oil)	3.125	6.25	3.125	3.125
Hexane	3.125	3.125	3.125	6.25
Chloroform	6.25	3.125	3.125	3.125
Chloroform Methanol (4:1 v/v)	1.56	1.56	1.56	1.56
Chloroform Methanol (1:1 v/v)	3.125	1.56	6.25	12.5
Methanol	1.56	1.56	1.56	1.56

Positive Control (Antidandruff shampoo) : ≤ 0.098

the chloroform fraction and essential oil. The methanol fraction was the most abundant in coumarins. Phenols, tannins, and flavonoids were present in all fractions, including the essential oil. None of the fractions nor the essential oil contained saponins. Quinones were present only in chloroform methanol (1:1 v/v), chloroform methanol (4:1 v/v) and methanol fractions. Finally,with the exception of essential oil, all fractions were high in alkaloids. Phytochemicals are important chemicals produced by plants during secondary metabolism and are usually responsible for their extensive pharmacological properties. Kumar et al. (2021) identified a plethora of such components in guava leaves. The detected phytochemicals are consistent with previous research done on the plant.

3.4. Quantitative Phytochemical Analysis

The TPC, TFC and TTC of the essential oil and each fraction were recorded in Table 5. The TPC of methanol and chloroform methanol (1:1 v/v) fractions was significantly higher compared with the chloroform methanol (4:1 v/v) fraction. Lower TPC was observed in hexane and chloroform fractions and essential oil. The TFC of chloroform methanol (4:1 v/v) and methanol fractions were greater compared with chloroform methanol (1:1 v/v). Lower TFC was observed in hexane and chloroform fractions and essential oil. The TTC of the methanol fraction was higher compared with the other fractions where relatively low TTC were observed. The lowest TTC was observed in the chloroform methanol (4:1 v/v) fraction.

Phenolic compounds are a diverse group of polyphenols comprising flavonoids, tannins, anthocyanins, phenolic acids and several other compounds. Being part of the alcohol family, they contain an OH group attached to a phenol ring, making them extractable. In a study conducted by Zahida et al. (2013), 368.61 ± 25.85 mg/100 g GAE phenolics and 162.92 ± 19.73 mg/100 g CAE flavonoids were present in the infusion of Malaysian guava leaves while Mailoa et al. (2013) found that the tannin content of guava leaves were 1.728 mg/g tannate acid equivalent. Wojdyło et al. (2007) revealed that the phenolic content of leaves varied in the range of 0.00 – 15.2 mg/100 g GAE dry weight. Ahmed and Abdulla (2014) reported that this variation is usually due to seasonal changes, especially the amount of rainfall in the particular area where the plant grows. The amount of phenolic compounds present in *P. guajava* leaves used in this study was in accordance with that of previous studies.

3.5. Antimicrobial Assay

The MIC values of *P. guajava* leaf extracts against isolates are represented in Table 6 . The solvents used in this study, showed no cytoxicity against the fungal isolates when tested with Iodonitrotetrazolium chloride (INT), a colorless compound used as an indicator to distinguish between viable cells in populations of respiring fungi. Hence, the change in color was wholly dependent on the fungal activity. As for the positive control, all wells treated with the commercial chemical based antidandruff shampoo showed no growth. MIC values of the essential oil, hexane and chloroform fractions against the fungal

isolates were between 6.25 1.35 mg/mL. Chloroform: methanol ratios and methanol alone showed a wider MIC range.

Padrón-Márquez et al. (2012) demonstrated that *P. guajava* leaf extracts induce numerous antimicrobial activities against dermatophytic fungi as a result of the presence of numerous phytochemicals. Prabhamanju et al. (2009) showed that the methanolic and chloroform extracts of selected plants against dandruff fungi was between 1-5 mg/ml. However, there is no earlier report on the antidandruff activity of guava leaf extracts. Bioactive compounds attack the proteins found in the cell walls and cell membranes of fungi, denaturing them and penetrating the cytosol to impair metabolism (Loi et al., 2020). Furthermore, the methanol fraction was found to be abundant in phytochemicals and had the highest phenolic concentration, justifying its lowest MIC. Chloroform: methanol (4:1 v/v) showed a considerable amount of phytochemicals as well, which equally contributed to its low MIC values.

4. CONCLUSION

The study validates and supports the traditional use of *P. guajava* leaf extracts by providing important evidence on their potential antidandruff properties. These extracts have the potential to be integrated into the cosmetic industry for the production of new hair products or as a supplement in existing products as, along with having good anti-fungal properties, the plant extracts are known to have conditioning properties and a sweet fragrance. Furthermore, the cultivation of the plant is effortless and cost-effective, especially in tropical continents like South Africa, as it grows fast and has the ability to adapt to a wide range of environmental conditions. It can form dense thickets and is even considered an invasive weed in many countries as its seeds are easily dispersed by avian and mammalian vectors. When in the right conditions, the plant takes only two years to reach the flowering stage and can bear heavily for up to 25 years. Although this was a pilot scale *in vitro* research, the findings and knowledge acquired can be further expanded to larger scales.

CONFLICTS OF INTEREST

None to declare.

ORCID

Cheetra Bhajan	0000-0003-1189-9800
Joyce Soulange Govinden	0000-0002-8119-1683
Vijayanti Mala Ranghoo Sanmukhiya	0000-0001-7767-276X

AUTHOR CONTRIBUTIONS

CB, JSG - Research concept and design, CB - Collection and/or assembly of data, CB, VMRS - Data analysis and interpretation, CB - Writing the article, JSG, VMRS - Critical revision of the article, JSG, VMRS - Final approval of the article.

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