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A COMPARATIVE STUDY ON THE ANTIOXIDANT POTENTIALS OF HYBRID AND GENERIC PAPAYAS AVAILABLE IN JAFFNA, SRI LANKA

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ABSTRACT

Generic papaya is a tropical plant abundant in Sri Lanka. Traditionally, it has been used therapeutically as it is a good source of polyphenols, carotenoids and an excellent source of vitamins that are powerful antioxidants. Specially, unripe fruit and leaf of papaya are recognized as a remedy for cancer and heart diseases due to their high antioxidant content. Recently, consumption of hybrid papaya fruits has increased as they are readily available in the local markets. However, the phytochemical constituents and quantity of these hybrid papaya varieties may differ from those available in the generic plant and hence their antioxidant potentials may also vary. Meanwhile there is concern within the community whether hybrid papaya has the same nutritional values as generic papaya. However, there have been no investigation done on the antioxidant activity of hybrid papaya available in Jaffna. Therefore, this study focused on investigating the in-vitro antioxidant potential of leaf and unripe fruit of generic and selected hybrid papaya varieties using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The unripe fruits and leaves of generic and selected hybrid (Red lady, Maradona, Tanin and Vega F1) varieties were collected from the Agricultural Research and Training Centre in Jaffna, Sri Lanka. The unripe fruit was sliced after removing the skin and seeds. The sliced unripe fruit and leaves were shade dried and powdered. The powdered plant materials were macerated with methanol and ethyl acetate separately for 48 hours at room temperature. The DPPH assay was carried out on the methanol and ethyl acetate extracts of the individual plant materials by employing ascorbic acid as the standard. The obtained data were analyzed using one-way ANOVA at 5% significance level and a significant difference between the antioxidant activities of the standard (IC_{50} value = 12.394 µg/mL) and the selected papaya extracts (IC_{50} values vary from 430.641 – 6,652.267 µg/mL) was found. Further, the selected hybrid papayas exhibited better antioxidant properties than the generic one; but, did not show a common trend within them. The leaf extracts were found to possess more antioxidant potential than the unripe fruit extracts of the same papaya variety. With respect to the solvents employed in extraction, methanolic extracts of the selected papayas showed better antioxidant activity than the corresponding ethyl acetate extracts. Preliminary phytochemical screening of the leaf and unripe fruit extracts of hybrid papayas revealed the presence of alkaloids, flavonoids, polyphenols and glycosides, which could be responsible for their antioxidant activity. The finding of the study revealed that hybrid papaya could be used as a raw material for functional food as generic papaya. However, there is a need to evaluate the other nutritional values to confirm it further.

KEYWORDS: *Antioxidant potential*, DPPH *assay, Generic papaya, Hybrid papaya, Leaf, Unripe fruit*

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1. INTRODUCTION

Papaya is a tropical plant found in Sri Lanka and widely available in all parts of the country. The fruit, peel, flower, seed, and leaf of the plant possess medicinal value as phytochemicals like enzymes (in latex), carotenoids (in fruits and seeds), alkaloids (in leaves), phenolic compounds (in fruits, leaves, and shoots), and glucosinolates (in fruits, leaves, and shoots) are found in the papaya plant (Zuhair *et al.*, 2013). Major groups of phytochemicals responsible for the antioxidant activity of papaya include polyphenols, carotenoids, and vitamins such as vitamin C & E (Foyzun and Aktar, 2017). As papaya is a highly consumed natural source, hybridization techniques are employed to increase its productivity.

Hybridization is the process of combining two genetically different parents to create new crop varieties (hybrid plants) with properties such as rapid growth, high yield, great disease resistance and longevity. Such hybrid plants might possess different phytochemical constituents compared to the generic plants. As such, the pharmacological activities of such hybrid varieties may vary (Addai, Abdullah and Mutalib, 2013).

Oxygen is essential for life to exist, but in certain instances it has serious deleterious impacts on the human body. Under normal physiological conditions, oxidative metabolism causes the production of free radicals like reactive oxygen species (ROS) (Chanda & Dave, 2009). They become harmful to humans, when they are not eliminated from the body by endogenous protective systems. Such imbalance between the generation of free radicals and endogenous protective mechanism leads to oxidative stress (Koju *et al.*, 2019).

Long-term accumulation of these free radicals in the human body can damage important biological molecules such as DNA, proteins, carbohydrates & lipids and result in a homeostatic disruption (Sivasankari, Poongothai, and Sudha, 2019). These cell damages cause numerous diseases and disorders like cancer, cardiovascular disease, neural disorder, Alzheimer’s disease, cognitive impairment, alcohol induced liver disease, ageing, atherosclerosis, *etc.* (Patel *et al.*, 2010).

The compounds which can prevent these cell damages are known as antioxidants. They can terminate the radical-based chain reactions by removing the free radical intermediates and inhibiting other oxidation reactions when present at low concentrations compared to that of the oxidizable substrate. Generally, the antioxidants serve as reducing agents and neutralize free radicals by oxidizing themselves. Several molecules play a role in antioxidant defence; they are either endogenous (internally synthesized) such as glutathione, α-lipoic acid, and coenzyme Q or exogenous (consumed) such as vitamin C, vitamin E, retinol, and phenols (Somogyi *et al*., 2007).

Antioxidants are prevalent in regularly consumed food products because they are either naturally occurring concurrent chemicals or synthetic antioxidants that are added during processing. Fruits, vegetables, tea, and other dietary sources contain various kinds of antioxidants.

Natural antioxidants are preferred because of their cost effectiveness, compatibility with food, and fewer adverse effects on the human body (Ahmed *et al.*, 2019). Also, it has been reported that they provide numerous health benefits including reduction of plaque development in the arteries and prevention of chronic diseases like cancer and heart disease (Dwivedi *et al.*, 2020). Meanwhile, consumers' desire for natural antioxidant sources has increased due to toxicity and carcinogenic nature of some synthetic antioxidants (Patel *et al*., 2010).

Currently, the consumption of hybrid papaya has increased in the country as they are readily available in the market. Also, cultivation of hybrid papaya is preferred over the generic variety as the hybrid plants are shorter in size and give higher yields. However, their phytochemical compositions and quantity may differ from the generic one as they are produced by cross pollination. Meanwhile the local community is concerned whether hybrid papaya possesses the same nutritional values as generic papaya. However, there have been no investigation done on the antioxidant activity of hybrid papaya available in Jaffna. Therefore, this study focused on investigating the *in-*

vitro antioxidant activity of leaf and unripe fruit of the selected hybrid (Red lady, Maradona, Tanin & Vega F1) and generic varieties by employing DPPH free radical scavenging assay to establish the antioxidant capacity of hybrid papaya in comparison with generic papaya. This study will provide a framework for the future antioxidant studies and to understand the nutritional values of the hybrid papaya.

2. METHODOLOGY

A. Determination of antioxidant activity and phytochemical analysis of leaf and unripe fruit extracts of generic and hybrid papayas

1. *Sample preparation*

Leaves, and unripe fruits of generic and selected hybrid (Red lady, Maradona, Tanin, and Vega F1) papayas were collected from the Agricultural Research and Training Centre in Jaffna, Sri Lanka and their authenticity were checked at the Department of Plant & Molecular Biology, University of Kelaniya, Sri Lanka. All raw plant materials were cleaned thoroughly with tap water followed by distilled water two to three times. The leaves were allowed to dry in the shade for a week. The unripe fruits were peeled off and seeds were removed, and the flesh was shredded into small thin pieces. The unripe fruit flesh slices were dried completely in the hot air oven at 50ºC for 2 days until a constant weight was attained. The dried leaves and unripe fruit flesh slices were milled separately, and the respective homogenous powders were obtained. The powdered samples were stored at 4ºC in airtight containers.

2. *Preparation of extracts from samples*

The powdered samples, 10 g each, were macerated separately with 200 mL of ethyl acetate and 200 mL of methanol at room temperature for 48 hours. Then, the macerated mixtures were filtered separately using Whatman No.1 filter papers with pore size 11.0 µm. The solvents were evaporated using rotatory evaporator (R3001, China) at 45ºC under reduced pressure and the resulting crude extracts were stored at 4ºC in the refrigerator.

3. *Preparation of DPPH solution*

The DPPH reagent (4 mg) was dissolved in 100 mL of methanol to prepare 0.004% (w/v) deep violet coloured DPPH solution which was stored in the dark at room temperature.

4. *Determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay*

Antioxidant activity of the crude extract was determined by adopting a slightly modified procedure of DPPH radical scavenging assay (Ahmed *et al.*, 2019) due to limited resources.

An amount of 3 mL of freshly prepared DPPH solution (0.04%) in methanol was added separately to 1 mL standard ascorbic acid solutions at different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL). The resulting mixtures were allowed to stand for 30 minutes in the dark. Absorbances of the said mixtures were recorded at 517 nm wavelength using a UV-Visible spectrophotometer (Spectro SC) and the standard curve for DPPH assay for ascorbic acid was plotted using the recorded data. Similarly, 1 mL solutions of each crude extract at different concentrations (50, 100, 200, 400 and 800 µg/mL) were added separately to 3 mL of freshly prepared DPPH solution (0.04%) in methanol, the resulting mixtures were allowed to stand for 30 minutes in the dark and absorbances were recorded at 517 nm wavelength. The same procedure was repeated with the mixture containing 1 mL of methanol and 3 mL of DPPH solution in methanol which was considered as the control. All experiments were carried out in triplicate. The degree of discoloration of the reaction mixture is proportional to the free radical scavenging efficiency of the crude extract. The following equation was employed to calculate the DPPH free radical scavenging activity (percentage of inhibition):

$$\text{Percentage of inhibition} = [(A_{control} - A_{sample}) / A_{control}] *100$$

$A_{control}$ = Absorbance of Control

A_{sample} = Absorbance of Sample

The percentage of scavenged DPPH was plotted against the concentration of each crude extract and the IC_{50} value of the respective crude extract was calculated.

5. Phytochemical analysis

The laboratory-based experiments to determine the presence/absence of phytochemicals (alkaloids, saponins, flavonoids, tannins, glycosides, polyphenols, gum & mucilage) were performed on the twenty crude extracts of hybrid papayas using standard protocols (Ahmed *et al*., 2019).

B. Data analysis

All experiments were carried out in triplicate and the recorded data were presented as mean ± standard deviation. The antioxidant activity was expressed as IC_{50} value. The statistical significance was evaluated by the analysis of variance (ANOVA) followed by Paired Sample T Test. Difference between the mean values was considered significant when p-value is less than 0.05 ($p < 0.05$).

3. RESULTS AND DISCUSSION

Yield percentages of crude extracts

10 g of dried and powdered leaf and unripe fruit flesh samples of generic and four different hybrid papaya varieties were separately extracted in methanol and ethyl acetate solvents by maceration. Though many extraction techniques are available, maceration process was chosen for the current study in order to extract the heat-sensitive phytochemicals along with others that may enrich the papaya extracts with more antioxidants. Moreover, heating may affect the extractability characteristics of the solvent (Ghori, 2013).

Table 1 shows the yield percentages of the corresponding crude extracts.

Table 1: Yield percentages of crude extracts of leaf and unripe fruit of generic and hybrid papayas in methanol and ethyl acetate.

Variety of Papaya	Plant part	Yield percentage of crude extract	
		Methanolic extract	Ethyl acetate extract
Generic	Leaf	12.0%	10.5%
	Unripe fruit	9.5%	7.0%
Red lady	Leaf	11.5%	11.0%
	Unripe fruit	8.5%	7.5%
Maradona	Leaf	6.0%	4.3%
	Unripe fruit	5.2%	3.8%
Tanin	Leaf	6.5%	4.5%
	Unripe fruit	4.8%	2.5%
Vega F1	Leaf	8.8%	5.3%
	Unripe fruit	6.8%	3.4%

Higher yields were obtained for leaf extracts than unripe fruit extracts. The highest yield (12.0%) was obtained for the leaf powder of generic papaya extracted in methanol. Meanwhile the lowest yield in percentage was observed for unripe fruit powder of Tanin papaya extracted in ethyl acetate with the value of 2.5%. In general, extraction was found to be more efficient in methanol than ethyl acetate which shows that polarity of the solvent employed for extraction influences yield percentage of the extract (Addai *et al.*, 2013). Further, the phytochemical compositions of plants vary according to the cultivar type and part of the plant, the yields of the corresponding crude extracts also differ.

In-vitro antioxidant activities of crude extracts

In this study, DPPH free radical scavenging assay was used to determine the antioxidant activity as it is a simple and rapid method and gives reliable information concerning the antioxidant ability of the tested plant material (Amira, 2013). When a DPPH free radical becomes paired with hydrogen from a free radical scavenging antioxidant, a DPPH-H species forms and the purple colour of DPPH fades rapidly to yellow. The degree of discoloration indicates the free radical scavenging potential of the antioxidant compound.

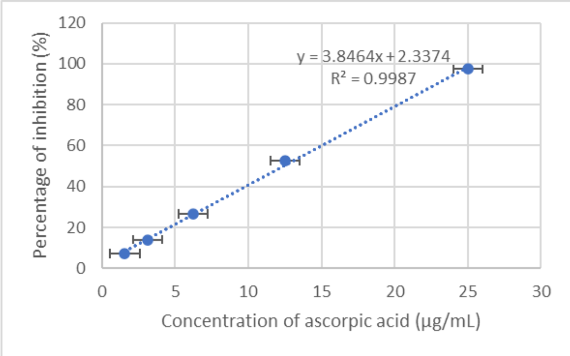

Figure 1: Standard curve for DPPH assay for Ascorbic acid

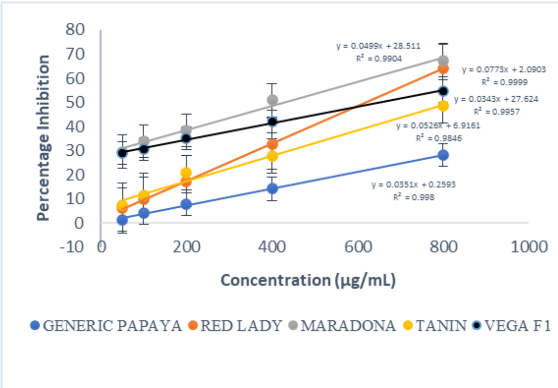

Figure 2: Percentage of Inhibition *vs* Concentration plots for Leaf extracts of Generic and Hybrid Papayas in Methanol

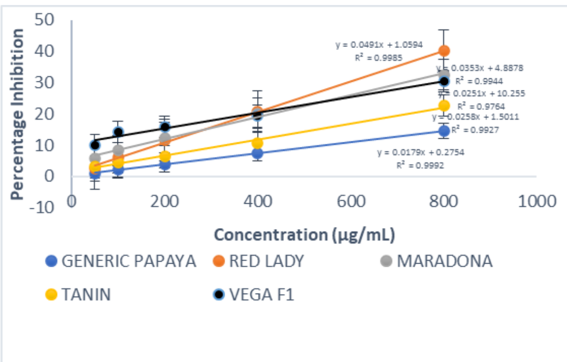

Figure 3: Percentage of Inhibition *vs* Concentration plots for Leaf extracts of Generic and Hybrid Papayas in Ethyl acetate

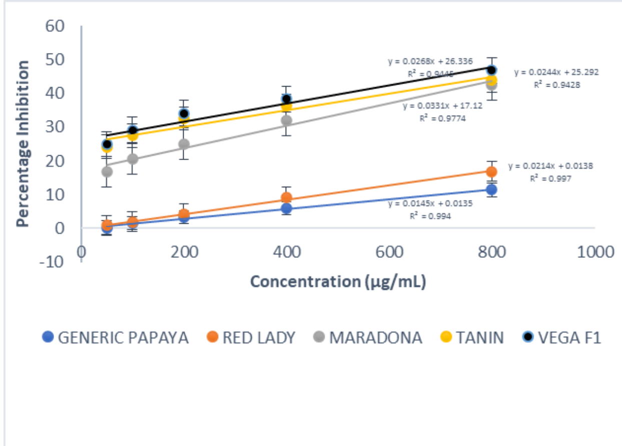

Figure 4: Percentage of Inhibition *vs* Concentration plots for Unripe fruit extracts of Generic and Hybrid Papayas in Methanol

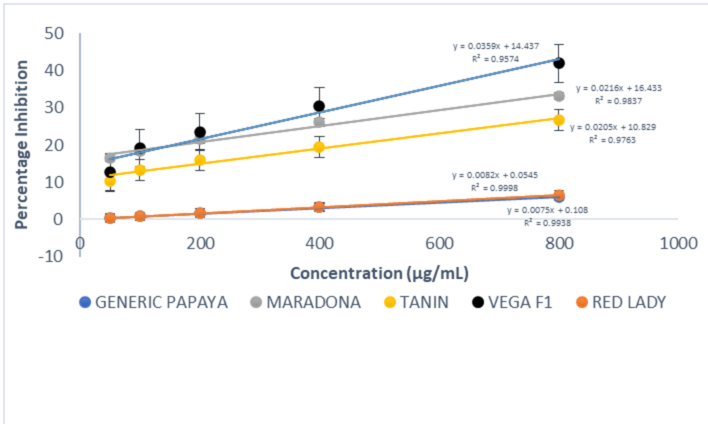

Figure 5: Percentage of Inhibition *vs* Concentration plots for Unripe fruit extracts of Generic and Hybrid Papayas in Ethyl acetate

Figure 1 shows the standard curve for DPPH assay for ascorbic acid. The DPPH free radical scavenging potentials (in percentage) of leaf extracts of generic and selected hybrid papayas in methanol and ethyl acetate solvents are depicted in Figures 2 and 3 respectively. Figures 4 and 5 illustrate the DPPH free radical scavenging activities (in percentage) of unripe fruit extracts of generic and selected hybrid papayas in methanol and ethyl acetate respectively.

The concentration of the plant extract required to reduce the initial DPPH concentration by 50% is known as the IC_{50} value of the respective plant extract. The IC_{50} values of all 20 crude extracts of papaya, determined from the regression analysis of the plots in Figures 2-5, are listed in Table 2.

Table 2: IC_{50} values of crude extracts of leaf and unripe fruit of generic and hybrid papayas in methanol and ethyl acetate

Crude Extract of Papaya			IC_{50} Value (µg/mL)
Solvent	**Plant Part**	**Variety**	
Methanol	Leaf	Generic	$1407.11 \pm 16.67^{e,f,g}$
		Red lady	$619.79 \pm 5.21^{g,h,i}$
		Maradona	$430.64 \pm 18.02^{h,i}$
		Tanin	$819.09 \pm 47.37^{f,g,h}$
		Vega F1	$652.36 \pm 20.24^{g,h,i}$
	Unripe fruit	Generic	3447.35 ± 197.90^{b}
		Red lady	$2335.80 \pm 322.20^{c,d}$
		Maradona	$993.35 \pm 62.76^{f,g,h}$
		Tanin	$1012.62 \pm 17.49^{f,g,h}$
		Vega F1	$882.99 \pm 38.58^{f,g,h}$
Ethyl acetate	Leaf	Generic	$2777.91 \pm 182.27^{b,c}$
		Red lady	$996.75 \pm 41.22^{f,g,h}$
		Maradona	$1277.97 \pm 76.98^{e,f,g}$
		Tanin	$1879.80 \pm 233.78^{d,e}$
		Vega F1	$1583.47 \pm 78.21^{d,e,f}$
	Unripe fruit	Generic	6652.27 ± 373.37^{a}
		Red lady	6090.92 ± 1006.37^{a}
		Maradona	$1554.03 \pm 127.93^{d,e,f}$
		Tanin	$1910.78 \pm 121.70^{d,e}$
		Vega F1	$990.61 \pm 21.47^{f,g,h}$

IC_{50} value of ascorbic acid is 12.39 ± 0.15^{j} µg/mL

Triplicate values are represented as mean ± SD; Values with different superscripts ($^{a-h}$) in the same column differ significantly ($P<0.05$)

The IC_{50} value of a plant extract is inversely related to its antioxidant activity. A low IC_{50} value indicates a high antioxidant activity of the plant extract and in turn a high percentage of inhibition of DPPH radicals.

In the present study, the IC_{50} value of the standard (ascorbic acid) was found to be 12.394 µg/mL whereas the IC_{50} values of the leaf and unripe fruit extracts of generic and selected hybrid papayas in methanol and ethyl acetate solvents varied from 430.641 – 6,652.267 µg/mL. Leaf powder of Maradona hybrid papaya extracted in methanol showed the highest IC_{50} value of 430.641 µg/mL, meanwhile unripe fruit powder of generic papaya extracted in ethyl acetate exhibited the lowest IC_{50} value of 6,652.267 µg/mL. A one-way ANOVA followed by Paired Sample T Test performed on the recorded data revealed a significant difference ($p>0.05$) between the antioxidant activities of ascorbic acid (standard) and the papaya extracts as well as between each and other extracts.

Based on the DPPH assay of the papaya varieties, the selected hybrid papayas exhibited better antioxidant properties than the generic one. However, a common trend could not be observed within the hybrid varieties.

When considering the plant parts, the leaf extracts were found to possess more antioxidant potential than the unripe fruit extracts of the same papaya variety selected for this study. A similar observation has been reported by Amira *et al.* (2013) where the analysis of antioxidant activities of different parts of the Carica papaya plant revealed that young leaves possess the highest antioxidant property followed by unripe fruit, ripen fruit, and finally seeds based on the type and quantitative availability of phenolic constituents.

This study further demonstrates that the polarity of the solvent used for extraction influences the extraction efficiency as well as antioxidant activity of the resulting extract. A separate study on the effect of solvents in the extraction of phenols responsible for antioxidant property of two papaya cultivars revealed high phenolic content in the cultivar extracted with 50% methanol (Addai *et al*., 2013). The findings of the current study were on a par with the literature where methanolic extracts showed better antioxidant activity than the corresponding ethyl acetate extracts (Foyzun & Aktar, 2017).

Phytochemical Analysis

In the current study, the phytochemical constituents of the leaf and unripe fruit extracts of the selected hybrid papayas were analyzed by adopting standard procedures (Ahmed *et al*., 2019) and the results are shown in Tables 3 and 4. However, quantitative determination of the identified phytochemicals responsible for the antioxidant activity could not be performed due to lack of facilities in the host institution.

Table 3: Phytochemical analysis of leaf extracts of selected hybrid papayas

Phytochemicals	RLM	RLEA	MLM	MLEA	TLM	TLEA	VLM	VLEA
Alkaloids	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	-	+	-	+	-
Tannins	-	-	-	-	+	-	+	-
Glycosides	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+
Gum & mucilage	-	-	-	-	-	-	-	-
Sterols	+	+	+	+	+	+	+	+

RLM = Red lady leaf methanolic extract; **RLEA** = Red lady leaf ethyl acetate extract; **MLM** = Maradona leaf methanolic extract; **MLEA** = Maradona leaf ethyl acetate extract; **TLM** = Tanin leaf methanolic extract; **TLEA** = Tanin leaf ethyl acetate extract; **VLM** = Vega F1 leaf methanolic extract; **VLEA** = Vega F1 leaf ethyl acetate extract
(+) indicates presence and (-) indicates absence

From the phytochemical screening of the leaf extracts of the selected hybrid papayas in methanol and ethyl acetate, it was found that all the crude extracts contain alkaloids, glycosides, polyphenols and sterols. While the presence of flavonoids was observed in the methanolic extracts of all hybrid papayas, those of Tanin and Vega F1 possess tannins additionally. The presence of flavonoids and tannins along with other antioxidants might have contributed to the enhanced i*n-vitro* antioxidant activities demonstrated by the methanolic extracts of leaf of the selected hybrid papayas. But none of the extracts exhibited the presence of saponins, gum and mucilage (Table 3). A phytochemical screening carried out by Aboobacker *et al* (2020) on the leaf extract of caccia papaya in methanol has revealed the presence of alkaloids, glycosides, flavonoids, saponins, tannins and carbohydrates. This observation is comparable to the present study.

Table 4: Phytochemical analysis of unripe fruit extracts of selected hybrid papayas.

Phytochemicals	RFM	RFEA	MFM	MFEA	TFM	TFEA	VFM	VFEA
Alkaloids	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+
Gum & mucilage	-	-	-	-	-	-	-	-
Sterols	+	+	+	+	+	+	+	+

RFM = Red lady unripe fruit methanolic extract; **RFEA** = Red lady unripe fruit ethyl acetate extract; **MFM** = Maradona unripe fruit methanolic extract; **MFEA** = Maradona unripe fruit ethyl acetate extract; **TFM** = Tanin unripe fruit methanolic extract; **TFEA** = Tanin unripe fruit ethyl acetate extract; **VFM** = Vega F1 unripe fruit methanolic extract; **VFEA** = Vega F1 unripe fruit ethyl acetate extract

While the unripe fruit extracts of the selected hybrid papayas in methanol and ethyl acetate were found to possess almost similar phytochemical compositions as those of the leaf extracts, a few exceptions were noted (Table 4). All the unripe fruit extracts contain flavonoids in addition to alkaloids, glycosides, polyphenols and sterols. Also, absence of tannins along with saponins, gum and mucilage were noted in all the unripe fruit extracts. It should be noted that not only the availability but also the quantity and extractability (extraction process and solvent) of available antioxidants determine the extent of antioxidant potential of the plant material. Further, quantity of phytochemicals in a plant material may vary according to the age of plant, time of collection, and the environmental conditions (Koffi *et al*., 2020). As such, lesser antioxidant activities of the unripe fruit extracts compared to the leaf extracts of papayas in the current study may be attributed to one or more of the above factors. The finding of the present study revealed that hybrid papaya could be used as a raw material for functional food as generic papaya.

4. CONCLUSION

This study has demonstrated a significant difference between the antioxidant activities of the standard and the selected papaya varieties based on statistical analysis. The selected hybrid papayas exhibited better antioxidant properties than the generic one but did not show a common trend within them. The leaf extracts were found to possess more antioxidant potential than the unripe fruit extracts of the same papaya variety. Methanolic extracts of the selected papayas showed better antioxidant activity than the corresponding ethyl acetate extracts. The presence of phytochemicals, such as alkaloids, flavonoids, polyphenols and glycosides, could be attributed to the antioxidant property of the papaya extracts. Overall, this study confirms that the antioxidant level of hybrid papaya is better than that of the generic papaya based on the DPPH assay, and hybrid papaya could be used for daily consumption as generic papaya. However, further studies are needed to understand the nutritional values of hybrid papaya.

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