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EFFECT OF EXTRACTION SOLVENT AND EXTRACTION METHOD ON BIOACTIVE COMPOUNDS FROM DIFFERENT PARTS OF *CARICA PAPAYA* L.

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ABSTRACT

Carica papaya L., commonly known as papaya plant is very commonly found in tropical regions. It is one of the common cultivated fruit around the world. During the recent years papaya plant found to be contain a very wide range of phytochemicals including phenolics, carotenoids, flavonoids and many more. The present study aimed to determine the effect of different extraction solvents on the total phenolic content, flavonoid content and salicylic acid content of different parts of papaya plant. The total phenolic content was determined by using folin-ciocalteau method. The total phenolic content, salicylic acid content, flavonoid content was found to be in case of leaves with 60% ethanol. In general ethanol and methanol was found to be act as good extraction solvents where as extraction with distilled water yields the lowest. Different solvents do play a greater role in boosting the extraction yield. The best extraction solvent for purification of specific bioactive compound may lead to better and promising future in case of medicines and drug discoveries.

Key words : *Carica papaya*, Extraction, Solvent, Bioactive compounds, Total phenolic content.

Introduction

Vegetables are the foundation of a balanced diet and are absolute necessity for maintain health in a balanced form. They contain rich source of vitamins, minerals, phytochemical compounds and dietary fiber content, which make the human body healthy and disease resistant. It is commonly accepted that fruits and vegetables are potent risk reducers of oxidative stress related diseases. Including them in the regular diet reduced the risk of oxidative stress related diseases and cancers, particularly gastrointestinal cancer by 50% (Asghar *et al.*, 2016).

Carica papaya L., which is commonly known as papaya belongs to the family Caricaceae and found mainly in tropical regions (Jagtap *et al.*, 2016). It is native to tropical America and introduced in India in 16^{th} century (Nariya and Jhala, 2017). Papaya is an evergreen plant having immense power house of nutrients. Parts of papaya including leaves, bark, roots and fruits can be used for medicinal purposes.

Papaya contains a broad spectrum of phytochemicals including enzymes (in the latex), carotenoids (in the fruit and seeds), alkaloids (in the leaves), phenolic (in fruits, leaves and shoots) and glucosinolates (in seeds and fruits). The fruit is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E (Foyzun and Aktar, 2017). Young leaves of papaya are potent source of flavonoids like myrectin, phenolic compounds *viz*., caffeic, chlorogenic acid and frolic acid, alkaloids *viz*., carpaine, pseudocarpaine (Jagtap *et al.*, 2016). Recent studies showed that papaya was used as an immune booster for the dengue fever (Asghar *et al.*, 2016). Unripe pulp of papaya is rich in carbohydrates, starch and also contains cardenolides and saponins that have medicinal value. The seeds used as emmenagogue, thirst quenchers, carminatives or for bites and stings of poisonous insects (Anuar *et al.*, 2008).

Papayas contain two important biologically active compounds chymopapain and papain which are widely used for digestive disorder. Folks use papaya latex to cure Dyspepsia and external burns and scalds (Kovendan, 2012). The fallen leaves of papaya plant are used variously for treatment of fever, pyrexia, diabetes, gonorrhea, syphilis, inflammation and dressing for foul wounds (Owoyele, 2008). Extraction solvents affect extraction efficiency of bioactive compounds from plant materials and their subsequent health benefits (Zhou *et al*., 2011).

The basic principle of solvent extraction is; the solute distributes itself in a fixed ratio between two immiscible solvents; which are usually water and an organic solvent. Solvent extraction helps in isolating compounds from plant materials and enables selective removal of components in a mixture so that they can be easily manipulated or concentrated. The grinding procedure during extraction often accompanied that production and volatile losses and chemical change so the solvents act as controlling temperature.

Previous studies have used methanol, petroleum ether, chloroform, ethanol, acetone, n-hexane and water as the solvents for extracting compounds of *Carica papaya* L. The present study aimed to study the impact of different common solvents on the extraction efficiency of bioactive compounds from different parts of papaya plant and to identify the most appropriate solvent for further extraction and isolation of bioactive compounds from papaya plant parts.

Materials and Methods

Sample collection : fresh leaves of papaya plant, ripe fruit and unripe fruit were collected from the campus of Fakir Mohan University, Vyasavihar, Balasore, Odisha, India. All the collected samples were washed with water. The leaves were dried under sun for 5-6 days. The sundried leaves were crushed to powder and stored in an air tight container. The unripe and ripe fruit were cut into small pieces and dried for 2-3 days.

Solvents used : Distilled water, Methanol, 70% Methanol, Ethanol, 60% Ethanol, Acetone and 50% Acetone.

Extraction : All the samples were homogenized with grinding by chilled mortar and pestle.

Plant parts used

Leaves : 1g of dried leaves powder (DLP) homogenized with 10ml distilled water (ddH_2O). The process was again repeated with 10ml of each of the six remaining solvents.

Ripe fruit : 1g of ripe fruit pulp weighed and homogenized with 6ml of ddH_2O. The process was again repeated with 6ml acetone, 5ml of 50% acetone, 5ml of ethanol, 5ml of 60% ethanol, 6ml methanol and 6ml of 70% methanol.

Unripe fruit : 1g of unripe fruit pulp weighed and homogenized with 6ml of ddH_2O. The process was again repeated with 6ml acetone, 5ml of 50% acetone, 5 ml of ethanol, 5ml of 60% ethanol, 6ml methanol and 6ml of 70% methanol.

The above homogenized materials were centrifuged at 10000 rpm for 18 minutes at 18 degree Celsius. The supernatants were collected and stored for further use.

Total phenolic content : Total phenolic content was estimated according to the method of Dihazi *et al*. (2003). The samples were taken out from refrigerator samples were thawed to room temperature before use. From each sample 1ml of supernatant was taken into a clean and dry test tube and volume was made up to 10ml with ddH_2O. From each diluted supernatant 1ml was taken into another test tube.10 different known concentrations of Gallic acid (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100mg/ml) were taken into 10 different test tubes. To each test tube 1ml of diluted Folin-ciocalteau reagent was added and mixed well by shaking. The tubes were incubated in dark for 3mins at room temperature. To each test tube again 1ml of 2.5% Na_2CO_3 was added and shaken to mix properly. The test tubes were again incubated at room temperature for 60mins in dark. The known concentration of Gallic acid was used to construct the standard curve. Absorbance of the contents of the tubes was read at 725nm using UV-VIS spectrophotometer.

Test for Salicylic acid : Salicylic acid was estimated following the method of Warrier *et al*. (2013).

In clean dry test tubes 1ml of the extracted supernatant was taken. To each test tube 1ml of 1% $FeCl_3$ was added and mixed properly. Then the volume was made up to 10 ml with ddH_2O. Known concentrations of salicylic acid (10, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 ìg/ml) were used to construct the standard curve. The absorbance was read at 540 nm in an UV-VIS spectrophotometer.

Test for flavonoid content : The total flavonoid content was estimated by following the method of Chang *et al*. (2002). 1ml of supernatant was taken in a clean and dry test tube. To the test tube 4.3ml of respective extraction solvent, 0.1ml of 10% $Al(NO_3)_3$ and 0.1 ml of 1M $C_2H_3NaO_2$ was added. The reaction mixture was incubated in dark for 30 minutes. Then the absorbances of the contents were recorded at 415 nm in a UV-VIS spectrophotometer. The amount of flavonoids was expressed as microgram of quercetin/g of the sample taking quercetin as the standard.

Results

Seven types of solvents were used as buffer to quantify the bioactive compounds like, total phenolic content, total flavonoid content and total salicylic acid content of different parts of papaya plant. Total phenolic content was observed to be highest in case of leaves as compared to ripe and unripe fruit (Fig. 1) in the order leaf > ripe fruit > unripe fruit. Highest yield in the leaves was obtained with 60% ethanol followed by absolute methanol and poorest by 50% acetone among organic solvents. As shown in Table 1, in case of ripe fruit and unripe fruit maximum yield was observed with absolute methanol. Normalized data obtained by taking ratio showed that highest and lowest yield in leaf was obtained when 60% ethanol and distilled water was used respectively. The extraction yield was obtained in the following order 60% ethanol > ethanol > acetone > methanol > 70% methanol > 50% acetone > distilled water.

Salicylic acid was observed to be highest in case of leaf of papaya (Fig. 2). However, the yield in case of

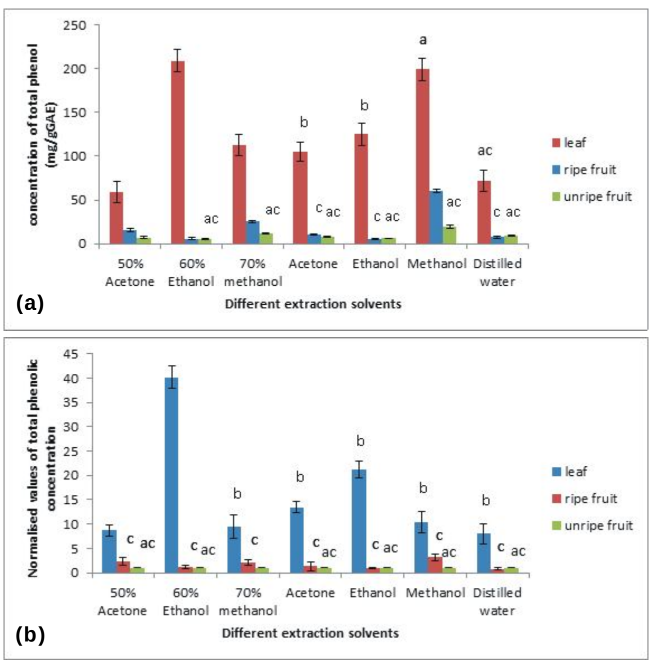

Fig. 1 : **(a)** show the effect of different extraction solvents on total phenolic content extracted from different parts of Papaya, **(b)** shows the normalized values of effect of different extraction solvents total phenolic content extracted from different parts of Papaya. Values represent mean ± SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

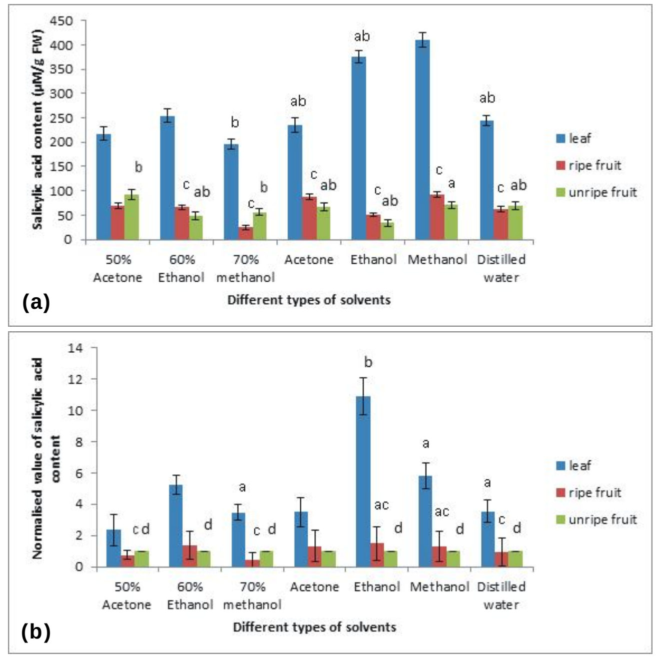

Fig. 2 : **(a)** show the effect of different extraction solvents on concentration of salicylic acid content extracted from different parts of Papaya, **(b)** shows the normalized values of effect of different extraction solvents salicylic acid content extracted from different parts of Papaya. Values represent mean ± SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

Table 1 : Effect of different extraction solvents on total phenolic content extracted from different parts of Papaya (b) Normalized values of effect of different extraction solvents total phenolic content extracted from different parts of Papaya. Values represent mean ±SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

Solvent	Concentration of total phenol (mg/gGAE)		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	104.73^{b} ±11.18	10.26^{c} ±1.14	7.77^{ac} ±0.95
50% Acetone	59.29^{ab} ±11.91	15.96^{c} ±1.84	6.85^{ac} ±1.08
100% Ethanol	124.94^{b} ±12.73	5.32^{c} ±0.76	5.88^{ac} ±0.31
60% Ethanol	209.19^{a} ±13.11	5.78^{c} ±0.98	5.2^{ac} ±0.8
100% Methanol	199.19^{a} ±12.97	59.97^{bc} ±1.96	19.11^{ac} ±1.83
70% Methanol	112.43^{b} ±12.18	25.06^{c} ±0.99	11.87^{ac} ±0.56
Water	71.72^{ac} ±11.71	7.28^{c} ±1.02	8.94^{ac} ±1.16

Solvent	Normalized value of total phenolic concentration (mg/gGAE)		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	13.47^{b} ±1.21	1.32^{c} ±0.95	1.00^{ac} ±0.1
50% Acetone	8.65^{b} ±1.17	2.32^{c} ±0.81	1.00^{ac} ±0.09
100% Ethanol	21.24^{b} ±1.73	0.90^{c} ±0.11	1.0^{0ac} ±0.05
60% Ethanol	40.22^{a} ±2.33	1.11^{c} ±0.31	1.00^{ac} ±0.1
100% Methanol	10.42^{b} ±2.19	3.13^{c} ±0.70	1.00^{ac} ±0.01
70% Methanol	9.47^{b} ±2.51	2.11^{c} ±0.55	1.00^{ac} ±0.08
Water	8.02^{b} ±2.07	0.81^{c} ±0.13	1.00^{ac} ±0.05

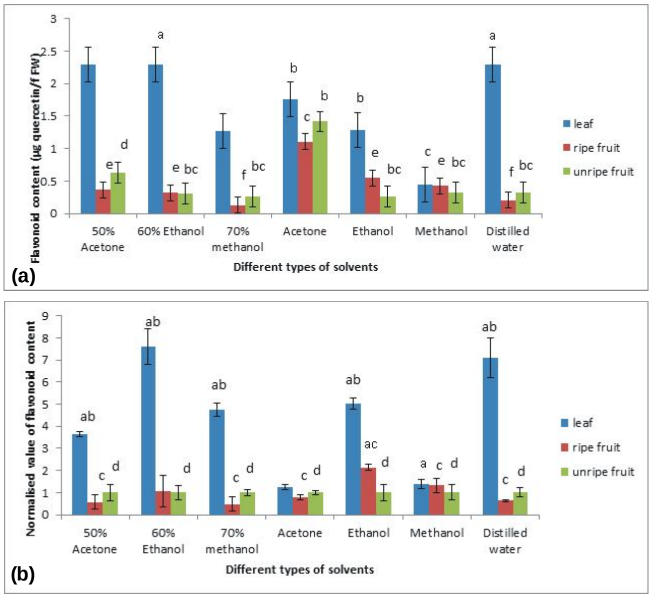

Fig. 3 : **(a)** show the effect of different extraction solvents on concentration of flavonoid content extracted from different parts of Papaya, **(b)** shows the normalized values of effect of different extraction solvents flavonoid content extracted from different parts of Papaya. Values represent mean ± SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

Table 2 : Effect of different extraction solvents on salicylic acid content extracted from different parts of Papaya, (b) Normalized values of effect of different extraction solvents on salicylic acid content extracted from different parts of Papaya. Values represent mean ± SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

Solvent	**Salicylic acid content (µg/g FW)**		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	235.96^{ab}±14.70	87.91^{c} ±6.06	3.49^{ab} ±7.61
50% Acetone	217.87^{b}±13.14	69.03^{c}±6.11	2.35^{b} ±9.9
100% Ethanol	375.21^{ab}±12.11	51.72^{c} ±5.29	10.90^{ab} ±6.69
60% Ethanol	254.37^{ab}±13.39	65.88^{c} ±5.13	5.23^{ab} ±8.81
100% Methanol	410.45^{a}±15.89	92.63^{c} ±5.18	5.81^{a} ±6.07
70% Methanol	196.31^{b}±10.09	24.97^{c} ±5.09	3.47^{b} ±7.66
Water	244.93^{ab}±10.97	62.73^{c} ±5.09	3.54^{ab} ±8.84

Solvent	**Normalized value of salicylic acid content (µg/g FW)**		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	3.49^{a} ±0.93	1.30^{c} ±1.01	1.00^{e} ±0.79
50% Acetone	2.35^{a} ±0.99	0.74^{c} ±0.31	1.00^{e} ±0.91
100% Ethanol	10.90^{b} ±1.19	1.50^{ac} ±1.07	1.00^{d} ±0.92
60% Ethanol	5.23^{a} ±0.59	1.35^{ac} ±0.9	1.00^{e} ±0.88
100% Methanol	5.81^{a} ±0.80	1.31^{ac} ±0.98	1.00^{d} ±0.99
70% Methanol	3.47^{a} ±0.49	0.44^{c} ±0.44	1.00^{e} ±0.83
Water	3.54^{a} ±0.70	0.90^{c} ±0.91	1.00^{e} ±0.78

Table 3 : Effect of different extraction solvents on flavonoid content extracted from different parts of Papaya, (b) Normalized values of effect of different extraction solvents on flavonoid content extracted from different parts of Papaya. Values represent mean ± SE of three separate experiments, each in triplicate. Sharing the same letter are not significantly different ($p<0.5$) using Student's t test.

Solvent	**Flavonoid content (µg quercetin/g FW)**		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	1.76±0.55^{b}	1.10±0.08^{c}	1.41±0.16^{d}
50% Acetone	2.29±0.77^{a}	0.36±0.04^{ac}	0.63±0.09^{ad}
100% Ethanol	1.29±0.58^{b}	0.54±0.05^{ac}	0.25±0.05^{e}
60% Ethanol	2.29±0.69^{a}	0.31±0.02^{ac}	0.30±0.09^{e}
100% Methanol	0.43±0.13^{ab}	0.42±0.01^{ac}	0.31±0.03^{e}
70% Methanol	1.27±0.49^{b}	0.12±0.03^{e}	0.26±0.09^{e}
Water	2.24±0.59^{a}	0.20±0.03^{ac}	0.33±0.02^{e}

Solvent	**Normalized value of flavonoid content (µg quercetin/g FW)**		
	Leaf	**Ripe fruit**	**Unripe fruit**
100% Acetone	1.24±0.11^{a}	0.78±0.13^{c}	1.00±0.36^{d}
50% Acetone	3.62±0.11^{ab}	0.57±0.32^{c}	1.00±0.31^{d}
100% Ethanol	5.01±0.26^{ab}	2.13±0.12^{ac}	1.00±0.12^{d}
60% Ethanol	7.59±0.81^{ab}	1.05±0.70^{ac}	1.00±0.10^{d}
100% Methanol	1.38±0.31^{a}	1.33±0.91^{c}	1.00±0.35^{d}
70% Methanol	4.76±0.3^{ab}	0.48±0.31^{c}	1.00±0.34^{d}
Water	7.10±0.05^{ab}	0.63±0.05^{c}	1.00±0.20^{d}

ripe and unripe fruit was comparable. Table 2 shows highest yield in the leaves was obtained when extraction was done with absolute methanol and poorest yield was achieved with 70% methanol. In case of ripe fruit and unripe fruit maximum yield was observed with absolute methanol and 50% acetone. Normalized data obtained by taking ratio showed that highest and lowest yield in leaf was obtained when ethanol and 50%acetone was used, respectively. The extraction yield was obtained in the following order ethanol > methanol > 60% ethanol > distilled water > acetone > 70% methanol > 50% acetone.

Flavonoid content was observed to be highest in case of leaves as compared to ripe and unripe fruit (Fig. 3). However not marked distinction was observed between the different solvents as observed in Table 3. Normalized data obtained by taking ratio showed that highest and lowest yield in leaf was obtained when 60% ethanol and acetone was used respectively. The extraction yield was obtained in the following order 60% ethanol > ethanol > 70% methanol > 50% acetone > methanol > acetone > distilled water.

Discussion

Carica papaya is found to be a good source of herbal medicines due to presence of wide range of phytochemicals. The study showed that the use of different extraction solvents has more or less effect on the extraction yield. Phenolic compounds yields higher in 60% ethanolic solvent whereas lowest in case of distilled water. Methanol appeared to be the best among all other organic solvents used in extraction of ripe and unripe fruit of papaya. According to Ali *et al.* (2011) extraction with methanol yields higher extraction yield in case of ginger fruit and tea and also supported by Addai *et al.* (2013). The study result of Asghar *et al.* (2016) shows quite similarity with this result which said ethanol and methanol as the most extractable solvents in case of phenolics. The present study found phenolics higher in case of leaves which concurred with the result reported by Yahia *et al.* (2010). The findings of the present study can be sequenced for the presence of phenolic compounds as leaves> ripe fruit> unripe fruit. However, the findings of Maisarah *et al.* (2013) reported that the sequence of extracted phenolic content was as follows: leaves> unripe fruit> ripe fruit.

The difference in the results might be due to the type of cultivars, growing condition, maturity stage and time elapsed before the fruits were analyzed. The other reasons for such variation can be attributed to the difference in sample preparation method.

Flavonoids are subclass of polyphenols which have role in antioxidative property of the veggies. The present study showed higher number of flavonoids present in the order leaves> ripe fruit (mesocarp)> unripe fruit (mesocarp). The highest yield was observed in extraction with 60% ethanol and lowest in distilled water. The result shows some similarity with Fatma *et al.* (2019) which said that, highest flavonoid content is observed in methanol and 70% ethanol whereas lowest in extraction with water. They reported higher content in the mature leaves than in young leaves. The content also affected by pest attack, disease, climate change, ultraviolet radiation, location, cultivar. The findings of the study of Asghar *et al.* (2016) shows higher flavonoid content in ethanol followed by methanol. The presence of flavonoids and phenols depends upon the maturity of the parts of the plants. The parameters that influence the extraction of secondary metabolites are solvent, temperature and extraction technique. So, choosing solvents should be carefully done with considering the possible toxicity associated with the solvent.

In the plants the salicylic acid acts to defend against harmful elements like fungus, insect and disease. In commercial purpose the salicylates show anti-inflammatory, anti-diarrhoea property. The present study showed that ethanol followed by methanol as the most efficient solvent for extraction of salicylic acid. In that case the highest content found when extracted with ethanol and lowest content, when extracted with 50% acetone. The highest amount of salicylic acid is found in leaf of papaya.

The varieties of result in the present study of measurement of phenolic compound, salicylic acid and flavonoids showed that it is difficult to obtain the most appropriate solvent for extraction and isolation of bioactive compounds from papaya parts. Ethanol and methanol acts well in extraction yield than acetone. Crude organic solvents show less strong action than the aqueous organic solvent. 60% ethanol yields more than any other. It shows addition of water to the solvents make a rise in the extraction yield. This might be due to the increase in bulge of plant material by water which increased the contact surface area between plant material and solvent. Increasing polarity of the solvent enhances the extraction power of certain solvent (Addai *et al*., 2013). The present study also showed that extraction with water has lower extraction yield than others. The reason might be due to high percentage of impurities.

The nutritional value of plant product depends upon their phenolic and flavonoid content. By including these in human diet their function is to show anti-inflammatory, anti-cancerous and anti-oxidative property (Asghar *et al*., 2016). The present study showed that papaya leaves are highly rich in phenolics, flavonoids and also in salicylic acid as compared to ripe fruit and unripe pulp. Further the present study indicated that extraction solvents play major role in optimization of extraction yield from different parts of the plant.

Conclusion

Though the organic solvents show better optimizing property, among the seven types of solvents used, 60% ethanol or the organic solvents with some water percentile (less than 50%) shows greater extracting property. The result concluded from the study is, different solvents do play a greater role in boosting the extraction yield. Presence of salicylic acid, flavonoids, phenolic content makes the papaya fruit a greater source of healthy diet. Nevertheless, further investigation is awaited for determining the most appropriate solvent for extraction, identification and isolation of bioactive compounds and their role in fight against the biotic stresses.

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