Investigation of Elemental Analysis, Antimicrobial Activity and Antioxidant Activity of the Male Flower of Papaya (*Carica papaya* L.)

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Abstract

This research deals with the investigation of elemental analysis, antimicrobial activity and antioxidant activity of the male flower of Papaya (*Carica papaya* L.). The sample was collected from Danbi Village, Hinthada Township, Ayeyarwady Region, during September, 2019. Preliminary phytochemical investigation of Papaya was carried out by test tube methods. The elemental analysis was determined by using EDXRF spectrometry. In addition, the soluble matter constituents of the sample in pet-ether, ethyl acetate, ethanol and water were done by solvent extraction method. Pet-ether, ethyl acetate, ethanol and water extracts of Papaya were subjected for screening antimicrobial activity against *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Candida albicans, Pseudomonas fluorescens, Agrobacterium tumefaciens, Staphylococcus aureus* and *Malassezia furfur* by agar well diffusion method. Furthermore, antioxidant property of ethanol and water extracts from the male flower of Papaya (*Carica papaya* L.) was investigated by using DPPH free radical scavenging assay method. The antioxidant activity of water extract (IC_{50}- 90.75µg/mL) was found to be higher than that of ethanol extract (IC50-94.84µg/mL).

Keywords: Papaya (*Carica papaya* L.), EDXRF, Antimicrobial activity and Antioxidant activity

Introduction

Papaya (*Carica papaya* L.) belonging to the family Caricaceae is properly a large herb growing at the rate of 6 to 10 ft the first year and reaching 20 or even 30 ft in height, with a hollow green stem becoming 12 to 16 inch or thicker at the base and roughened by leaf scars. The leaves emerge directly from the upper part of the stem in a spiral on nearly horizontal petioles 1 to 3.5 ft. long hollow, succulent, green or more or less dark purple. The life of a leaf is 4 to 6 months. Both the stem and leaves contain copious white milky latex. The 5-petalled flowers are fleshy, waxy and slightly fragment. Some plants bear only short stalked pistillate (female) flowers, waxy and ivory-white while others may bear only staminate (male) flowers, clustered on panicles of 5 or 6 ft. long. Male and female flowers are borne in the leaf axils, the males in multi-flowered dichasia, the female flower is few-flowered dichasia. This change of sex may occur temporarily during high temperatures in midsummer. Male or hermaphrodite plants may completely change to female plants after being beheaded. When the fruit is green and hard it is rich in white latex. As it ripens, it becomes light or deep-yellow externally and the thick wall of succulent flesh becomes aromatic, yellow, orange or red (Morton, 1987).

Papaya (*Carica papaya* L.) is believed to have originated in the lowlands of eastern Central America, from Mexico to Panama. Papaya plant is grown in tropical and subtropical countries, which includes 57 countries like India, Brazil, Indonesia, Mexico, Nigeria and Africa (Maisarah, 2013).

Papaya (*Carica papaya* L.) contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion (Bouanga-Kalou, 2011). Fruit contains linalool, benzylisothiocynate, cis and trans 2,6-dimethyl-3,6-expoxy-7-octen-2-ol,α-carpaine, benzyl-β-D-glucoside. Seed also contains

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carpaine, benzylisothiocynate, benzylglucosinolate, glycotropacolin, benzylthiourea, β-sitosterol and an enzyme myrosin. Root contains arposide and an enzyme myrosin. Leaves contains carpaine, pseudo carpaine, carposide, vitamin C and E. Latex are reported to contain papain, chymopapain, glutamine cyclotransferase, chymopapain A, B and C, pepidase A and B and lysozymes (Maisarch, 2013).

Papaya (*Carica papaya* L.) contains two biological active compounds which are chymopapain and papain which are widely useful for digestive disorders and gastrointestinal disturbance. Papaya leaves are used traditionally in treatment like jaundice, malaria, dengue and antiviral activity. Young leaves have medicinal properties like anti-inflammatory, hypoglycemic, abortifacient, hepatoprotective, wound healing, antihypertensive and antitumor activities. Flowers are also used to treat jaundice febrifuge and pectoral properties. In India, male flowers are used as herbal tea which acts a dietary supplement for consumption. Seeds have pharmacological activities like anthelmintic, contraceptive, anti-inflammatory, analgesic and antimicrobial property. Its roots can be used as medicine for renal and urinary bladder problem (Anitha, *et al*, 2018).

In the present work, the male of flower of Papaya was chosen for elemental analysis, antimicrobial activity and antioxidant activity.

Figure (1) Photographs of the plant and flowers of Papaya (*Carica papaya* L.)

Materials and Methods

Sample Collection and Preparation

The male flower of Papaya (*Carica papaya* L.) was selected for investigation of elemental analysis, antimicrobial activity and antioxidant activity. This sample was collected from Danbi Village, Hinthada Township, Ayeyarwady Region, during September, 2019. The collected dried sample was ground into powder by grinder. The dried powdered sample was stored in the air-tight containers to prevent the moisture and other contaminations.

Phytochemical Investigation of the Male Flower of Papaya (*Carica papaya* L.)

Preliminary phytochemical examination was carried out on dried powder of Papaya to determine the presence or absence of alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponin, starch, flavonoids, phenolic compounds, tannins, steroids and terpenoids according to the test tube method.

Some Elemental Analysis of the Male Flower of Papaya

For this measurement, pellets of the sample were first made X-Ray spectrometer permits simultaneous analysis of light element to heavy element. Energy dispersive X-Ray fluorescence spectrometer (Shimadzu EDX-700) can analyze the elements from Na to U under vacuum condition. Analysis of some elements in the male flower of Papaya was measured by EDXRF method using EDX-700 instrument at the Universities' Research Centre (URC), Mhonyor University. The results obtained are shown in Table 1.

Preparation of Various Crude Extracts of the Male Flower of Papaya

25g of dried powered sample was percolated with 75cm^3 of pet-ether for 24 hours and filtered. The filtrate was placed in a weighed porcelain basin and then evaporated to dryness on a water-bath until it was completely dried. Pet-ether extract was obtained. Ethyl acetate and ethanol extracts from the male flower of Papaya were done in the same procedure. In addition, watery extract of dried powder sample was prepared by boiling 25g of sample with 75cm^3 of distilled water for about 6 hours and filtered. The filtrate was evaporated to dryness on a water-bath until it was completely dried.

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method (Collins, 1965)

Screening of antimicrobial activity of different crude extracts such as pet-ether, ethyl acetate, ethanol and water extracts of the male flower of Papaya were determined against eight strains of microorganism such as *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Pseudomonas fluorescens, Agrobacterium tumefaciens, Staphylococcus aureus, Candida albicans* and *Malassezia furfur*. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The inhibition zones of crude extracts against eight microorganisms tested are shown in figure 4. The study of antimicrobial activity was performed by agar well diffusion method. Nutrient agar was prepared according to the method described by Collins, C.H.

Screening of antioxidant Activity by DPPH Assay Method

The DPPH radical scavenging method was used to evaluate the antioxidant property. Standard solution was used as ascorbic acid. The control solution was prepared by mixing 1.5cm^3 of 60µM DPPH solution and 1.5cm^3 of 95% ethanol using shaker. The sample solution was also prepared by mixing thoroughly 1.5cm^3 of 60µM DPPH solutions and 1.5cm^3 of test sample solution. The solution was allowed to stand at room temperature for 30 minutes. After 30 minutes, the reduction of the DPPH free radical was measured by reading the absorbance at 517nm by a UV-Visible spectrophotometer. The results were shown in tables (3) and (4), and figures (5), (6) and (10).

Results and Discussion

Phytochemical Investigation of the Male Flower of Papaya

The phytochemical constituents of male flower of Papaya was investigated by test tube method. Alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, reducing sugars, saponins, starch, flavonoids, phenolic compounds, tannins, steroids and terpenoids were found to be present in the male flower of Papaya.

Some Elemental Analysis of the sample by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrometry

The element contents present in the male flower of Papaya were determined by EDXRF Spectrometer. The EDXRF spectrum of the sample was shown in Figure 2 and data was listed in Table 1. According to the results, potassium is the most abundance. A few amount of phosphorus, calcium and sulphur were observed. In addition, iron, manganese, zinc, copper, rubidium and platinum were found as trace elements in this sample.

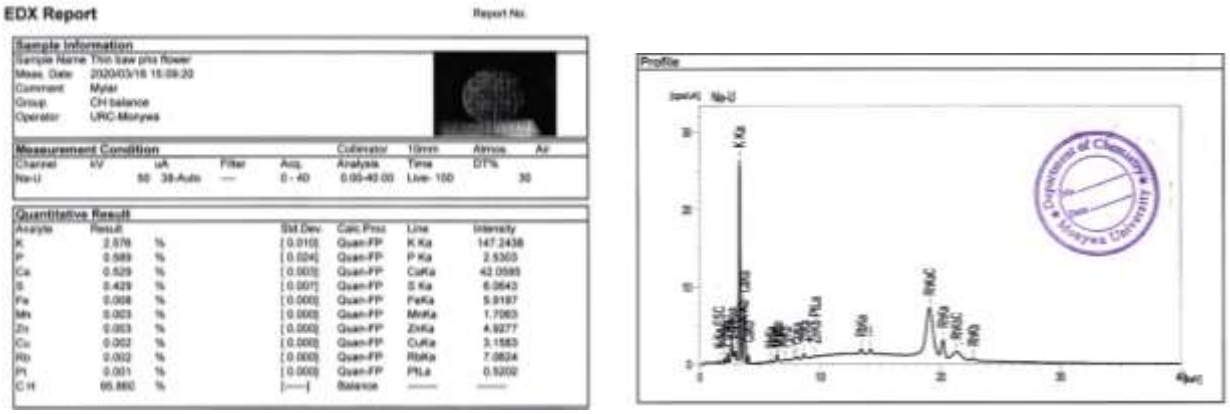


Figure (2) EDXRF Spectrum of the Male Flower of Papaya

Table (1) Relative Abundance of Some Elements in the Male Flower of Papaya by EDXRF Spectrometry

No	Elements	Relative Abundance (%)
1	Potassium (K)	2.576
2	Phosphorus (P)	0.589
3	Calcium (Ca)	0.529
4	Sulphur (S)	0.429
5	Iron (Fe)	0.008
6	Manganese (Mn)	0.003
7	Zinc (Zn)	0.003
8	Copper (Cu)	0.002
9	Rubidium (Rb)	0.002
10	Platinum(Pt)	0.001
11	Hydrocarbon (C/H)	95.860

Investigation of Soluble Matter Contents of the Male Flower of Papaya

In this research work, the soluble matter contents of the male flower of Papaya were done with different polarity of pet-ether, ethyl acetate and ethanol by solvent extraction method. However, water extract was performed by boiling on a water-bath. The yield percent of petroleum ether extract (2.8%), ethyl acetate extract (5.2%), ethanol extract (14.0%) and water extract (22.8%) were obtained. From the results, it was observed that the amount of polar constituents were higher than that of nonpolar constituents in the male flower of Papaya.

Table (2) Soluble Matter Contents of the Male Flower of Papaya

No	Extracts	Yield (%)
1	Petroleum ether (PE)	2.8
2	Ethyl acetate (EA)	5.2
3	Ethanol (EtOH)	14.0
4	Water	22.8

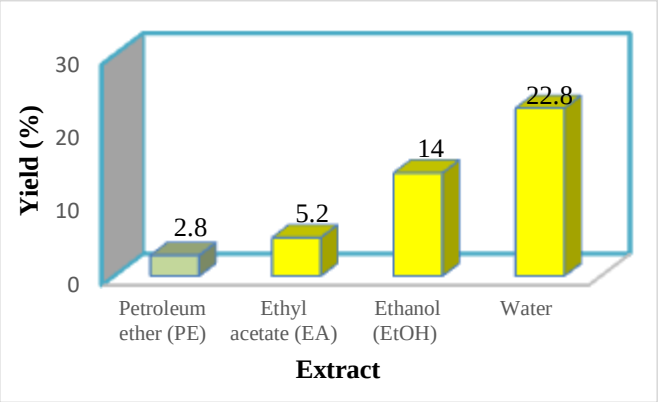
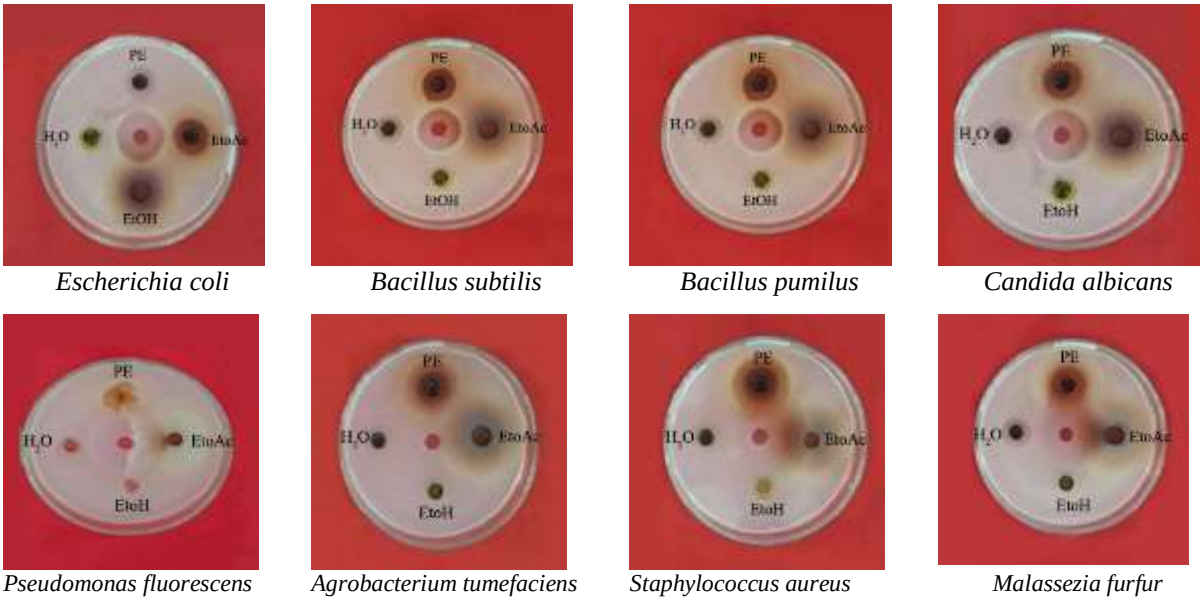


Figure (3) A bar graph of soluble matter contents of the male flower of Papaya

Screening of Antimicrobial Activity of Various Crude Extracts by Agar Well Diffusion Method

Screening of antimicrobial activity of different crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts from the male flower of Papaya were determined against eight strains of microorganism such as *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Candida albicans, Pseudomonas fluorescens, Agrobacterium tumefaciens, Staphylococcus aureus* and *Malassezia furfur*. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger inhibition zones diameter is the more antimicrobial activity on the tested microorganisms. The inhibition zones of crude extracts against eight microorganisms tested are shown in figure (4). It was found that pet-ether extract of the male flower of Papaya was not observed antimicrobial activity against all test microorganisms. Ethyl acetate extract of the male flower of Papaya showed a moderate antimicrobial activity against *Escherichia coli, Bacillus subtilis, Bacillus pumilus, Candida albicans, Malassezia furfur.* Ethanol extract of the male flower of Papaya recorded a moderate antimicrobial activity against *Bacillus pumilus, Malassezia furfur, Escherichia coli, Bacillus subtilis, Candida albicans, Pseudomonas fluorescens, Agrobacterium tumefaciens* and *Staphylococcus aureus* with inhibition zone diameter ranged in 12 mm to 19 mm. In addition, water extract of the male flower of Papaya exhibited only for the fungus, *Candida albicans and Malassezia furfur*, and there was no zone recorded antimicrobial activity against *Escherichia coli, Bacillus subtilis, Bacilus pumilus, Pseudomonas fluorescens, Agrobacterium tumefaciens and Staphylococcus aureus.*



Escherichia coli *Bacillus subtilis* *Bacillus pumilus* *Candida albicans*

Pseudomonas fluorescens *Agrobacterium tumefaciens* *Staphylococcus aureus* *Malassezia furfur*

Figure (4) The Images of Inhibition Zones of Various Crude Extracts Against Eight Microorganisms

Determination of Antioxidant Activity of Ethanol and Water Extracts from the Male Flower of Papaya (*Carica papaya* L.)

The present study was carried out to investigate the radical scavenging activity of ethanol and water extracts by using DPPH assay according to the spectrophotometric method. In this experiment, six different concentrations (25, 50, 100, 200, 400, 600) µg/mL of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and DPPH solution was applied for control. Ethanol without crude extract was employed as blank solution. Determination of absorbance was carried out at wavelength 517nm using UV visible spectrophotometer. Each experiment was done triplicate.

From these experimental results, it was found that as the concentrations increased, radical scavenging activity of crude extracts (usually expressed in term of % inhibition) increased. From the average values of % inhibition, IC_{50} (50% inhibitory concentration) values were calculated by linear regressive excel program shown in tables (3) and (4), and figures (5) to (9). From the results shown in figure (10), it can be clearly seen that IC_{50} values were found to be 94.84µg/mL for ethanol extract and 90.75µg/mL for water extract. Among these extracts, since the lower the IC_{50} showed the higher the free radical scavenging activity, water extract was found to be more effective than ethanol extract in free radical scavenging activity.

Table (3) Perfect Inhibition of Standard Ascorbic acid by DPPH Scavenging Assay Method

No	Concentration (µg/mL)	Ascorbic acid (Standard)
1	3.125	41.51
2	6.5	87.01
3	12.5	88.91
4	25	89.92
5	50	90.10
6	100	93.55

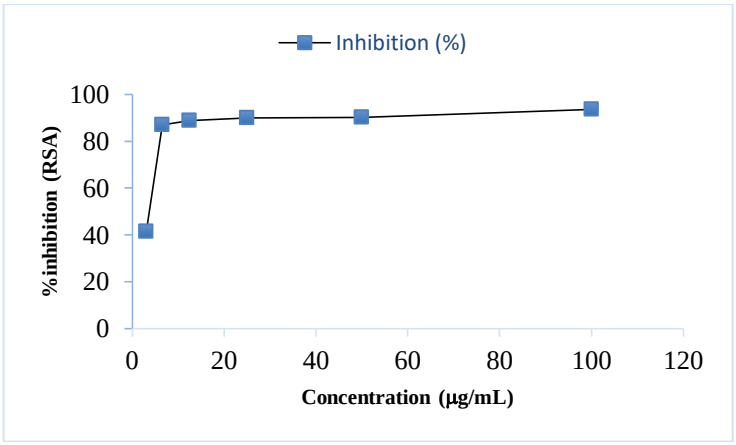


Figure (5) Antioxidant activity of Standard ascorbic acid

Table (4) Perfect Inhibition of Ethanol and Water Extracts from the Male Flower of Papaya by DPPH Scavenging Assay Method

No	Concentration (µg/mL)	Ethanol extract	Water extract
1	25	15.84	29.92
2	50	28.35	38.23
3	100	52.49	52.67
4	200	86.09	79.53
5	400	93.4 4	86.88
6	600	93.88	89.15

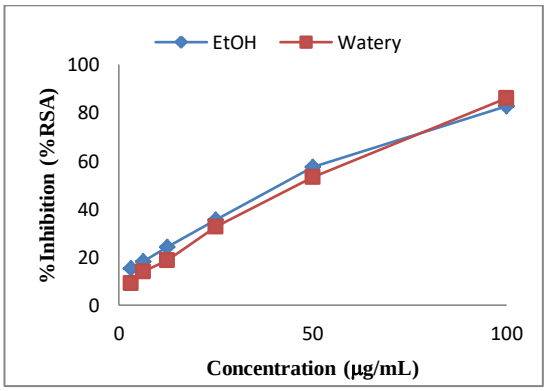


Figure (6) Antioxidant activities of ethanol and water extracts for the male flower of Papaya

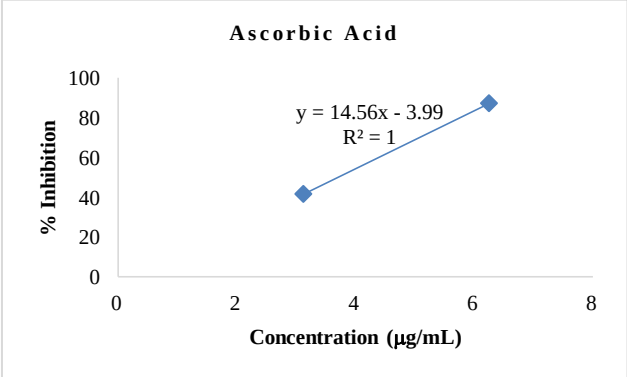


Figure (7) Linear regression equation for antioxidant activity (IC_{50}) of standard ascorbic acid

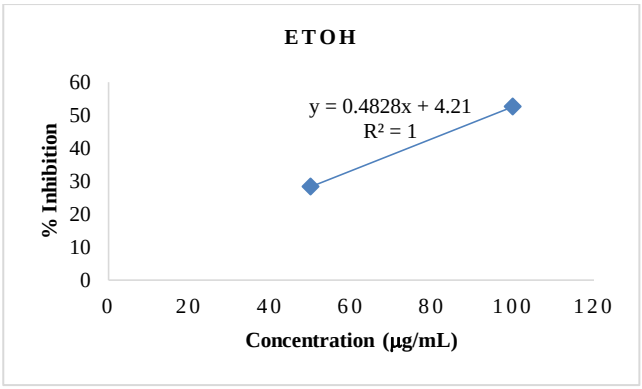


Figure (8) Linear regression equation for antioxidant activity (IC_{50}) of ethanol extract from the male flower of Papaya

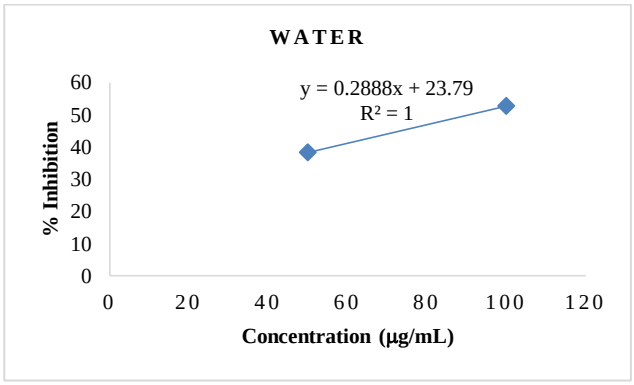


Figure (9) Linear regression equation for antioxidant activity (IC_{50}) of water extract from the male flower of Papaya

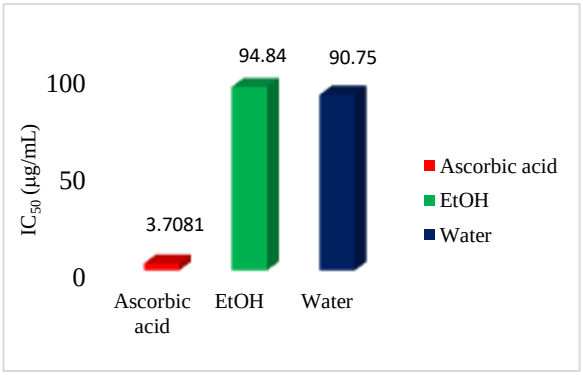


Figure (10) IC_{50} values of ascorbic acid, ethanol and water extracts from the male flower of Papaya

Conclusion

From overall assessment of the present work, concerning with phytochemical investigation, elemental analysis, antimicrobial activity and antioxidant property of the male flower of Papaya (*Carica papaya* L.), the following inferences could be deduced. The preliminary phytochemical screening of the male flower of Papaya revealed the presence of alkaloids, α-amino acids, carbohydrates, glycoside, organic acids, reducing sugars, saponins, starch, phenolic compounds, flavonoids, tannins, steroids and terpenoids. According to the

EDXRF elemental analysis, it can be observed that the relative abundance of potassium was observed to be highest. Phosphorus, calcium, sulphur, iron, manganese, zinc, copper, rubidium and platinum were found as trace elements in this sample. The soluble matter of the sample in pet-ether, ethyl acetate, ethanol and water were found to be 2.8%, 5.2%, 14.0% and 22.8% respectively. Thus, it was found that the amount of polar constituents were higher than that of nonpolar constituents in this sample. From the result of antimicrobial activity by agar well diffusion method, pet-ether extract from the male flower of Papaya has showed that they are not effect against all test microorganisms. Ethyl acetate and ethanol extracts from the male flower of Papaya showed a moderate antimicrobial activity against all test microorganisms. In addition, water extract of Papaya exhibited only for the fungus, *Candida albicans* and *Malassezia furfur*, and there was no zone recorded antibacterial activity. In the screening of antioxidant activity, the order of antioxidant activity was observed as water extract (IC_{50}-90.75µg/ml) › ethanol extract (IC_{50}-94.84µg/mL). From these observations, the radical scavenging activity of water extract from the male flower of papaya was found to be more effective than ethanol extract.

Outcome

The male flower of Papaya may be used to treatment muscle contractions, maintain healthy nerve function, diarrhea, excessive sweating and blood loss due to the presence of potassium high content. Water extract of the male flower of Papaya does not show effective against the bacterial activity although antifungal activity of this male flower showed. Ethyl acetate and ethanol extracts of the male flower of Papaya showed a moderate antimicrobial activity. Water and ethanol extracts of male flower of Papaya are found to possess antioxidant property. The presence of tannins and flavonoids may play the role of antioxidant in the male flower of Papaya. Therefore, male flower Papaya can be used as traditional medicine.

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