Microwave assisted extraction Papaya leaf and investigation on antioxidant activity

ABSTRACT:

Microwave assisted extraction (MAE) has gained lot of attention due to its advantages such as less solvent consumption, short time period, higher extraction efficiency, therefore serves as better alternative for conventional extraction methods of plant materials. Plant phenolic compounds are important constituents responsible for reducing the oxidative stress that induces tissue damage which is the one of the major causative factors assosciated with the chronic disease. Papaya plant is a well known medicinal plant which was recently became popular for the treatment of dengue fever due to its property for elevating white blood cells, platelet count. Considering the current medicinal importance of the papaya plant, the present study was aimed at microwave assisted extraction of phenolic content from papaya leaf using ethanol, water as solvent and investigate their antioxidant potential

In order to compare the extraction efficiency of phenolic compounds, Conventional extraction method i.e, Soxhlet extraction and Microwave assisted extraction methods were used to prepare the extracts. The prepared extracts were subjected to preliminary phytochemical analysis and FTIR. The total phenolic content was determined by using Folin-Ciocalteu method and in-vitro antioxidant activity was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

The alcoholic and aqueous extracts of papaya leaf showed the presence of steroids, alkaloids, saponins, carbohydrates, phenolic compounds by preliminary phytochemical analysis. FTIR spectrum of both aqueous and ethanolic extract showed characteristic peak at 3314.62 cm^{-1}, 1635 cm^{-1} which provide evidence for presence of phenolic compounds. The total phenolic content of the alcoholic and aqueous leaf extracts from MAE was found to be 43.583mg and 80.58 mg/g papaya leaf powder of the Gallic acid equivalent (GAE), respectively. High total phenolic content was observed in aqueous extract of papaya leaf in comparison with ethanolic extract. Papaya leaf contains appreciable quantity of phenolic content and aqueous solvent is best for extraction of phenolic content from papaya leaf. Microwave assisted extracts showed higher phenolic content and high antioxidant activity. Therefore, papaya leaf is a good candidate to be used as a natural antioxidant for the treatment of various diseases.

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Key words: Papaya, Anti-oxidant, Total phenolic content, phytochemical analysis, UV spectroscopy, FTIR, DPPH assay

INTRODUCTION

The is a useful research in the medical field and treatment of disease. The researcher chose papaya leaves in the research. Is there any research report or theory that papaya leaves can inhibit, prevent disease? Add to research conclusion and introduction.

In recent years, global trend is increasing towards the use of natural antioxidant in the area of food science and complementary medicines in comparison with synthetic antioxidants which are toxic to human health [1] Plants acts as rich source of natural antioxidants due to the presence of secondary metabolites mainly poly-phenolic compounds and flavonoids. Phenolic compounds acts as a reducing agents due to their redox potential, thereby acting as antioxidants, thereby have important role in lipid peroxidation [2].
Several extraction techniques such as Microwave extraction (MAE), supercritical fluid extraction, solvent extraction, Soxhlet extraction, refluxation methods are used for extraction of antioxidant constituents such as Polyphenolic compounds from Plant [3-6]. Among these MAE is popularly being used due to its higher efficiency of extraction [7]. Many reports have shown that MAE has more extraction potential than conventional method of extraction [8,9].

Carica papaya,commonly known as papaya belonging to the family Caricaceae,is a small, sparsely branched tree which is usually five to ten m tall with a single stem, spirally arranged leaves at the top of the trunk. . Young leaves of papaya is used for the treatment of jaundice, urinary complains, urinary tract infection, gonorrhoea, dressing wounds, vermifuge in colic, fever, beriberi etc [10]. Papaya leaf extract were also reported to have antimalarial and anti-plasmodial activities [11]. Significant use of leaf juice is found to be the capability to increase platelets and WBC and also repairs the liver [12]

Papaya leaf consist of many active constituents such as papain, chymopapain, cystatin, tocopherol, ascorbic acid,cyanogenic glucoside, flavonoids and glucosinolates [13].Carpaine, dihydrocarpaine, and cyanogenic glycoside are the other active components of alkaloid family. Bitter taste of papaya leaves is due to the presence of pseudocarpaine and dehydrocarpaine [14]. Papaya also contains important flavonoids namely kaempferol, myricetin, quercetin etc [15].

The extract and isolated constituents of plant are known to possess various biological activities such as anti-hyperlipidemic, antidiabetic, anti-inflammatory and also act as free-radical scavengers. Further it has been proven that free radicals play a key role in the development of metabolic disorders and thereby affects the quality of life [16].

Various researchers have reported that phenolic compounds and flavonoids are one of the important secondary metabolites which act as very potent free radical scavengers. Phenolic compounds act as a reducing agent due to their redox potential, thereby acting as antioxidants [17]. Hence phytoconstituents with high amount of phenolic compounds are known to show protective effects in biological system against oxidative stress.

Hence considering the medicinal importance of papaya leaf, in the current study an effort was made to extract the phenolic compound by MAE including investigation of antioxidant potential of the extract.

MATERIALS AND METHODS:

Approximately how many fresh papaya leaves were collected by the researcher and how many papaya trees were collected // how many times they were collected, indicated in the research method.

Collection and preliminary processing of plant material

Fresh green leaves of *Carica papaya* were collected from locality of Surathkal, Mangalore. The plant was identified and authenticated by an expert botanist at St. Aloysius College, Mangalore. The leaves were washed thoroughly with distilled water and then chopped into pieces.

Extraction of plant material

Extracts of the papaya leaves were prepared separately by conventional method (Refluxation) and by microwave assisted extraction (MAE) method.
Aqueous and ethanolic extracts were prepared separately by subjecting 10gms of chopped leaves to refluxation for a period of 8 hours with 200 ml of distilled water and ethanol respectively as the solvents.

Microwave Assisted Extraction (MAE) was done by using 10 grams of the leaves was in a microwave oven (CATA-R) working at a 800W irradiation power and 2450MHz frequency. MAE was done using ethanol and water as solvent at a temperature of 50°C for a period of 5mins [18].
After the extraction, solutions were filtered, filtrate was evaporated and concentrated using rotary flash evaporator to get dry extracts. The extracts obtained by soxhlation and MAE compared for the percentage yield and amount of phenolic content, thereby indicating their antioxidant activity.
After the extraction, solutions were filtered; filtrate was evaporated and concentrated using rotary vacuum evaporator to get dry extracts. The percentage yields of aqueous and ethanolic extracts were calculated.

Preliminary Phytochemical Screening (Qualitative Analysis)

All the papaya leaf extracts of were subjected to various phytochemical tests to determine the presence of various phyto-constituents [19].

Estimation of phenolic content

Phenolic content in papaya leaf extracts were estimated by Folin-Ciocalteau method [20]. Test solution was prepared by taking 100mg of extract was dissolved in 100ml of phosphate buffer (pH 6.8). 10ml of the above solution and diluted up-to 100ml with phosphate buffer. From this 4ml was transferred to 25ml volumetric flask to which 1.25ml of FC reagent and 2.5ml of sodium carbonate was added. The final mixture volume was adjusted with distilled water. For the calibration curve, Gallic acid was used as standard and standard solution of 50µg/ml was prepared in phosphate buffer (pH 6.8). Aliquots of 2, 4, 6, 8, 10 and 12 ml were taken in 6 different 25ml volumetric flasks from the stock solution. Into each, 1.25 ml of Folin-Ciocalteau reagent and 2.5ml of 20% sodium carbonate. The resulting blue colour was evaluated for absorbance at 765 nm on UV-Visible spectrophotometer (Shimadzu 1700) after keeping in dark at room temperature for 30min. Using the linear equation obtained from the standard plot, the phenolic content was estimated and depicted as gallic acid equivalent per gram of the plant material.

Fourier transfer Infrared spectroscopy

Fourier Transform Infrared spectroscopy can be considered as a powerful tool for identifying functional groups present in compounds. Extract from MAE was encapsulated in KBr pellet of FT-IR to prepare translucent sample discs. The spectrum of these samples was recorded using Bruker FTIR spectrophotometer.

Determination of antioxidant activity by DPPH assay

The radical scavenging activity was determined by the use of DPPH free radical [21]. Ascorbic acid was used as a standard by dissolving 10mg in 10ml of methanol as diluent. Serial dilutions were prepared using 10 µl, 20 µl, 30 µl, 40 µl and 50 µl of this standard and the volume made up-to 50 µl using methanol. To these 100 µl of DPPH was added and the absorbance was noted at a wavelength of 517nm after 30 minutes of incubation against blank taken as 50 µl of the diluent (methanol). The test solutions were also prepared in a similar manner using 10mg of the 4 plant extracts and dissolving in 10ml of methanol. 100 µl of DPPH reageant and 50 µl of methanol were used as the control. Using the following equation, the percentage inhibition activity was calculated:

$$\% \text{ Inhibition} = [(Ao\text{-}A1)/Ao] \times 100 \quad \text{.....(i)}$$

Where *Ao* stands for the absorbance of the control, and *A1* denotes the absorbance of the extract/ standard. All the readings were performed in triplicates.

RESULTS AND DISCUSSION

Extraction of plant material and Phytochemical Analysis

Aqueous and ethanolic extracts of papaya leaf were prepared by refluxation method. The yield of ethanolic and aqueous extract was found to be 4.05%and 6.6% respectively. The yield of the leaf extract of from MAE was found to be 26.67% and 46.45% using ethanol and water as solvents respectively.

Preliminary phytochemical analysis of papaya leaf extract confirmed the presence of steroids, alkaloids, saponins, carbohydrates, phenolic compounds

Estimation Total phenolic content by Folin-Ciocalteau method

Amount of polyphenolic content in papaya leaf extracts were estimated by applying the Folin-Ciocalteu method where gallic acid is opted as the standard. Absorbance of different concentration of gallic acid solutions was measured at 765 nm for preparation of standard plot and results of which is shown in **Fig.1.**

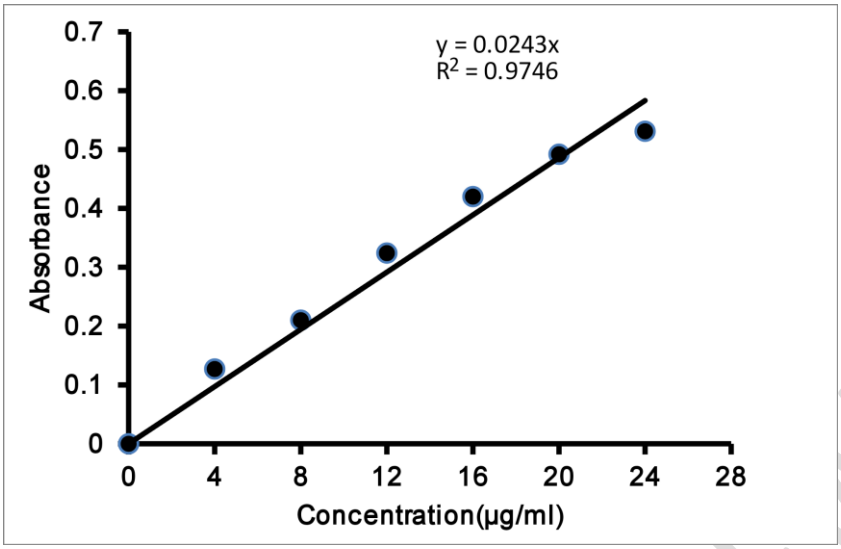

Fig. 1: Standard plot of gallic acid in phosphate buffer pH 6.8 at 765nm

From the calibration equation, Refluxation method resulted in extraction of 39.583mg and 65.58 mg/g Gallic acid equivalent (GAE)of polyphenolic content from papaya leaf extracts by using ethanolic and aqueous solvents respectively.

Ethanolic and Aqueous extracts obtained from MAE produced 43.583mg and 80.58 mg/g Gallic acid equivalent (GAE)of polyphenolic content respectively. Hence MAE using aqueous solvent was found to be superior for getting high extraction efficiency of phenolic content than refluxation method.

Fourier transfer infrared spectroscopy: The FTIR analysis of extracts obtained from MAE was done to determine the important functional groups present. FTIR spectrum of Parijata and Tamarind leaf extracts are shown in **Fig 2&3**

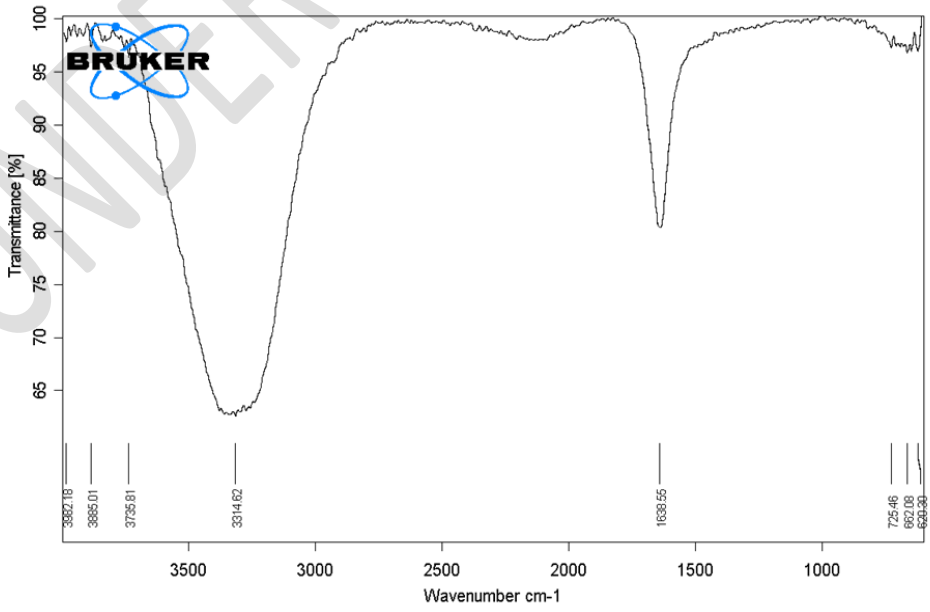

Fig.2: FTIR spectrum of Papaya leaf ethanolic extract obtained by MAE

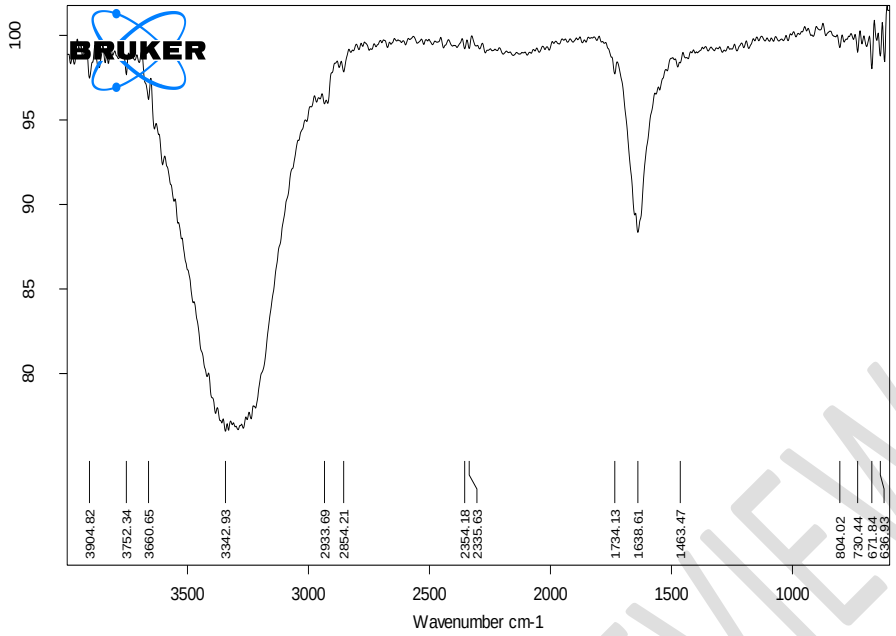

Fig.3: FTIR spectrum of Papaya leaf aqueous extract obtained by MAE

The FTIR spectrum of both aqueous and ethanolic leaf extract shows characteristic band at 3342cm-^{1} corresponds stretching vibration of –OH and-H bonded alcoholic and phenolic groups. Band at 1638 mainly due to aromatic C=C and C=O vibrations [22].

DPPH radical scavenging activity:
Free radical scavenging activity using the DPPH method of different concentration of papaya leaf extracts are shown in **Table 1**. Result showed that as the concentration of the extracts was increased, DPPH radical scavenging activity also increased. At highest concentration (100µg/µl), papaya aqueous extracts obtained from MAE showed higher percentage of DPPH scavenging activity i.e.75.08%. Extracts. The extracts from MAE showed higher polyphenolic content and this can be correlated with their antioxidant activity.

In the results of the research in Table 1, highlight which one was extracted and which had the best results.

Table 1: Antioxidant activity of Papaya leaf extracts by DPPH assay

Samples	**Concentration µg/µl**	**% DPPH Scavenging activity**
Aqueous extracts from MAE	20	32.30
	40	40.90
	60	67.47
	80	73.19
	100	75.08
Ethanolic Extracts from MAE	20	9.20

	40	23.78
	60	37.24
	80	49.11
	100	68.53
Aqueous Extracts from Refluxation	20	12.11
	40	32.29
	60	38.60
	80	49.46
	100	65.34
Ethanolic Extracts from Refluxation	20	8.207
	40	21.60
	60	35.67
	80	41.78
	100	56.78

CONCLUSIONS

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Papaya leaves contain appreciable quantity of phenolic content, and act as potent free radical scavenger, hence it can be used as a source of natural antioxidants which will have higher potential in the treatment of various diseases arising due to involvement of free radical and hence could lead to a new field of future research. In comparison to refluxation method, MAE showed higher extraction efficiency for phenolic compound and hence showing higher antioxidant potency. It has been proved that polarity of the solvent, nature of the extracted compounds and extraction process highly affects therapeutic activities of the plant extracts. The antioxidant behaviour of the plant mainly depends on the amount of phenolic content and it enhances the total antioxidant capacity of medicinal plants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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