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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIOXIDANT ACTIVITIES OF PAPAYA *CARICA PAPAYA* L.

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ABSTRACT

This study aimed to determine the antioxidant activity as well as the phytochemical constituent of *Carica papaya* leaves. Extraction was performed by successive maceration methods using soxhlet solvent. Antioxidant activity was evaluated by DPPH radical scavenging assay. Based on the phytochemical analysis, it showed that extract of C*arica papaya* contains alkaloids, phenolic, flavanoids, tannin and its derivative anthraquinone. The result showed that papaya extract plant samples displayed, these phytochemicals has the tendency to inhibit the infections microorganism the minimum inhibitory concentration. The inference depicts that the papaya leaf phytoextracts do not have the bacterial effect else the bacteria S*taphylococcus aureus* more resistant to the phytoconstituents. The phenolic content achieved also a satisfactory result which also aid in antioxidant benefit.

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INTRODUCTION

Plants are naturally gifted for their ability to synthesize various medicinal and bioactive compounds. *Carica papaya* is a plant species belonging to Caricaceae family that is distributed in tropical areas. Papaya originated in Central America. Papaya plant is small evergreen, short-lived picturesque, straight growing. Papaya as a folk medicines from the time immemorial. The whole plant including leaves, barks, roots, fruits and their juices used as a traditional medicine (Parle Milind *et al*., 2011). Photochemical as plant derived chemicals, which are beneficial to human health and disease prevention (Anderson, G.D. *et al*., 2004). Antioxidant compounds in food play an important role as a health protecting factor. Accordingly, natural antioxidant products obtained from medicinal herbs, such as phenolic compounds, vitamins and terpenoids have been widely applied in food and medicinal fields (Cai *et al*., 2004 Leandro *et al*., 2012 Sadiq, 2014. As a papaya is generally available the application of the leaf extract was aimed to study the phytochemical analysis in antioxidant activities of *Carica papaya* L. was investigated.

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MATERIALS AND METHODS

Collection of plant materials: Fresh papaya green leaves were obtained from home garden in Ethiraj province, Villupuram,Villupuram district, Tamil Nadu and was dried by applying the leaves over shade for about a week .The dried leaves were grinded using a blender to a fine powder. The total weight of the leaf powder was measured as 408.4g

Samples preparation and extraction: Five hundred grams of papaya samples were cleaned and washed with tap water. They were chopped into small pieces and homogenized using a blender for 2 minutes. The homogenized samples were kept in the freezer maintained at 800°C for three days. Later, all the samples were grounded into fine powder using a dry grinder and stored in a freezer at 200°C before extraction. Ten grams of samples were homogenized in 250 ml 80% (v/v) ethanol and chloroform at room temperature. The mixture was shaken using shaking incubator at 200 rpm for 120 min at 500°C. The mixture was then centrifuged at 3000 rpm for 15 min at room temperature and the supernatant was taken. This supernatant was stored at 200°C until further analysis.

Phytochemical analysis: This was done on the extracts particularly to ascertain the presence of different bioactive compounds present in leaf as compared to the seed.

The presences of alkaloids, saponins, tannins, flavonoids, phenolic and anthraquinones were determined.

Test Solution

Determination of antioxidant activity (Brand-Williams *et al*., 1995 and Ayoola *et al*., 2006): Antioxidant compounds in food play an important role as a health protecting factor. 0.1M DPPH v/v in methanol the molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; 1) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. Prepare 0.1 M DPPH in methanol and allow it to completely soluble .Wrap the vial using foil as it is light sensitive. Transfer 1 ml of 0.1M DPPH alone in a tube and 1 ml of respective sample's in a separate tube. Take a fresh tube and add 1ml of respective sample and I ml of 0.1M DPPH. Mix well. Incubate the mixture in dark for 30 minute at room temperature. Measure the absorbance at 517nm using 0.1 M DPPH as standard and methanol as blank. Repeat the procedure for different dilution or samples.

Representation of DPPH protocol: The radical scavenging activity was calculated using the following formula:

% inhibition = {[Ab-Aa]/Ab} x 100

Where,Ab is the absorption of the blank sample
Aa is the absorption of the extract

Determination of hydrogen peroxide scavenging activity (Ruch *et al.,* 1989): Oxygen, an element indispensable for life, can under certain circumstances, adversely affect the human body. It is produced by plants during photosynthesis, and is necessary for aerobic respiration in animals. The oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). They are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. ROS include free radicals such as superoxide anion radicals (O2-), hydroxyl radicals (OH·) and non free-radical species such as hydrogen peroxide (H_2O2) and singlet oxygen (1O2)1–3. ROS are continuously produced during normal phtyisologic events and can easily initiate the peroxidation of membrane lipids,leading to the accumulationof lipid peroxides.

Because of their toxicity, the development and isolation of natural antioxidants from plant species especially from edible plants –polyphenols, flavanoids ,alkaloids etc., (Farombi *et al*., 2008). The ability of the Plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (2.5mg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of Carica papaya leaf extracts and standard compounds were calculated

FORMULA

% Scavenged [H_2O2] = [(AC – AS)/AC] x 100

Where, AC is the absorbance of the control and AS is the absorbance in the presence of the sample of *C. papaya* leaf extracts or standards.

Determination of total phenolic content singleton and rossi 1965 method: Phenolic compounds are a large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants. These phenolics are the most abundant secondary metabolites in plants and can be classified into non soluble such as tannins, lignin, cinnamic acid, phenolic acids, flavanoids and quinines. These secondary metabolites play a avital role in plant fertility and reproduction and in various defence reactions to protect abiotic and biotic stress (Singlet *et al*., 1999; Weishhaar and Jenkins, 1998; Winkel –Shirley, 2001; Forkmann and Martens, 2001).

The total Phenolic content (TPC) was determined by Folin-Ciocalteu assay using tannic acid as standard. One hundred microliter of extract concentration (using extract solvent) containing 0.2mg extract was dispensed into a test tube, 100µl of distilled water and 2.5ml of Folin-Ciocalteau reagent was added respectively and shaken thoroughly, after 3 minutes, 2.0 ml of 7.5% sodium carbonate solution was added and the mixture was incubated at 45°C in a water bath for 40 minutes. Absorbance was measured at 760nm against a blank. The same procedure was repeated to all standard tannic acid solution. The blank is a mixture of 0.2ml of distilled water, 2.5ml of folin-ciocalteau reagent and 2.0ml of 75% sodium carbonate. The total phenolic content was expressed as tannic acid equivalent (mg of TAE/g sample) through the calibration curve of tannic acid.

FORMULA

= Sample OD\STD OD*100

RESULTS AND DISCUSSION

In the present investigation has been carried out to analysis the phytochemical, antioxidant and antibacterial activities of *Carica papaya* L. against *Staphylococcus aureus.* Papaya (*Carica papaya* L.) plants were collected from home garden in Ethiraj province, shown in (figure 1). The leaves were collected and dried. The dried leaves were than powdered (figure 2). Quantity of phytochemicals obtained after soxhlet extraction shown in Table 1. Qunatity taken for ethanol extract is 5 gram and the total quantity of phytochemicals is 0.97g. While chloroform extract is 5gram and the total quantity of phytochemicals is 0.52g.The phytochemcial leaf extracts (ethanol and chloroform) shown in figure3.

Phytochemical analysis: *Carica papaya* L. commonly were selected for testing of their chemical compounds by phytochemical screening shown in figure 4. The results of the phytochemical analysis of the leaf extracts of *Carica papaya* L. shown in Table 2. The result revealed chloroform extract showed positive results with 1% ferric chloride of polyphenols, flavanoids and glycosides. And also sowed negative results for saponin, tannin, alkaloid and anthraquinone while the ethanol extracts contained showed negative results with 1% ferric chloride of polyphenolic compound, and also showed positive results for flavanoids, glycosides, saponin, tannin, alkaloid and anthraquinone.

Figure 1. Papaya plant and its leaf

Figure 2. Papaya leaf powder

Figure 3: Phytochemical Extracts

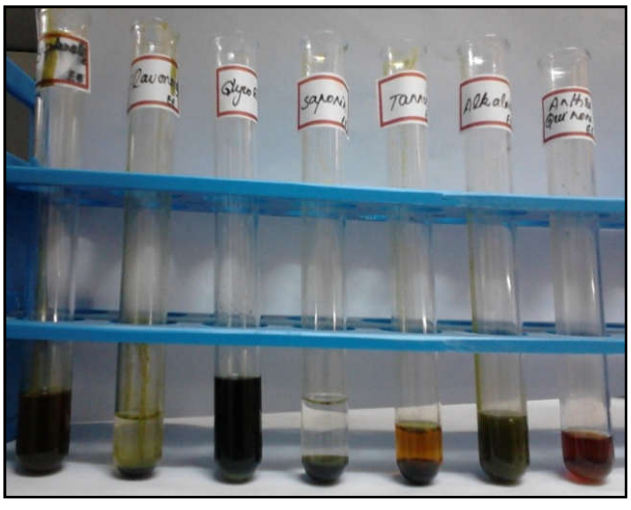

Figure 4: Test's for Phytoconstituents

Phytochemical activity over *Staphylococcus aureus:* The diluted extracts along with the bacteria on nutrient agar plate shows that there is growth in both the plates which indicates the phytochemicals do not have antibacterial property i.e the bacterium resist over the phytoconstitutents obtained from papaya leaf (figure 5).

Antioxidant activity: DDPH (1,1 diphenyl-2- picrylhydrazyl) The results of antioxidant activity by DPPH method shown in Table3. Graph. No.1 ethanol extract of *Carica papaya L.* showed the free radical scavenging activity (0.59mg/g). It was followed by chloroform extract of *Carica papaya* L. indicates the highest amount of free radical

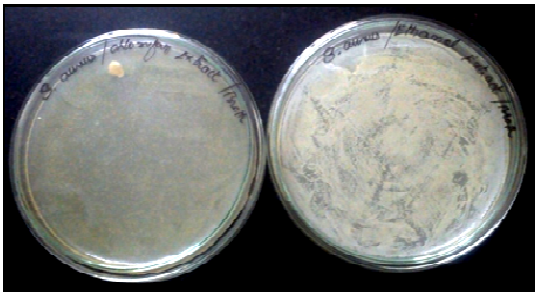

Figure 5. Diluted Extract and S. aureus on Nutrient agar plate

Table 1. Quantity of phytochemicals obtained after Soxhlet extraction

Extract	Quantity taken for extraction	Total quantity of phytochemicals
Ethanol	5 g	0.97 g
Chloroform	5 g	0.52 g

Table 2. Phytochemical analysis of leaf extract

Test	Chloroform Extract	Ethanol Extract
Polyphenols	+	-
Flavanoids	+	-
Glycosides	+	+
Saponin	-	-
Tannin	-	+
Alkaloid	-	+
Anthraquinone	-	+

Table 3. Antioxidant activity using DPPH

S.No	Extract	Interpertation
1	Ethanol	0.59±mg/g 19.6
2	Chloroform	0.72± mg/g 24

scavenging activity (0.72mg/g).The mean values of *Carica papaya* was mg/g. The lower values indicated the strongest ability of the extracts to act as DPPH scavengers. All the sample extracts exhibited significant does dependent inhibition of DPPH activity that rapidly increase. However, the difference in extraction methods to be taken into account in making the generalization. Scavenging effect increases as the concentration of the sample increased until reached. The dose of young leaves extracts that required in reducing the absorbance of DPPH.

Total phenolic content: Total phenolic content was determined by the Folin-ciocalteau method shown in Table 4. Graph no 2. The extract of *Carica papaya* showed the highest amount of phenolic compounds. The ethanol and chloroform extracts showed a high quantity of phenolic compounds (0.95 mg/1g and 1.21mg/1g). This study showed that the papaya leaf varied significated. The total phenolic contents was observed and the results also indicates that the leaves contained high phenolic content that may provide good source of dietary antioxidant.

Determination of hydrogen peroxide scavenging activity: The ethanolic extract of *Carica papaya L.* leaf extracts showed a significant dose –dependent hydroxyl radical scavenging activity and it reached up to (70.38%) at the concentration of ethanolic extract of *Carica papaya L.* leaf by 2.5mg/ml. However, vitamin C which was used as a positive control showed better radical scavenging effect (90% at the concentration of 2.5mg/ml). The chloroform extracts of *Carica papaya* leaf extract showed a significant dose-dependent hydroxyl radical scavenging activity and its reached upto (67.61%) at the concentration of chloroform extract of *Carica papaya* leaf by 2.5mg/ml. However, vitamin which was used as a positive control showed better radical scavenging effect (90% at the concentration of 2.5mg/ml) shown in Table 5. Graph no.3.

Statistical analysis: All data were presented as means ± standard deviations. A significant difference was considered at the level of p=0.05.

ANTIOXIDANT ASSAY

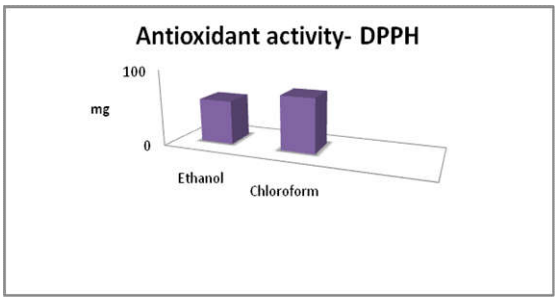

Chart 1

Table 4: Total quantity of phenolic substances

S.No	Extract	Interpertation
1	Ethanol	0.95±mg/1g 31.6
2	Chloroform	1.21± mg/1g 40.3

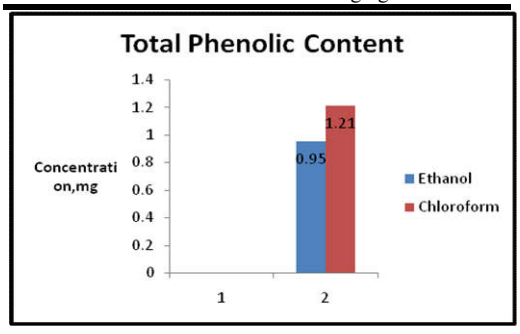

Chart 2.

Table 5. Percentage of Scavenging activity

S.No	Extract	Interpertation
1	Ethanol	70.38 % 234
2	Chloroform	67.61 % 225

Determination of hydrogen peroxide scavenging activity

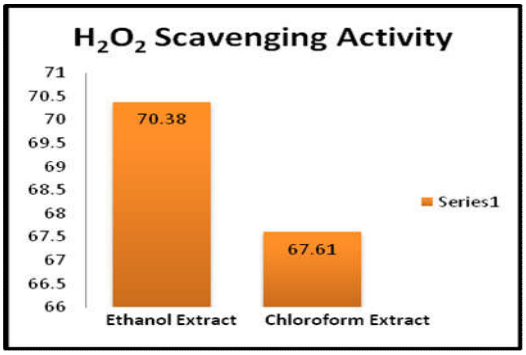

Chart 3.

Table 6. Representation of Scavenging activity

Description	Volume of sample (ml)	Volume of 40mM Hydrogen peroxide (ml)
Phosphate Buffer	0	1
Ethanol extract	1	0
Chloroform extract	1	1
Incubation	At room temperature for 10 minute	
Absorbance	230 nm	

Table 7. Representation of Phenolic content

Description	Volume of sample (ml)	Volume of Distilled water (ml)	Folin Ciocalteau reagent (ml)	7.5% Sodium carbonate (ml)
Ethanol extract	0.1	0.1	2.5	2
Chloroform extract	0.1	0.1	2.5	2
Incubation	At 45 º C for 40 minute in a water bath			
Absorbance	760 nm			

Conclusion

The study with different experiment approach concludes that the phytochemical extracted with different solvent ie. Ethanol and chloroform do not have the antibacterial activity as mentioned in references. Moreover the bacterium applied in the experimental procedure was multiple drug resistant bacteria which naturally make the bacteria to be viable in the presence of phytochemicals. The difference in the type of phytochemicals obtained through qualitative test revealed the presence of bioactive. The phenolic content achieved also a satisfactory result which also aid in antioxidant benefit.

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