Current Trends in **Pharmaceutical Research** 2017, 4(1):16-25
ISSN 2310-4820
 www.dibru.ac.in/ctpr

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *CARICA PAPAYA* (AMITA) LEAF EXTRACTS

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Abstract

The screening of antioxidant and antimicrobial activity of plant extracts indicates plant's potential as a therapeutic agent, which could play a very important role in drug development and as a health supplement. This study was designed to explore the antioxidant and antimicrobial property of Carica papaya (Amita) leaf extracts. The extraction was performed by the process of cold maceration using solvent methanol, water and chloroform. The antioxidant activity was evaluated by using DPPH and H_2O_2 radical scavenging method. The plant extracts were tested for antibacterial activity against microorganisms like Staphylococcus aureus, Escharichia coli, Bacillus subtilis and Enterobacter aerogenes. The phytochemical tests revealed the presence of alkaloids, glycoside, saponin, resins, flavonoids, phenols and tannins. The chloroform leaf extracts of C. Papaya exhibited maximum antibacterial activity against Enterobacter aerogenes. The water and methanolic extracts were found to be less active than the chloroform extracts against E. Aerogenes and E. Coli.. In DPPH radical scavenging method the scavenging of C. papaya extract reached to 96% in methanol, 96% in water and 98% in chloroform and the standard gallic acid was found to be 83%. The percentage of H_2O_2 scavenging activity of C. papaya extracts reached to 82% in methanol, 95% in water and 66% in chloroform and the standard gallic acid was found to be 95%. The extract showed good antimicrobial activity against microbes with highly moderate antioxidant activity which signifies that papaya leaf is a good source of health promoting constituents that can be used for therapeutic purposes.

Keywords: *Carica papaya,* Amita, antioxidant, antimicrobial, phytochemical.

Introduction

An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates, and inhibit other oxidation reactions by reducing stress responsible for many degenerative disorders. Antibacterial is an agent that interferes with the growth and reproduction of bacteria. All antibiotic drugs have antibacterial properties. Phytochemicals and their derived products have been an extraordinary source of compounds with therapeutic and drug development potential (De D et al., 2012).

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Carica papaya is a single stemmed herbaceous perennial tree with large leaves (20-60 cm long) belongs to the family Caricaceae and popular as Amita in Assam. The entire India is a residence of this tree including Assam. It is also found in Holland, France, Australia, Brazil and UK with different names like tree melon, papaya, Paw paw, Mamao etc. Papaya offers not only the luscious taste but is also a rich source of antioxidant nutrients, vitamin C and flavanoids, the vitamin B complex, folate and pentothanic acid, the minerals like potassium and magnesium and fibre. These nutrients promote the cardiovascular system functions and also provide protection against colon cancer. On the contrary, it is reported that *C. papaya* leaf is having therapeutic effect on dengue and malaria (Bhande V et. al. 2014) and anti-inflammatory actions (Owoyele et. al., 2008). The fruit is valued for its proteolytic enzymes including *papain*, which is used as *bromelain* to treat sports injuries, causes of trauma and allergies. Biochemically, its leaves and fruits are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications. *Carapain*, an alkaloid present in papaya, can be used as a heart depressant, amoebicides and diuretics. The leaves have antibacterial and antifungal activities which is useful in skin ulcer. The purpose of the study was to study the phytochemical and antimicrobial properties of *Carrica papaya* L. leaves followed by the evaluation of *in-vitro* antioxidant activity of the water, methanol and chloroform extracts.

Materials and Methods

Plant materials

The leaves were collected from Nagaon, District- Nagaon, Assam in the month of January 2016. The plant was identified by Dept. of Botany, ADP College, Nagaon, Assam. The leafs were washed and cleaned, preserved in small polythene bags with 70% alcohol which was poured into these bags to kept the leaves fresh and then dried in shade condition.

Preparation of extract

The extraction was performed by the process of cold maceration using methanol, water and chloroform as solvents. The marc of the extracts were collected by filtering through Whatman No.1 filter paper, dried on water bath, covered, abed and subjected towards the screening of various phytochemicals and tests.

Phytochemical screening

Phytochemical evaluation has been carried out for all the extracts according to standard protocols [Shah C.S and Quardry J.S (1996) and Kokate C.K (1994)].

In-vitro Antioxidant assay

DPPH scavenging method

The antioxidant activity of plant extract and standard antioxidant were measured *in-vitro* in terms of hydrogen donating or free radical scavenging ability by using the 1,1 diphenyl-2-picryl hydrazyl (DPPH). DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract (Blois, 1957). The purple DPPH becomes yellow diphenyl picryl hydrazine while reduced. Extract solution was prepared by dissolving 1mg of dry plant extract in 1ml methanol. An aliquot of 2.5ml of 75 µM DPPH solution in methanol and 0.5ml of plant extract in methanol at various concentrations were mixed and incubated at room temperature for 90 minutes and absorbance of the test mixture was read at 517 nm using a UV-VIS spectrophotometer (Systronic 119) against a DPPH control containing 1ml of methanol measurements. The experiment was done in triplicate. Gallic acid was used as standard.

Percentage scavenging activity was calculated as follows:

$$\text{Scavenging } \% = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Scavenging of hydrogen peroxide

H_2O_2 itself is not very reactive, it is a weak oxidizing agent, but sometimes it can be toxic to cell because it may give rise to hydroxyl radical in the cells. It can cross cell membrane rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer solution (pH- 7.4) (0.6 ml, 40mM). Absorbance of hydrogen peroxide was determined 10 minutes later at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging of both plant extracts and standard compounds were calculated as:

$$\text{Scavenging } \% = \frac{\text{AC} - \text{AS}}{\text{AC}} \times 100$$

Where,

AC = the absorbance of control

AS = the absorbance in the presence of the sample`s extracts and standards

Antibacterial Activity Study

Collection of bacterial species

Tested bacterial species were procured from MTCC, Chandigarh, Punjab, India with standard code.

Sl. No.	MTCC Code	Name of bacteria
1	96	*Staphylococcus aureus*
2	432	*Enterobacter aerogenes*
3	441	*Bacillus subtilis*
4	739	*Escherichia coli*

Preparation of culture media

A. 200ml nutrient agar media

For the preparation of 200 ml, nutrient agar media the chemical compositions was taken in grams as follows:

Beef extract = 1 gm
Agar = 3 gm
Peptone = 0.6 gm
Distilled water = q.s.

B. 200ml nutrient broth media

The chemical composition of broth media was as follows:

Beef extract = 1 gm
Peptone = 0.6 gm
Distilled water = q.s.

All the above mentioned chemical ingredients were taken separately into two 250 ml conical flask and mixed with distilled water to volume make up to 200 ml. After dissolving the composition top of the conical flask was closed by an airtight cotton cork. The air tight conical flask was then transferred in plastic wares and sterilized by autoclave at temperature of 121ºC at 15psi for 15-40 minutes. After 30 minutes the culture media was taken out from the autoclave and then stored in aseptic room for tests. 20mg tuber extracts of different solvent (methanol, chloroform and water) taken in 1ml eppendrof and were dissolved in 1ml of DMSO to give a concentration of 0.02mg/ml and mixed by vortex at constant rotation for 1-2 hours. The agar well diffusion assay was performed under laminar air flow which was pre sterilized by passing UV light for 30 minutes before starting the experiment. The assay method was carried out by preparation of three wells of 5mm diameter for each extract using a sterile cork borer in agar plate aseptically. The agar cylinders were removed using a sterile loop. The wells were grouped as the test well and controlled well was filled with 50, 80 and 100µl from the stock solution of the test material and the control well was filled with the same

amount of solvent i.e. DMSO. One standard antibacterial drug, chlorophenical was used as the positive control. The wells were kept for 15 minutes for drying by passing the blower in LAF. The plates were then taken out from the LAF aseptically when all the agar plates become completely dry and labelled and incubated at 37ºC overnight. The inhibition zones were recorded in the test as well as the control well. The assay was repeated twice.

Results and Discussion

The plant *Carica papaya* leaves has been investigated in a systematic way covering initial pharmacognostical, phytochemical study to rationalize its use as a drug.

Phytoconstituents

Successful evaluation of botanical phytocompounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Hence, extracts were screened to find out the presence of various phytoconstituents in *Carica papaya* leaves. The results of qualitative screening of phytochemical components in leaves of *Carica papaya* revealed the presence of alkaloids, glycoside, saponin, resins, flavonoids, phenols and tannins presented in Table 1.

Table 1: Phytochemical screening of *Carica papaya* leaf extracts:

Constituents	**Tests**	**Extracts**		
		water	**methanol**	**chloroform**
Alkaloids	Dragendorff`s Test	+	+	-
	Mayer`s Test	+	+	-
Carbohydrates	Benedict`s Test	-	-	-
	Fehling`s Test	-	-	-
Flavonoids	Lead Acetate Test	+	+	-
Phenol	Ferric Chloride Test	+	-	-
Glycoside	Legal Test	+	+	-
	Killer- Killani Test	+	+	-
Protein	Xanthoproteic Test	-	-	-
	Warming Test	-	-	-
Tannin	Gelatin Test	+	+	-
	Ferric Chloride Test	+	+	-
	Lead Acetate Test	+	+	-
Steroids	Salkowski`s Test	+	-	-
Saponin	Foam Test	+	-	-
	Froth Test	+	-	-
Resin	Acetone- Water Test	+	+	-
Fixed Oil & Fat Test	Stain Test	-	-	-

Where, (-) sign indicates absence of the constituent
(+) sign indicates presence of the constituent

Antioxidant study

In DPPH free radical scavenging method, the scavenging activity of *Carica papaya* leaf extract at concentration of 1mg/ml reached to 96%, 96% and 98% in methanol, water and chloroform extracts respectively. On the other hand the Gallic acid at same concentration had 83% of scavenging activity. The data presented in Table 2 and figure 1 depicts that the extracts possess the scavenging character in accordance with the standards and showed their activities at different concentration. The study reveals that the extract of *Carica papaya* exhibits the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Table 2: Antioxidant study with DPPH Radical Scavenging Activity:

Concentration (µg/ml)	**Extracts**			**Standard (Gallic acid)**
	Methanol	**Water**	**Chloroform**	
10	62.33±2.5	52.66±2.5	75±2.6	51.33±1.5
50	83.33±3.0	74±3	82.66±3.7	55.66±1.5
100	83.67±1.5	82.66±3.05	87±2	58.33±2.08
150	84±3	85±2	92.33±1.5	66.33±2.5
200	92.66±2.0	87±1.5	93.66±1.5	77.33±2.08
250	93.66±2.5	87.33±2	97.66±1	78.33±1.5
300	96.66±1.5	96.33±0.57	98±1.5	83.66±1.5

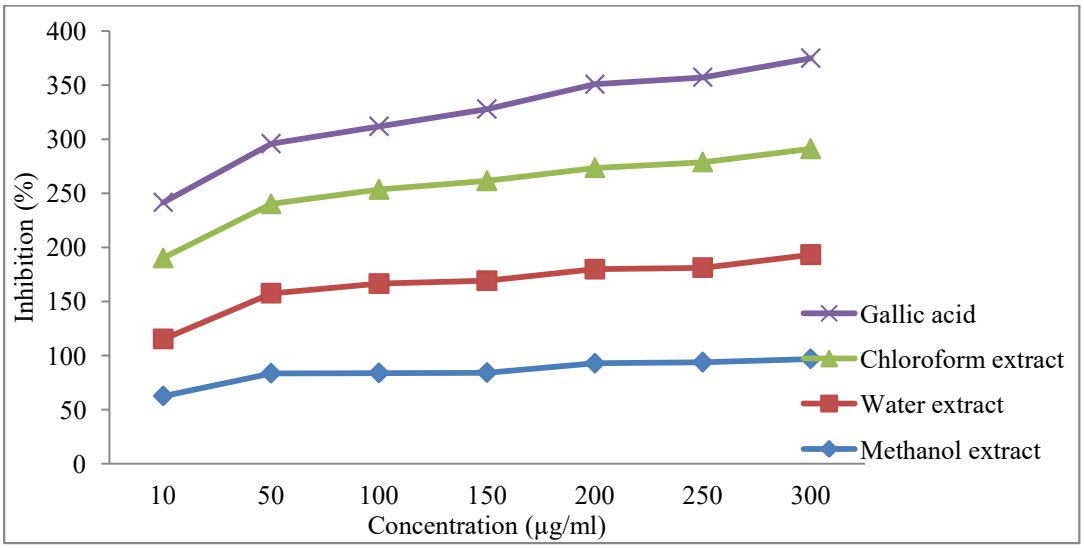

Figure 1: Antioxidant study with DPPH Radical Scavenging Activity

Hydrogen peroxide scavenging activity of the *Carica papaya* extract on hydroxyl radical is shown in Table 3 and figure 2. H_2O_2 is highly important because of its ability

to penetrate biological membranes. The correlation between total flavonoids content and hydrogen peroxide in this study was found positive (r = 0.932). The percentage of H_2O_2 scavenging activity of *Carica papaya* extracts reached to 82%, 95% and 66% in methanol, water and chloroform respectively. And the standard used i.e. Gallic acid was found to be 95%.m It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. *C. papaya* extract also showed hydrogen peroxide decomposition activity in a concentration dependent manner. The decomposition of H_2O_2 by methanol, water and chloroform extracts of *C. Papaya* and free radical scavenging activity resembles with the report described by Srikanth et al., 2010.

Table 3: Antioxidant activity with H_2O_2 Free Radical Scavenging Activity

Concentration (µl/ml)	**Extracts**			**Standard (Gallic acid)**
	Methanol	**Water**	**Chloroform**	
10	62.78±0.38	65.04±0.27	61.06±0.37	83.65±0.23
50	70.94±0.65	65.38±0.38	64.5±0.77	85.74±0.13
100	71.1±1.01	71.21±0.57	64.24±0.37	87.94±2.05
150	74.76±0.40	75.83±0.17	65.55±0.57	92.98±0.72
200	76.87±0.17	86.87±0.20	65.56±0.19	94.24±0.27
250	81.35±0.33	87.13±0.91	66.34±0.36	94.51±0.44
300	82.29±1.04	95.19±0.26	66.74±0.25	95.8±0.14

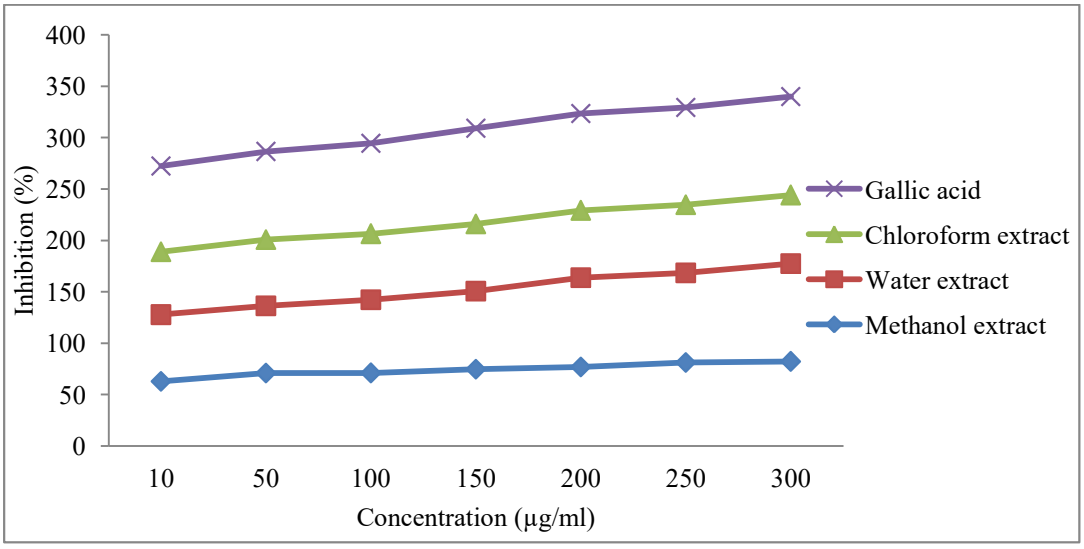

Fig 2: Antioxidant study with H_2O_2 Free Radical Scavenging Activity

Antimicrobial Assay

Methanol, chloroform and water extracts of *Carica papaya* Linn. leaves were tested against various Gram-negative and Gram-positive bacteria (Table.4). In anti microbial assay the zone of inhibition were found to be 9-18mm, 10-19mm and 12-21mm in diameter for the extracts of methanol, water and chloroform respectively with correspond to moderate and maximum activities. Among the extracts assayed, the chloroform leaf extracts of *C. Papaya* exhibited maximum activity against *Enterobacter aerogenes* at 100mcg/ml conc. for example, 21mm was recorded as diameter of zone of inhibition. This was followed by water extract against *Enterobacter aerogenes* with zone of inhibition 19mm in diameter and methanol extract against *B. subtilis* with zone of inhibition 18.33mm in diameter at 100mcg/ml conc. The least activity was recorded for methanol extracts against *B. subtilis* with zone of inhibition 9mm in diameter. Activities of the various extracts were comparable to those of standard antibacterial agent chloremphenical. In the present antimicrobial activity of plant extract towards drug resistant or clinically significant microbes are reported and it was observed that active constituent of plant material seep out in organic solvent to deliver biological activity (Sumathi R et al., 2014). Further phytochemical studies for identification and elucidation of active compound in plant parts tested in expected to serve as lead in the development of novel bioactive antimicrobial components.

Table 4: Antibacterial Activity of *Carica papaya* leaf extracts

Test Micro-Organisms	**Zone of Inhibition (%)**									**Standard drug (Chloramphenical)**		
	Methanol			**Water**			**Chloroform**					
	30µl	50µl	100µl	30µl	50µl	100µl	30µl	50µl	100µl	30µl	50µl	100µl
Staphylococcus aureus	-	9.66	10.33	-	-	10.33	-	-	12.33	29.66	34.66	35.66
Escherichia coli	11.66	13.66	16.33	11	12.33	12.78	13.66	15.66	16.31	31	36.33	38.33
Enterobacter aerogenes	9.6	11	15	11.666	14.33	19	14.31	15	21	30.33	34.33	40.33
Bacillus subtilis	9	12.3	18.33	12.33	15	16.33	10.53	12.33	17	30.33	36.66	37.66

Conclusion

It can be concluded by saying that the plant *Carica papaya* is a very precious gift for human being for the bearing of some important phytoconstituents. The phytochemical tests confirmed that the plant is a store house of glycoside, alkaloids, tannins, flavonoids, saponin, phenol and resin etc. The result suggests that the methanolic and aqueous extracts show the presence of these constituents more distinctly than the chloroform extract. The plant extracts exhibited predominant antibacterial activity against microorganisms. The chloroform leaf extracts of *C. Papaya* exhibited maximum antibacterial activity against *Enterobacter aerogenes.* The water and methanolic extracts were found to be less active than the chloroform extracts against *E. Aerogenes* and *E. coli.* At various concentrations of the extracts the scavenging activity of *C. Papaya* extract reached to 96% in methanol, 96% in water and 98% in chloroform in DPPH

radical scavenging method against the standard Gallic acid found to 83%. The percentage of H_2O_2 scavenging activity of *C. papaya* extracts reached to 82% in methanol, 95% in water and 66% in chloroform and the standard used i.e. Gallic acid was found to 95%. The extract showed good antimicrobial activity against microbes with highly moderate antioxidant activity which signifies that papaya leaf is a good source of health promoting constituents that can be used for therapeutic purpose.

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How to cite this article:

Banik B, Sahu N, Chetia N, Saikia M, Boruah P. Evaluation of antioxidant and antimicrobial activity of *Carica papaya* (Amita) leaf extracts. *Curr Trends Pharm Res*, 2017, 4(1):16-25.