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Original Article

PHYTOCHEMICALS SCREENING, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CARICA PAPAYA LEAF EXTRACTS

PRASHANT PUROHIT*, MAHESH KUMAR KATARIA

Department of Pharmaceutics, Seth G. L. Bihani SD College of Technical Education, Shriganganagar-335001, Rajsthan, India
***Corresponding author: Prashant Purohit; *Email: prashantskip@gmail.com**

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ABSTRACT

Objective: The objective of the present study aimed at investigating the phytochemical antioxidant and anti-microbial properties of *Carica papaya* leaf extracts.

Methods: As phytochemicals are biologically active compounds and a powerful group of plant chemicals believed to stimulate the immune system along with antioxidants, the molecules which hinder oxidation of other molecules by the process of inhibiting or by generating the oxidizing chain reactions and preventing diseases. The anti-microbial activity on various gram-positive and gram-negative bacteria were determined using zone of inhibition and antioxidant by the 2,2,1-diphenyl-1-picrylhydrazyl method.

Results: Phytochemical screening, antioxidant and antibacterial potential were determined using different aqueous and organic solvents in addition to the determination of trace element in leaves of C. papaya. Antioxidant activity using DPPH free radical scavenging assay indicated leaves extracts leaves showed inhibition of per oxidation. The result showed that the ethanolic extract of *C. papaya* possessed a significant antioxidant activity as compared to methanol and aqueous extract. The antibacterial study showed leaves extract is the best to cope infectious action of bacteria.

Conclusion: This study was conducted to test the medicinal profile of *C. papaya* by extracting secondary metabolites with organic and aqueous solvents. Ethanol was found to be the best solvents of choice to extract natural products to get maximum medicinal benefits and could be used to medicinal formulation against different infectious diseases.

Keywords: Carica papaya, Phytochemicals, Extraction, Antioxidant, Antibacterial

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INTRODUCTION

A proper extraction process of herbal plants for isolation of its bioactive compounds is essential in pharmaceutical, food and cosmetic application. Richard (1998) stated that two important points should be considered during the extraction process; 1) the purpose in conducting the extraction, and 2) the nature of the targeted compound that needs to be isolated. Many contributing factors play a part in a successful extraction procedure, such as part of the plant used, types of solvent, and extraction technique. The selection of a suitable solvent is critical as it greatly affects the active compounds that need to be isolated. "Like dissolves like" is the basic principle in which the solvent polarity influences the solubility of active compounds. If the compound of interest belongs to the polar compound; hence, a polar solvent is the most appropriate choice to use and vice versa. Studies have reported that extracting bioactive compounds using a different type of solvent significantly affect phytochemical content and biological activities. Moreover, a good solvent should possess a less toxic effect, can preserve the active compound and enhance the extraction yield and its bioactivities [1, 2].

Carica papaya belongs to the genus *Carica* and the family of Caricaceae. It is the most cultivated plants in tropical and subtropical countries such as India, Indonesia, Australia, Brazil, and China. The young leaves of *C. papaya* exert the most prominent antioxidant activities and contained the highest total phenolic and flavonoid content as compared to other parts of the plant [3, 4].

Various literature studies have reported on the effect of *C. papaya* for its wound-healing activities. The wound healing properties have been attributed to its antioxidant, anti-inflammatory and antimicrobial activities. Wound healing is a complex and dynamic process involving overlapping interactions among cellular structures, tissue layers, and different types of cells. The wound can be interpreted as a disruption of the functional continuity of cells and tissues due to physical, chemical, microbial infection or immunology process. In general, the wound healing process comprises three distinct phases; inflammation, proliferation and remodeling [5, 6].

To date, most of the extract preparations of *C. papaya* for wound healing potential used varieties of solvents from polar to mid-polar. Nevertheless, it is difficult to find a relationship of *in vitro* study based on the effect of different solvent against antioxidant and wound healing activities. Therefore, the present study was aimed to examine the effect of different extraction solvents on the antioxidant and antimicrobial activities of *C. papaya* leaves extracts [7, 8].

MATERIALS AND METHODS

Materials

The fresh leave of Carica papaya collected from the swami Keshwanand Rajasthan Agriculture University, Bikaner, Rajasthan. The sample was identified and authenticated by Department of Botany, Government College Kolayat, Bikaner, Rajasthan.

Preparation of the extracts

The method of extraction was conducted according to Vuong *et al.* (2013) with slight modification. Initially, the leaves were washed with tap water and dried in the oven for two (2) days at 50 °C. Afterward, the dried leaves were ground using blender (Model: CB15V, India) to obtain a fine powder [8, 9]. 7.5 g of *C. papaya* leaves powder was subjected to a reflux extraction system for 20 min (70 °C) using three different solvents; methanol, ethanol, aqueous and hydro alcohol (100 ml). Extracts were filtered and concentrated using a rotary vacuum evaporator at 60 °C. The dried extracts were stored until further use [9, 10].

Phytochemicals screening of the *C. papaya* leaf extracts [11-15]

Alkaloids

Wagner's test–For 1ml of the sample solution, few drops of Wagner's

reagent was added along the sides of the test tube. The appearance of a reddish-brown precipitate indicates the presence of alkaloids.

Carbohydrates

Benedict'stest–for 1 ml of the sample solution, few drops of Benedict's reagent were added and heated for 2 min. The appearance of a colored precipitate indicates the presence of carbohydrates.

Amino acids

Ninhydrin test–for 1 ml of the sample solution, two drops of ninhydrin reagent was added. The formation of purple color indicates the presence of amino acids.

Glycosides

Kellar-Killiani test–for 1 ml of sample solution 1 ml of glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid were added. The appearance of a reddish-brown ring at the junction of liquids indicates the presence of glycosides.

Phenolic compounds and tannins

Ferric chloride test–for 1 ml of extract, few drops of neutral l5% ferric chloride were added. The appearance of a dark green color indicates the presence of phenolic and tannin compounds.

Protein

Biuret test–for 1 ml of extract, one drop of 2% copper sulfate and 1 ml of ethanol and potassium hydroxide were added. The presence of the pink color of the ethanolic layer indicates the presence of protein.

Saponins

For 1 ml of extract, a few drops of distilled water were added and shaken vigorously. The appearance of foam indicates the presence of saponins.

DPPH radical scavenging activity

The free-radical scavenging activity was determined by DPPH proposed by Zadeh *et al.*, 2008, with slight modifications.

A standard solution of ascorbic acid was prepared in the concentration of 2 mg/ml in methanol. The standard calibration curve was obtained using different working standards and was made up of 3 ml with ethanol. DPPH solution (500 ?l) was then added and mixed vigorously. Similar steps were followed for the sample extract. The reaction mixture was incubated for about 45 min in dark conditions and absorbance was measured at 517 nm using a spectrophotometer. All determinations were made in triplicates and the standard curve was obtained using ascorbic acid [16-18].

The % DPPH which was scavenged (% DPPHsc) was calculated using the formula:

$$(\%)\ \text{Inhibition of DPPH scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and

A_1 is the absorbance of the sample extract or standard.

Antimicrobial activity

Antimicrobial activity of the extract was evaluated against bacterial strains such as *Staphylococcus epidermidis* (MTCC-3382), *Salmonella typhi* (MTCC-733) and *Salmonella typhimurium* (MTCC-3224) and fungal strains such as *Candida krusei* (MTCC-9215) and *Aspergillus fumigatus* (MTCC-10561). Agar disc diffusion system was executed to evaluate the antimicrobial activity of plant extract *(Ahlam et al., 2013)*. All concentrations of both plant extract were prepared using dimethyl sulphoxide (DMSO). For the inoculums (10^8 cfu/ml), test bacteria and fungi were grown in sterile Muller–Hinton broth and sabouraud dextrose broth tubes, respectively, overnight. The inoculums were aseptically plated using sterile cotton swabs into petri dishes and filter paper disc was impregnated with different concentrations to obtain 200, 150, 100, 50 µg/disc samples and placed on the prepared agar surface [19]. The petri dishes were pre-incubated at room temperature, allowing the complete diffusion of the samples and incubated at 37 °Cfor 24 h (for bacteria) and 48 h (for fungi). Tetracycline and Nystatin were used as standard antibacterial and antifungal drugs, respectively. The experiments were performed in triplicate. The inhibitory potential of the extract was quantified after incubation by gauging the diameter of the zone of inhibition in mm. Antimicrobial activity was assessed using the parameters according to *Quinto and Santos, 2005*; i.e. inhibition zone<10 mm, inactive; 10-13 mm, partially active; 14-19 mm, active; >19 mm, very active [20-23].

RESULTS AND DISCUSSION

Qualitative analysis of *Carica papaya* leaves extract: phytochemical screening.

Phytochemical screening of *Carica papaya* leaves extracts shown in table 1, in the ethanolic extract, all the bioactive compounds such as alkaloids, glycosides, phenol, tannin, saponin and carbohydrates are present.

The overall result shows that ethanol extracts possess a greater number of bioactive compounds when compared to other solvents.

Table 1: Qualitative analysis of *Carica papaya* leaves extract: phytochemical screening

S. No.	Phytochemicals	Ethanol	Methanol	Water	Water+Methanol
1	Alkaloid	Present	Present	Present	Present
2	Carbohydrate	Present	Present	Absent	Present
3	Amino acid	Present	Present	Absent	Absent
4	Glycoside	Present	Present	Present	Present
5	Phenols	Present	Present	Absent	Absent
6	Protein	Present	Absent	Present	Present
7	Saponin	Present	Present	Absent	Absent

DPPH radical scavenging activity of carica papaya leaves

IC_{50} (Inhibitory Concentration) of the sample extract was determined by comparing it to the standard value of ascorbic acid. The result of the antioxidant activity of *C. papaya* with ethanolic extract by DPPH assay shows that the presence of free radicals is more and directly proportional to the concentration of the sample. It means higher the concentration higher will be the percentage of free radicals in the *C. papaya* leaves ethanolic extract.

The Graph 1 indicates that the percentage of radical scavenging activity of the ethanolic extracts at different concentrations with 50 % of DPPH scavenging activity was found to be 80 µg/ml, as the concentration of the sample extract was evaluated compared to the standard value. The results show that ethanolic solvent extracts analyzed for the DPPH scavenging activity show the higher radical inhibition activity, which can be comparable to standard ascorbic acid.

Antimicrobial activity

The results of the antimicrobial determinations for all the organic extracts of both plant against the five bacterial species *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Salmonella*

typhimurium, Staphylococcus thermophilus are investigated in a disc-diffusion assay represented in table 2 showing the antibacterial property of ethanolic extract that produced zones of inhibition against all five bacterial strains.

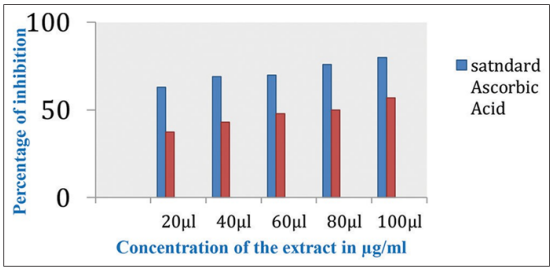

Fig. 1: 2,2,1-Diphenyl-1-picrylhydrazyl scavenging activity of ethanolic extracts of *Carica papaya* leaves. (Values are expressed as mean±SE mg of ascorbic acid per gram of dry weight, that is, extract triplicates of each sample extract were recorded)

Table 2: Zone of inhibition (mm) of the *Carica Papaya* against the test microorganisms

Bacterial strains	Volume in µl of the plant extract (*Carica Papaya*) ethanol				Standard drug (Ciprofloxacin)
	20	40	60	80	5 µg
	Diameter of zone of inhibition (mm)				
Escherichia coli	10	12	14	19	31
Salmonella typhi	10	11	12	14	30
Staphylococcus aureus	14	14	12	14	30
Salmonella typhimurium	12	12	15	19	30
Staphylococcus thermophilus	11	12	14	18	31

The resulting data about the antimicrobial potential of the whole plant extract is shown in table 2. The maximum zone of inhibition exhibited (80µg/disc) was in the order *Escherichia coli, Salmonella typhimurium>Staphylococcus thermophilus>Salmonella typhi>Staphylococcus aureus*.

CONCLUSION

Bioactive compounds can be studied by extraction and isolation, also with defining their structure and by analyzing it in laboratory models as *in vitro* and *in vivo* studies and importance was given for the identification and characterization of the specific phytochemical, which is primarily responsible for biological activity.

The current study which was aimed at investigating the presence of biologically active phytochemicals and antioxidant properties of *C. papaya* leaves extract reveals that samples with various solvents such as methanol, ethanol, water and hydro alcohol have shown the presence of phytochemicals constituents such as alkaloid, carbohydrates, and amino acids.

Among the used solvent extracts, the *C. papaya* leaves with ethanolic fraction showed the presence of more phytochemicals and exhibited strongest antioxidant properties, which can effectively scavenge reactive oxygen species compared to other solvent extracts used.

Hence, *C. papaya* can be considered as an important and potential natural source for various pharmaceutical and medicinal applications. Interestingly, the broad spectrum of phytochemicals, antimicrobial agent and antioxidants presents in them can be regarded as the reservoir of naturally occurring diverse bioactive molecules and papaya as herbal medicine can be furnished for the quantitative and qualitative extraction for exploring the new promising biomolecules for pharmaceutical applications.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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