Phytochemical Investigation and Antimicrobial Activity of the endocarp of unripe fruit of Carica papaya

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ABSTRACT

Carica papaya L., belong to family, viz., Caricaceae. The plant seed, leaf, flower and fruit contain a number of medicinally important compounds. In the present study, we comparatively screened the presence of certain phytochemicals e.g. - alkaloids, anthroquinone, cathanol, flavonoids, phenols, saponins, steroids, terepenoids, and tannins present in the endocarp of unripe fruit pulp with other explants of Carica papaya. The antibacterial activity of the leaf/flower/endocarp extracts of Carica papaya, against pathogenic bacteria like gram positive (Bacillus subtilis, Bacillus cereus, Micro coccus luteus and Staphylococcus aureus), and gram negative (Escherichia coli 101 and 119) bacteria were studied by in-vitro agar well diffusion method. The plants aqueous endocarp extracts showed pronounced inhibition than leaf extracts. Leaf extracts showed more inhibitory action on Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumoniae. The unripe endocarp acetone extracts demonstrated higher activities against all the gram-negative bacteria, while the highest activity was demonstrated against Micrococcus luteus. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts was analyzed and found that unripe fruit’s endocarp concentration of active particle on 4 times dilution showed maximum inhibitory activity. Carica papaya may be used for the treatment of gastroenteritis, urethritis, otitis media, typhoid fever and wound infections.

Key words: Carica papaya, antibacterial activity, unripe endocarp, gram positive and gram negative, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration

INTRODUCTION

World wide spending on finding new anti-infective agents (including vaccines) and the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. Plants have an almost limitless ability to synthesize aromatic substances, most are secondary metabolites (Phytochemicals), previously with unknown pharmacological activities, has been extensively investigated as a source of medicinal agents (Krishnaraju et al., 2005). Now-a-days natural products of plant sources as the main source of new, safer and more effective bioactive compounds with antimicrobial properties due to the raising problems of side effects and limited efficacy (Gupta et al; 1998; Corazo et al; 1999) of the available antibiotics.

There has been a revival of interest in herbal medicines, due to increased awareness of limited ability of synthetic pharmaceutical products to control major diseases and need to discover new molecular structures as lead compounds from the plant kingdom (Nair et al; 2004). Any part of the plant may contain active components. Initial screening of potential antibacterial and anti-fungal compounds from plants may be performed with pure substances, or crude extracts. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin et al., 1985).

Carica papaya belongs the family caricaeace is well known for its various medicinal properties to treat various ailments such as asthma, rheumatism, diarrhea, boils and hypertension. It is also used to treat ailment in human colic, boils and to increase milk production. It yields a milky sap often called latex, complex mixture of chemicals. Every part of the Papaya tree has a medicinal use. Because of this specificity, papaya has attracted a lot of scientist to isolate important metabolites. Numerous references are reported related to antimicrobial activity of papaya. Most of terpenoids and polyphenol present in papaya have been reported for their antimicrobial properties. These antimicrobial compounds have been previously reported from leaf, roots, flowers, fruit and latex of papaya. Chemical profile of unripe fruit pulp of Carica papaya has also been reported by Oloyede (2005) ,but no-one has emphasized phytochemical and antimicrobial study of the papaya unripe fruit (endocarp) compare with other explants of papaya. The aim of the present study was to screen for their potential antibacterial and antifungal activity against selected group of bacteria and fungi available in our laboratory and to compare their effectiveness against the other explants of papaya and standard antibiotics.

MATERIAL AND METHODS

Preparation of Explants Extracts

Apparently healthy plant leaves, flower and seeds of Carica papaya plant were collected, washed thoroughly in tap water followed by distilled water and dried at room temperature. All explants were weighed 5 gram in 50 ml each for different solvents: acetone, ethanol (70%) and methanol. Weighed explants were crushed carefully in pestle and mortar under sterile condition. The crude extracts were filtered through cheesecloth and the extracts were concentrated using water bath at 40°C. The volume of each extract was reconstituted. The solvent extract was stored at 4°C in refrigerator and used as per the requirement of the experimental need.

Phytochemical Screening of Explants Extracts

The phytochemical components of the Carica papaya, leaves, seeds, flower and unripe endocarp were screened for the presence of alkaloids, saponins, glycosides, tannins, flavonoids, and anthraquinones by using the methods of Jigna et al., (2006). Phenolic compounds present in unripe endocarp were extracted and identification of the compounds was followed by thin layer chromatography. The components qualitatively analyzed were alkaloids, anthroquinone, flavonoids, phenols, saponins, steroids, triterpenoids and tannins.

Inoculums:

The test micro-organisms, gram positive (Bacillus subtilis, Bacillus cereus, Micro coccus luteus, Pseudomonas aeruginosa and Staphylococcus aureus , gram negative (Escherichia coli) bacteria , and fungi Aspergillus niger and Fusarium moniliforme were obtained from MTCC, Chandigar. All the stock culture of bacterial and fungal strain were prepared and inoculated into LB (Luria bertini), and PDA medium respectively and incubated at 37°C for overnight. The bacterial cells were harvested by centrifuging at 5000 g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, ph 7.4). The bacterial cells were diluted to approximately 10⁶ CFU ml before use (Owais et al., 2005).

Antimicrobial assay:

Antibacterial and antifungal of leaf extract:

The antibacterial activity of the Carica papaya leaves were assessed using the antibacterial and antifungal activity of the leaf, flower, seed and unripe endocarp extracts were determined using agar well diffusion method by Perez et al., (1990). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media similarly PDA media was inoculated with fungal spores. Wells (5 mm diameter) were punched in the agar. Then 100µl of each three solvent were added to the wells separately. The plates were incubated at 37°C for 24 hours for the antibacterial and 72 hours for the antifungal activity and it was assessed by measuring the diameter of the zone of inhibition in millimeters against bacterial and fungal growth respectively. Minimum inhibitory concentration (MIC) and the minimum bacterial concentration (MBC) of the unripe pulp (endocarp) of papaya were determined.
of the preliminary phytochemical analyses of the different explants extracts of Carica papaya. Flower, while in case of leaf, on 2 times dilution plant extract did not show inhibitory activity. Table-1 shows the results comparing with standard, MIC was determined for 2 times dilution of seed & unripe endocarp methanol extract. Acetone extract of unripe seeds had shown higher zone of inhibition for E. coli aureus. Methanolic flower extract showed highest zone of inhibition for Escherichia coli. The extracts of Carica papaya in methanol (100µg/ml) exhibit relatively higher zone of inhibition than comparison ethanolic and acetone extract of leaf respectively. While none of the crude leaf extracts showed that methanol extract possesses antibacterial activity against tested gram positive (Bacillus subtilis) and gram negative (Escherichia coli) bacteria. The extracts of Carica papaya in methanol (100µg/ml) exhibit relatively higher zone of inhibition than comparison ethanolic and acetone extract of leaf respectively. While none of the crude leaf extracts showed highest zone of inhibition for Bacillus subtilis. Methanolic extract showed antibacterial against A. niger and negative for Alternaria. Antimicrobial activity of unripe seeds extract: Acetone extract of unripe seeds had shown higher zone of inhibition for E. coli-101 and Micrococcus luteus. Methanolic extract showed antifungal against A. niger and negative for Alternaria. Antifungal of Unripe endocarp extract: Endocarp extract showed good inhibition zone against Aspergillus niger and Fusarium. The results of this study showed that all part of papaya have antimicrobial activity. Unripe seeds and endocarp showed more antimicrobial activity than flower and leaf. Flowers showed much activity against Bacillus cereus. Bacillus subtilis , E. coli 119 in methanol solvent while activity seems lower in acetone solvent than methanol. This may be due to the better solubility of the active components in organic solvents (de Boer et al;2005). Endocarp extract are more active in acetone solvent while seed extract showed activity in ethanol solvent. Leaves are active in methanol solvent. Among fungi Aspergillus niger were more susceptible to the extracts. There may be several factors that will predispose microbes to antimicrobial agents such that previous encounter with the agent or nature of medium, which may affect the diffusability of the agent. The activity of the extracts was comparable to standard antibiotics. Based on the recent observation, it is possible to suggest that unripe endocarp of carica extracts showed broad spectrum activity but less than streptomycin.

The preliminary photochemical screening of the different papaya explants leaves, fruits, seeds, pulp, revealed the presence of carbohydrates, alkaloids, steroids, flavonoids and tannins. Saponin inhibits Na+ efflux, by the blockage of the entrance of the Na+ out of the cell. This leads to higher Na+ concentration in cells, activating a Na+ - Ca2+ antiporter producing elevated cytosolic Ca2+ which strengthens the contractions of heart muscle and thereby reducing congestive heart failure (Schneider and Wolfling, 2004). The separation and identification of steroids by Thin Layer Chromatography in the plant part (endocarp of unripe fruit pulp) revealed the presence at 3 spots from which two flavonoids namely quer cetin and apigenin were identified in endocarpic part of pulp. With regard to the results obtained from the flavonoid test, it can be said that flavonoids are present in all samples. For the presence of tannins the ferric chloride test gave positive results indicating the presence of catechol tannins in all samples except seeds. In general, these results suggest the validity of the therapeutic effect of aqueous extract of unripe pulp of Carica papaya.

Present study was conducted to investigate the comparative antimicrobial potential of Carica papaya unripe endocarp with other explants. The results obtained are encouraging as the methanolic, ethanol and acetone extracts have shown considerable antimicrobial activity. The antibacterial activity of the plant is appreciable, considering the importance of the micro-organism in infection. Preliminary results encouraged us to further purification and bio-assay of the active components in organic solvents (de Boer et al;2005).

REFERENCES:

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