In vitro Anticancer and Antimicrobial Properties of Carica papaya against Biochemically Characterized Clinical and Standard Microbes

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors NZ, FA, SS and GP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RR, MM, QA and MSR managed the analyses of the study. Authors QA and AM managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

The bacterial pathogens have established various defense system against antimicrobial agents. The main mechanism of action of antibiotic resistance is obtained by pathogenic microorganisms, which directly involved in the diagnosis of various therapeutic plants with their significant antimicrobial properties. The current study investigated the antimicrobial and anticancer properties of Carica papaya. Fresh sample of C. papaya was collected in the native area of Punjab and selected isolate were tested against the aqueous, chloroform, ethanol, methanol extracts of C. papaya and anti-microbial activity by disc diffusion method. Anticancer activity was carried out in the HeLa cell line in above mention fractions of the extract at different concentration. The present study concluded, the extracts of the specific plants, particularly the ethanol and methanol extracts

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established the potential role against bacterial strain and HeLa cell line. It has also been concluded that these extracts might be implicated as natural products and serve to mediate as novel pharmaceuticals and therapeutic drugs.

Keywords: Carica papaya antifungal; anticancer; drugs; pathogens; hela cell lines.

1. INTRODUCTION

Various plants are used for the management of numerous diseases. The therapeutic importance of pharmaceutical plants resides as a secondary metabolites are significantly smaller as compared to primary molecules, which are mainly comprised of lipids, carbohydrates and lipids. These natural products are significantly involved to synthesize novel antifungal and antimicrobial chemicals. The clinical significance of plant compounds over the synthetic products is that they are distinguished by the eukaryotic system and have harmful consequences on human health [1-6]. The World Health Organization (WHO) has recommended that world’s population primarily based on conventional medicines, which are considered to be as traditional therapies comprising of different plant extracts or active metabolites. Natural byproducts such as pure compounds comprised of lipids, carbohydrates and lipids. The clinical significance of plant extracts and pure compounds have unrestricted opportunities for novel drug leads [7-11]. C. papaya is also called as kapaya, lapaya, tapaya, papayas and European named as tree melon. In 2010, figures of 60 countries explained approximately 13.01 million metric tons of papaya fruit that were synthesized with the length of 438,588 Ha [12]. C. papaya is effectively treats and improves all types of digestion and abdominal disorders. Green fruits are used for several veterinary and human diseases such as diabetes mellitus, intestinal helminthes, malaria and jaundice. Moreover, it has also been used for contraceptive purpose through traditional healers in Sri-Linka, India and Pakistan [13-19]. Leaves, stem and roots Carica papaya was collected in February 2016 from Lahore division. They were collected very carefully and kept in labelled paper bags to avoid decay. The bags were kept at room temperature till the plant part was preceded for extraction.

2. MATERIALS AND METHODS

2.1 Plant Extraction and Isolation of Bacterial Strain

The cold maceration method was used for the extraction of plant material. Seven bacterial strains namely Bacillus sp, Proteus vulgaris, Pseudomonas aeruginosa, and Micrococcus luteus, S. aureus, Klebsiella oxytoca and E.coli have isolated from clinical samples. All these isolates along with ATCC, P. aeruginosa (25922) E.coli (35151) and S.aureus (29213) were verified on the basis of morphological and biochemical characteristics as noted in selective and differential media including tryptic soya agar, Shigella agar, Muller Hinton agar, nutrient agar, Salmonella, Mannitol salt agar, and MacConkey agar. The cultures were preserved in glycerol and a working stock was prepared to work with.
Fig. 1. The graphical representation indicates the % viability of the standard drug (paclitaxel) and *Carica papaya* fruit with the aqueous extract in different concentrations. Mentioned graph (a,b,c,d) indicated the cytotoxic effect on aqueous(a), methanol(b), ethanol(c) and chloroform(d) extract of fruit of *Carica papaya* in different concentration. The most effective cytotoxic effect was seen in the 200µg/ml concentration in comparison with standard drug (paclitaxel).

Fig. 2. Graphical representation indicates % viability of standard drug (paclitaxel) and papaya leaves with the aqueous extract in various concentrations. The above mention graph (a,b,c,d) indicated the cytotoxic effect of *C. papaya* on aqueous (a), methanol (b), ethanol (c) and chloroform(d) extract of leaves in different concentration. At 200µg/ml effective cytotoxic effect was seen as compared to other part of the fruit.
The Antimicrobial activity of each extract was measured by an improved Kirby-Bauer disc diffusion method [19]. Ciprofloxacin (an antibiotic) was measured as positive control, while DMSO was measured as negative control. All these assays were carried out in triplicate to minimize errors. After overnight incubation, the plates were placed in incubator set at 37°C and zone of inhibition were measured (in mm).

2.2 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was evaluated according to the method of micro dilution, using different serially diluted plant extracts by NCCLS protocol [20]. The extracts were diluted to form different series of concentrations from 6.25-100mg/ml in nutrient broth. In these broth dilutions, the microorganism suspension of 100 µl (0.5 McFarland turbidity standard) was added and incubated for 18 hours at 37°C. Minimum inhibitory concentration of each extract was taken as the lowest concentration which providing invisible bacterial growth. It was observed that all extracts exhibited zone of inhibition above 20 mm against isolates. MIC was measured for extracts, which established a zone of inhibition of 20mm or more.

2.3 Antifungal Activity

Fungus from rotten potato, onion and bread were isolated and grown on Sabouraud Dextrose Agar (SDA). For mounting lactophenol cotton blue solution was used. Aspergillus niger, Aspergillus flavus and Saccharomyces cerevisiae upon verification were stored on SDA or Nutrient broth. Antifungal activity of plant extract was determined in the same manner as antibacterial activity with the exception that SDA was used instead of MH agar. The plates were analysed for results up to three days.

2.4 Anti-Cancer Activity

The MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole protocol were used for anticancer properties of Carica.papaya. Four fraction (aqueous, ethanol, methanol and chloroform) of Carica.papaya extracts with dilution of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5µg/ml and 6.5 µg/ml were taken in this experiment. One confluent T-25 flask was subjected to trypsinization to attain cell for plating in 96 well plates. Trypsinization includes treatment of cells with trypsin 1x after washing with PBS 1x. Detached cell were centrifuged in a sterile 15 ml falcon tube at 4000rpm in the swinging rotor for 5 min at 4°C. Supernatant was discarded to get pellet of detached cells. In 1.0 ml of complete media, the pellet was suspended after completing washing. Total cells were counted and recorded with Neubauer chamber. Complete media was dilute cells. Cells were plated at the density of 7x10³ cell per well of 96 well plate and incubated overnight in a carbon dioxide incubator. On second day varying concentration of Carica papaya extracts (leaves and fruit) were administered in triplicate. After 24 hours 20µl of 5mg/ml MTT reagent in PBS1x was added to each well and then incubated again for 2h in CO₂ incubator. All incubations were carried out under standard aseptic conditions (37°C, 5% CO₂ and 90% humidity) set of HeLa cells. The MTT salt was condensed into blue color and the water insoluble Formazan crystals incubated for 2hour. Medium was decanted out and finally 100µl of solution containing 50% DMSO and 20% SDS as extraction buffer was added to every single well to solubilize Formazan crystals. Optical density was determined at the wavelength of 570 nm.

3. RESULTS

Microbial infection and cancer are a widespread issue in the world. Although, Carica papaya has been known to be medicinal but no reports were found about the effect of their organic and inorganic extract on clinical isolates in the local area of Lahore, where the present study was conducted. This study determined the anticancer and antimicrobial properties of Carica papaya extracts (leaves and fruits). It also qualitatively illustrated the tests for phytochemical analysis to determine the alkaloids, phenolic, flavonoid, carbohydrates and saponine contents of the plants parts.

3.1 Carica papaya with Its Antimicrobial Activity

In order to understand the antifungal and antibacterial properties of Carica papaya extracts were analysed against various strains of bacteria such as, Bacillus sp, Proteus vulgaris, E. coli, Klebsiella oxytoca, Pseudomonas aeruginosa, S. aureus and against fungal strains such as C. albican, A. niger, A. flavus and S. cerevisiae. The minimum inhibitory concentration (MIC) can be seen in the table given below showing different fraction (water, methanol, ethanol and chloroform) collected from leaf and fruit. Against different fractions of C. papaya, S.
4. DISCUSSIONS

A. niger showed the most effective antibacterial activity except CP.F (H₂O) CP.F (Chloroform). P. aruginos showed the inhibitory action against the aqueous extract of both leaves and fruit part of this plant. Methanol and ethanol fraction of these parts of the plant were effective against E. coli. Bacillus sp found sensitive against aqueous extract of leaves and methanol fraction of fruits. Proteus showed no sensitivity and the entire fungal strains showed negative result with the extract of plant.

3.2 Anticancer Activity of Carica papaya

MTT protocol was used to find out the cytotoxic activity of different extract of Carica papaya on HeLa cell line. Basic procedure of this assay was already discussed in the content of materials and method on various fractions (water, ethanol, methanol, and chloroform) of Carica papaya was seen by using different concentration of each fraction with standard drug (paclitaxel).

4. DISCUSSIONS

Ze-You-Lie [21] determined the anticancer activity (H69 cell line) of C. papaya leaves and measured the IC50 to be 6.5 µM/L. In the present study the effective apoptotic action was at 200µg/ml. Leaves part were found to have better anticancer activity as compared to the fruit part and organic fraction (ethanol and methanol) have better effective as compared with other. Maisarah [22] worked with a different alcoholic concentration of C. papaya (root, seed, shoot) on A. niger, A. flavus, C. albicans. The higher concentrations showed the significant antifungal result. In the recent study the same strains showed to be sensitive at an extract concentration of 20mg/ml (5µl in each disc) in each fraction. Jyotsna [23] determined the antimicrobial activity of aqueous, methanol, ethanol, acetic acid and petroleum ether C. papaya extract and effective result were seen in aqueous and ethanol extract but methanol, acetic acid and petroleum ether showed the sensitive result. In present work, the selected different strain also showed the significant antimicrobial activity against E-coli having the MIC 15mm with the methanol extract of fruit. S. aureus showed the maximum antibacterial activity in each fraction of the C. papaya leaves and fruit except the chloroform and aqueous fraction of the fruit. P. aeruginosa is sensitive only to C. papaya leaves and fruit with the aqueous extract. In E-coli, the inhibitory result is seen in the methanol and ethanol extract [24-25]. P. vulgaris was found to be sensitive against each fraction. Bacillus showed the inhibitory effect against aqueous extract of leaves and fruit and also with the methanol fraction of the fruit. K.oxytoca showed inhibitory in growth by the action of an ethanol fraction of fruit. Chavez-Quintal et al., [26] worked with ethanol extracts of the leave against R. stolonfer, Fusarium spp and C.gloeosporides of C. papaya leaves. In present work the fungal strains A. niger, A. flavus, S. cervicca and C. albican showed the sensitive result in set protocol of C. papaya. Anibijuwon, [11] worked with the fresh and dried leaf extract C. papaya and comparative analysis were in favour of the organic extract than the aqueous extract. Fresh extract was found more effective in Gram negative bacteria in comparison to the dried extract. In the present work the dried extract exhibited the significant result from qualitative analysis of the photochemicals which detected the saponine, flavonoid, phenol and carbohydrates. Antimicrobial activity also explained the broad spectrum of the organic extract of C. papaya with maximum 15 mm zone of inhibition in E-coli with methanol extract. According to the study of Asish et al., [16] suggested in vitro studies performed by SRB method with DU-145 cell lines showed that different extracts of fruit of Carica papaya (MLE, ELE andCFE) have anti-oxidant properties. In the current study, it has been concluded that MLE, ELE and CFE have significant role to damage cancerous cell in comparison with standard drug 5-FU. In present study methanolic and ethanolic extract of leave showed the comparative result to the paclitaxel similarly in fruit extract chloroform solvent is shown more anti proliferative response towards standard drugs. Saurabh et al., [9] bioassay has fractions of medium polarity LJP resulting in different sub fractions of MP-LJP, which explained specific anti proliferative activity against PCa cells, including benign hyperplasia as compared to non-cancerous cells of prostate origin. Examination of several combinations of these sub-fractions consequently lead to highly refined FC-3 extracts that account for bulk of the potent and selective anti proliferative responses of MP-LJP [27-30]. In present study methanolic and ethanolic extract of leave showed the comparative result to the paclitaxel similarly in fruit extract chloroform solvent is shown more anti proliferative response towards standard drugs Paclitaxel. The current study was performed to determine the antibacterial and anticancer properties of C. papaya leaves and fruit in different solvents (water, methanol, ethanol and chloroform). It has been concluded
Table 1. Qualitative phytochemical analysis of the *Carica papaya*

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Alkaloid contents</th>
<th>Phenolic contents</th>
<th>Flavonoid contents</th>
<th>Saponine content</th>
<th>Carbohydrates content</th>
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<tr>
<td>CP.L (H₂O)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CP. L (Ethanol)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CP. L (Methanol)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CP. L (chloroform)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CP.F (H₂O)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CP. F (Ethanol)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CP. F (Methanol)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CP. F (Chloroform)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Table 2. Antimicrobial activity of *C. papaya*

<table>
<thead>
<tr>
<th>Names of isolates</th>
<th>CP.L (H₂O)</th>
<th>CP.L (MET)</th>
<th>CP.L (ETH)</th>
<th>CP.L (CHLO)</th>
<th>CP.F (H₂O)</th>
<th>CP.F (MET)</th>
<th>CP.F (ETH)</th>
<th>CP.F (CHLO)</th>
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<tr>
<td>P. aeruginosa ATCC(25922)</td>
<td>10mm</td>
<td>-</td>
<td>-</td>
<td>11mm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>E. coli ATCC(35151)</td>
<td>10mm</td>
<td>11mm</td>
<td>8mm</td>
<td>14mm</td>
<td>15mm</td>
<td>12mm</td>
<td>10mm</td>
<td>7mm</td>
</tr>
<tr>
<td>S. aureus ATCC(29213)</td>
<td>10mm</td>
<td>13mm</td>
<td>10mm</td>
<td>12mm</td>
<td>10mm</td>
<td>7mm</td>
<td>6mm</td>
<td>-</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bacillus. sp</td>
<td>9mm</td>
<td>-</td>
<td>-</td>
<td>6mm</td>
<td>7mm</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>K. oxytoca</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6mm</td>
<td>-</td>
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<tr>
<td>C. albicans(ATCC)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>A. niger</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>A. flavus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. cervicaceae</td>
<td>-</td>
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from the results obtained that the plant part fractions were significant in possessing antibacterial, antifungal and anticancer activities. Among all, the ethanol and methanol fractions were found to be the most important fractions at their higher concentrations.

5. CONCLUSION

The recent study was performed to determine the anticancer and antibacterial properties of *C. papaya* leaves and fruit in different solvents (water, methanol, ethanol and chloroform). It was concluded from the findings obtained by plant part fractions were significant in possessing antibacterial, antifungal and anticancer activities. Among all, the ethanol and methanol fractions were found to be the most important fractions at their higher concentrations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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