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RESEARCH ARTICLE

COMPARATIVE IN VITRO STUDIES OF ANTIMICROBIAL ACTIVITIES OF RAW AND RIPEN PULP OF *CARICA PAPAYA* AGAINST SOME HUMAN PATHOGENS.

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Abstract

Present study evaluates the antibacterial activity of *Carica papaya* fruit (unripe and ripe) extracts obtained in different solvents (Petroleum ether, Chloroform and Ethyl acetate). The extracts were studied against various diseases causing pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica* and *Micrococcus luteus* using agar well diffusion method. The ethyl acetate extract of raw papaya pulp was found more active against *S. enterica* with zone of inhibition 20 mm. Extract of petroleum ether and chloroform also showed activity against *P. aeruginosa* and *S. enterica* with inhibition zone 15 mm and 17 mm respectively. Extracts of ripen pulp showed no or less activity against any of these tested pathogens. Chloroform showed moderate activity with the inhibition zone 15 mm against *S. enterica* followed by *P. aeruginosa* with inhibition zone 14 mm. When these results were compared to the results of standard drugs, raw papaya pulp was found more effective against pathogens than the other. Minimum inhibitory concentration was calculated and maximum values lie between 10- 20 mg/ml. This research concludes that raw papaya has potential natural antibacterial compounds which can aid in further investigation.

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Introduction:-

Present time, life-threatening infections caused by pathogens has increased worldwide. It was suggested that some microorganisms cause majority of infections in both the community and the hospital environments. Although many antimicrobial agents have been discovered, the indiscriminate use of antibiotics has resulted in many microorganisms developing resistance to them (Sofowora, E.A. 1982). Some human pathogens like *Pseudomonas*, *Salmonella* and many others causing hazardous infections arise due to contaminated water or food. *Pseudomonas aeruginosa* is a strain of bacteria found widely in the environment; the most common type causing infections in humans in the hospital and/or with weakened immune systems (Lyczak JB., et al. 2000). *Salmonella enterica* is responsible for the majority of the virulence factors of the bacterium (de Jong, H.K., Parry, C.M., van der Pol, T). *Staphylococcus aureus* is a commonly known pathogen that is responsible for infections like pneumonia, diabetes, cancer, vascular disease,

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and lung disease have developed resistance to most classes of antibiotics (Enright MC.2003).Several studies prove the presences of antimicrobial agent as primary or secondary metabolites in medicinal plants which can be used against these resistance developing pathogens.

Carica papaya (family Caricaceae) is rich in antioxidant nutrients such as carotenes, flavonoids and vitamin C, as well as vitamin B (foliate and pantothenic acid). Papain is an endolytic plant cysteine protease enzyme has been used in medicine to treat ulcers, arthritis and reduce skin adhesions and studies have shown that it has anti-allergic, anti-inflammatory, antimicrobial properties (Uhlir, H.1998 and Anibijuwono, I. I. and Udeze, A.O. 2009). It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine. Chymopapain compounds present in *C. papaya* are used in bloating and conditions of chronic indigestion and Carapine, an alkaloids can be used as a heart depressant, amoebicide and diuretic.

The investigation is carried out to overcome the problem of bacterial resistance and identify the antibacterial activity of compounds present in papaya pulp (unripen and ripen) against different types of pathogens.

Materials and Method:-

Plant materials collection and extraction:-

Both fresh raw and ripen pulp of *Carica papaya* was collected from the area of Balawala, Dehradun. Washed, dried under the shed and blended into fine powder. Three different solvents were used for the extraction process of powdered material viz., petroleum ether, chloroform, ethyl acetate sequentially using hot maceration method. Crude extracts of both pulps were obtained by evaporating the solvent extracts using water bath at 55°C.

Standardization of extracts:-

Stock solution used for antibacterial test was prepared at a concentration of 30 mg/ml (w/v) by dissolving 30 mg of each crude extract in 1 ml of 99.5% Dimethylsulfoxide (DMSO). Antibiotic disc (Vancomycin, Rifampicin, Erythromycin, and Nitrofurantoin) and DMSO used as positive and negative control, respectively.

Test pathogens:-

To assess the antibacterial properties, pure cultures of *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella enterica* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, SBSPGI, Dehradun. Pathogens were grown on nutrient agar slants at 37°C. For antibacterial assays, Loop full bacteria were inoculated into nutrient broth and incubated overnight at 37°C.

Screening of papaya pulp extracts for antibacterial activity:-

Antibacterial properties of various extracts of raw and ripe papaya pulp were demonstrated by well diffusion method against infection causing bacteria.

Agar well diffusion method:-

Muller's Hilton agar media was used to determine the antibacterial activity of pulp extract of papaya. 25 µl of 24 hrs fresh culture of each pathogen was evenly spread over sterile agar poured plates with the help of sterile glass spreader. After spreading, four wells were made in each plate using sterile cork borer. With the help of micro-pipette, wells were filled with raw and ripe pulp extract. Separate spread plate was used for positive control (antibiotic) and negative control (DMSO). These plates were left for diffusion of extracts for 30 minutes and then incubated in the upright position at 37°C for 24 hours. The formation of clear zone of inhibition around the well indicating the presence of antibacterial activity plates were observed and measured.

Minimum Inhibitory Concentration (MIC):-

The MIC value of pulp extract is considered the lowest concentration of extract which exhibit maximum antibacterial activity. Various concentrations of both the pulp extract were prepared to demonstrate the minimum inhibitory concentration of antibacterial substance. To the culture tubes contains 9 ml fresh culture broth, 1 ml of the concentration ranged between 5 – 30 mg/ml was added. No plant extract was added to the control tube containing 9 ml of culture broth. After this, sealed the tube with sterile cotton plug and were incubated at 37°C for overnight. Tube with no or little growth of organism after 24 hours compared with the turbidity of control tube was considered as the MIC value.

Relative percentage inhibition:-

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula.

$$\text{Relative percentage inhibition} = \frac{100 \times (a - b)}{(c - b)}$$

(c - b)

Where,

a: total area of inhibition of the test extract

b: total area of inhibition of the solvent

c: total area of inhibition of the standard drug

Result:-

The present study was done to evaluate the antibacterial efficacy of papaya pulp (unripe and ripe) against human pathogens Table 1. Among all three extracts of raw papaya pulp, ethyl acetate extract was more effective against *S. enterica* with inhibition zone 20 mm and relative per cent inhibition of 133.3 followed by *S. aureus* having 18 mm inhibition zone and 225 relative per cent inhibition. The relative per cent inhibition was calculated and given in Table 2. In ripen papaya, chloroform pulp extract showed strong inhibitory effect on the pathogens with the inhibition zone of 15 mm and 100 per cent inhibition against *S. enterica* followed by 14 mm inhibition zone and 233.3 per cent inhibition against *P. aeruginosa*. Petroleum ether and ethyl ether extract found no or little effect against test organisms. Fig show that all the pathogens were found resistance to the antibiotic drug used as positive control except Vancomycin and Nitrofurantoin, which were moderately active against *S. enterica* and *M. luteus* respectively. When the results were compared between the inhibition zone of antibiotics and the two sample extracts, all the three extracts of raw papaya pulp found more effective. The MIC values are presented in fig 1. The lowest concentration values ranging between 5 – 30 mg/ml. The MIC of ethyl acetate extract of raw pulp against *S. aureus* and *P. aeruginosa* was 10 mg/ml and 5 mg/ml against *S. enterica*. The MIC of ethyl acetate extract of ripe pulp against *S. aureus*, *S. enterica* and *P. aeruginosa* was 10, 25 and 15 mg/ml respectively.

Table 1:-Antibacterial activity of papaya pulp extract against pathogens.

Test pathogens	Zone of Inhibition (mm) at 30 µg							
	Raw pulp			Ripe pulp			+ve Control	
	PE	Ch	EA	PE	Ch	EA	Standard discs	
<i>S. enterica</i>	15	17	20	10	15	8	Vancomycin	15
<i>P. aeruginosa</i>	15	16	16	10	14	14	Rifampicin	6
<i>S. aureus</i>	14	13	18	14	12	9	Erythromycin	8
<i>M. luteus</i>	13	16	14	11	14	15	Nitrofurantoin	13

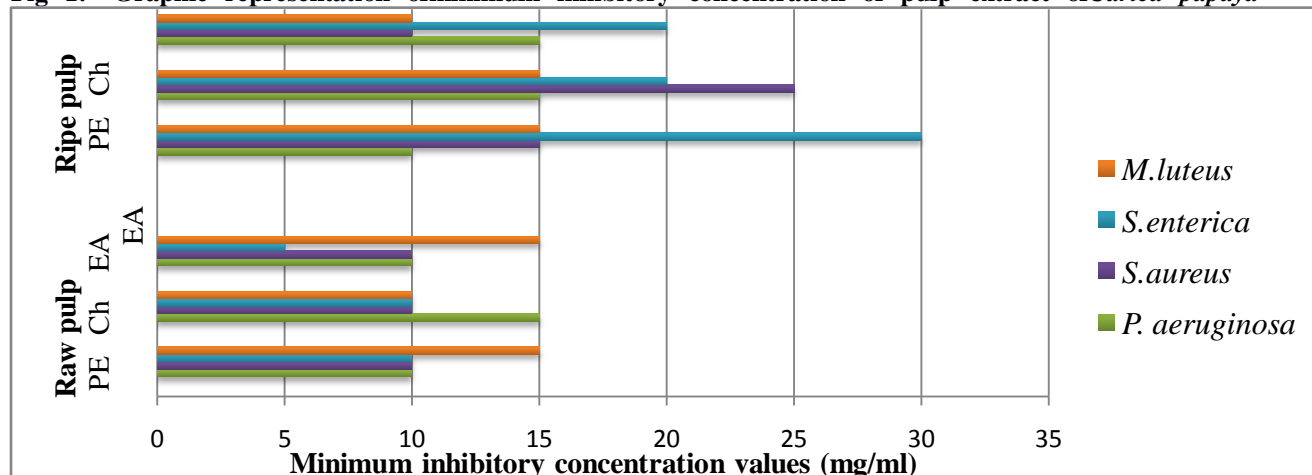
PE- Petroleum ether, Ch- Chloroform, EA- Ethyl acetate, µg - microgram, +ve - positive

0-12 = Resistance; 13-17 = Moderate; above 18 = Sensitive

Table 2:- Relative Percentage inhibition (%) of fresh pulp extract of *C. papaya* against different strains of pathogen.

Microbes used	Relative percentage inhibition (%)						
	Raw pulp			Ripe pulp			
	PE	Ch	EA	PE	Ch	EA	
<i>S. enterica</i>	100	113.3	133.3	66.6	100	53.3	
<i>P. aeruginosa</i>	250	266.6	266.6	166.6	233.3	233.3	
<i>S. aureus</i>	175	162.5	225	175	150	112.5	
<i>M. luteus</i>	100	123.1	107.7	84.6	107.7	115	

PE- Petroleum ether, Ch- Chloroform, EA- Ethyl acetate

Fig 1:- Graphic representation of minimum inhibitory concentration of pulp extract of *Carica papaya*Fig 2: Photos showing zone of inhibition of ripen pulp extracts of *C. papaya* against tested pathogensFig 3: Photos showing zone of inhibition of raw pulp extracts of *C. papaya* against tested pathogens

Discussion:-

India is one of the twelve mega biodiversity centre's having more than 45,000 plant species. Use of plants as a source of medicine has been inherited and is an important component of the health care system. Plants are the important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona, L.*et al* 1998). Present investigation was designed to obtain information on the antibacterial activity of the two samples of *C. papaya*. Among papaya fruit extracts, the extracts of raw pulp showed higher inhibition of the bacterial growth than the extracts of ripen pulp. This is due to the presence of an enzyme called Papain which is not found in ripen papaya fruits, shows extensive proteolytic activity towards proteins, short chain peptides, amino acid esters and amide links and is applied extensively in the fields of food and medicine (Tsuge, H.*et al*. 1999).

Previous studies confirms that the unripe fruits extract treated wounds were found to heal faster which induced complete healing in shorter period (13 days) than that required by ripe papaya (17 days).

(Ocloo, A. *et.al.* 2012).In unripen pulp extracts, ethyl acetate extract showed highest activity and inhibited all the pathogens than petroleum etherextract. This was believed due to the presence of sinigrin and caricin, both glycosides and the enzyme myrosin. The wound healing property of this plant can be attributed to the presence of bioactive compounds like alkaloids, flavinoids, carbohydrates, glycosides, saponins, phenolic compounds and tannins and cardenoides(Pandey P., Garg F.C., Tripathi L.K. 2016).In antibiotic sensitive test, Vancomycin showed activity against *S. enterica* due to its unique glycopeptide structurally unrelated to any currently available antibiotic. There is also evidence that Vancomycin alters the permeability of the cell membrane and selectively inhibits ribonucleic acid synthesis(Mansfield, 1985).The low MIC value observed for *S. enterica* is a good indication of high efficacy against this bacterium. High MIC may be an indication of low efficacy or that the organisms have the potential for developing resistance to the bioactive compounds(Draughon FA. 2004). Therefore, this systemic screening of plant extracts for antibacterial activity is a continuous effort to find new antibacterial compounds.

Conclusion:-

The present study focused on antibacterial activity of *C.papaya* against both gram-negative and gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. These results supports the traditional application of the plant that possess compounds with antibacterial properties can be used as antibacterial agents in drug manufacture by the pharmaceutical industries.

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