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Antimicrobial activity evaluation of the root of *Carica papaya* Linn.

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Abstract: Carica papaya Linn. Belonging to family *Caricaceae* is a well known medicinal plant. Carica papaya root extracts of the plant were tested for their in vitro antimicrobial activity by Continuous Hot Extraction (Soxhelation) method. The test organisms were *P.vesicularis, sterptococcus faecalis, Aeromonas hydrophilia, Salmonela typhae, Stphylococcus cohni, Serratia ficaria and E.coli*. The Zone of inhibition was determined for concentration ranging from 12.5mg/ml to 50mg/ml. (12.5mg/ml, 25mg/ml, 37.5mg/ml, and 50mg/ml). Antibacterial activity tested for well diffusion method.

Key words: Carica papaya Linn ,Antimicrobial activity, Root of Carica papaya Linn.

INTRODUCTION

Plants are the main source of food. They are rich in nutrients. They are also rich in compounds which have pain relieving and healing abilities. From earliest times itself, plants were used for treatment of disease without knowledge about the compounds present and their mode of action. Over the centuries societies around the world have developed their own tradition to make sense of medicinal plants and their uses. The wide spread use of herbal remedies and health care preparations obtained from commonly used traditional herbs and medicinal plants have been raised due to the occurrence of natural products with medicinal properties. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs bv

microorganisms has also increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections occur in hospitals resulting in high mortality¹. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and immunocompromised patients in mortality in developing countries². The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new

infection-fighting strategies³. For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties⁴. Carica papaya is an evergreen, tree-like herb, 2-10 m tall, usually unbranched, although sometimes branched due to injury, containing white latex in all parts. Stem cylindrical, 10-30 cm in diameter, hollow with prominent leaf scars and spongy-fibrous tissue. Has an extensive rooting system. Leaves spirally arranged, clustered near apex of trunk; petiole up to 1 m long, hollow, greenish or purplish-green; lamina orbicular, 25-75 cm in diameter, palmate, deeply 7-lobed, glabrous, prominently veined; lobes deeply and broadly toothed. C. papaya grows satisfactorily in a wide range of areas from the equatorial tropics to temperate latitudes. However, it must be grown in warm, sunny sites sheltered from wind; preferably below 1500 m. Strong winds are detrimental, particularly on soils that cannot make up for large transpiration loss. C. papaya is not frost hardy; exposure to frost or cold wind usually results in leaf damage and subsequent death of the tree. Roots are very sensitive to waterlogging, and even short periods of flooding can kill the plant. Carapine, an alkaloid present in papaya, can be used as a heart depressant, amoebicide and diuretic. The fruit and juice are eaten for gastrointestinal ailments; a fresh leaf poultice is used to treat sores. The fresh root with sugarcane alcohol can be taken orally or as a massage to soothe rheumatism. A flower decoction is taken orally for coughs, bronchitis, asthma and chest colds. In some countries, the seeds are used as an abortifacient and vermifuge.

The relatively lower incidence of adverse reactions to plant preparation compared to modern conventional pharmaceuticals, coupled with their reduced cost is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthesis drugs⁵. Infectious diseases account for high proportions of health problems in the developing countries like India. Micro organisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of diseases⁶ infectious .Bacterial resistance to antimicrobial drugs is a worldwide problem that has emerged even among the common poultry pathogens. Nowadays, the use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized using herbs. Herbal medicines are in great demand in both developed and developing countries as a source of

primary healthcare owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and will overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs were known to prevent cancer. Some herbs have antibacterial and antifungal properties that are useful for clinical use. Some of the *in vitro* studies have been conducted, in which herbal extracts were given to clinical drug resistant strains and different serotype strains of infection⁷. Hence the present study reports on the antibacterial activity of *Carica papaya*.

MATERIALS AND METHODS:

Plant collection

Healthy disease free, mature fresh plant root sample were collected locally from Bhopal (M.P.), India. Fresh root were washed thoroughly 2-3 times with running tap water and once with sterile water, shadedried without any contamination. The dried leaves were then powdered using an electric mill.

Preparation of Extracts

Root was washed; air dried under shade and powdered with the help of electric mill at Pinnacle Biomedical Research Institute (PBRI), Bhopal. Powdered leaves were weighed and packed in soxhlet. Solvent used for Soxhelation was mixture of methanol and acetone in the ratio of 70:30 respectively. Extraction was continued at the temperature of 50°C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40°C. Concentrated extract was dried at 40°C in hot air oven. Dried extract was packed in an air tight container.

Collection and maintenance of Microbial culture

The strains were collected from the Pinnacle Biomedical Research Institute (PBRI), Bhopal. The bacterial strain such as (P.vesicularis, sterptococcus faecalis, Aeromonas hydrophilia, Salmonela typhae, Stphylococcus cohni, Serratia ficaria and E.coli.) were inoculated in a nutrient broth at 370C for 24 hour in incubator. The 36g of Muller Hinton agar (Himedia) was mixed with distilled water and then stabilized in autoclave at 15lbs pressure for 15 min. The sterilized media was poured into Petri dishes; the solidified plates were bored with 5mm diameter cork bearer. The plates with wells were used for the antimicrobial studies. The various extracts were tested against the P.vesicularis, sterptococcus faecalis, Aeromonas hydrophilia, Salmonela typhae, Stphylococcus cohni, Serratia ficaria and E.coli for antimicrobial activity. Wells of equal size were cut and the antibiotic was

added into it for positive control; respective solvents acting as a negative control. The plates were incubated at 370 C, overnight.

Antibacterial sensitivity

The antibacterial activity of crude plant extracts of *Carica papaya* were determined by well diffusion method. Plates were prepared by pouring sterile Muller Hinton agar (Himedia) into sterile petri dishes that were previously autoclaved. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gaps in the medium and the plant extracts were added into it. Then the plates were incubated at 37oC and observed for zones of growth inhibition after 24 hours.

RESULTS AND DISCUSSION

The results of antibacterial sensitivity of various solvent extracts of Carica papaya leaves by well diffusion method are in depicted below graph 1 and table 1. The results reveal that all extracts are potent antimicrobials against all the pathogenic organisms studied. The antibacterial activity was screened from the zone of inhibition. The diameter of inhibition zones for each of the samples were compared with (positive control). In negative control has not shown any inhibitory effect. The emergence of antibiotic resistance has its roots in the injudicious use of antibiotics and the subsequent transfer of resistance genes and bacteria among animals, animal products and environment. Extra chromosomal genes associated with plasmids were found to be responsible for these antibacterial resistant phenotypes that may impart resistance to an entire antibacterial class⁸. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid and are producing a better opportunity for testing wide range of microorganism. Preliminary phytochemical analysis during the present study also

ascertains the presence of some potential group of bioactive substances⁹ the potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials¹⁰. Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites, of which at least 12,000 have been isolated. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The property of active phyotoconstituents responsible for the antibacterial activity cannot be (The nature of active phytochemical altered. responsible for antibacterial activity cannot be ascertained). Continued further exploration of plantderived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies. In conclusion, Caesalpinia pulcherrima extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of new clinically effective antibacterial finding compounds and formulated reparations for enhancing potency and stability are needed to recommend Carica papaya in control of several bacteria associated diseases.

The zone of inhibition for different extract is reported in Table. No. 1.

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	14.25 ± 2.04	18.12±1.62	10±1.62	7.95±0.33
sterptococcus faecalis	7.25±0.5	7.5±1.35	6.75±0.64	8.9±0.66
Aeromonas hydrophilia	7.12±1.31	7.15±1.3	9.17±0.90	7.17±0.43
Salmonela typhae	7.75±1.70	7.25±1.04	7.45±1.31	6.95±0.49
Stphylococcus cohni	7.25±1.25	7.12±0.85	6.75±0.43	9.97±0.30
Serratia ficaria	10±1.63	7±0.1	14.1±0.95	15.02±1.48
E.coli	6±0.6	6.37±0.47	7.52±0.41	8.5±0.40

Extract 1 AE Table 1.1

Extract 2 AE-Tables 1.2

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	6.87±0.62	6.95±0.31	8.1±0.2	8.9±0.66
sterptococcus faecalis	6±0.1	6±0.2	7.12±0.25	8.02±0.41
Aeromonas hydrophilia	6.175±0.20	6±0.1	6±0.11	6±0.1
Salmonela typhae	6.17±0.17	6.05±0.1	6±0.11	6.1±0.2
Stphylococcus cohni	7.97±0.57	9.87±1.03	6.82±0.55	6.9±0.62
Serratia ficaria	6±0.2	6.07±0.09	6.05±0.05	6.05±0.05
E.coli	9.1±0.75	10.45±0.61	12.05±0.05	10±0.05

Extract 3 PEE Tables 1.3

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	12.6±0.62	6.95±0.30	8.1±0.2	7.92±0.51
sterptococcus faecalis	6±0.05	6±0.21	7.12±0.25	6.02±0.05
Aeromonas hydrophilia	6.17±0.20	6±0.2	6±0.31	6.97±0.40
Salmonela typhae	6.17±0.17	6.05±0.1	6±0.2	6.37±0.18
Stphylococcus cohni	7.97±0.57	9.87±1.03	6.82±0.55	7±0.05
Serratia ficaria	6±0.07	6.07±0.09	6.05±0.05	7.02±0.38
E.coli	9.1±0.75	10.45±0.61	12.05±0.1	7±0.53

Extract 4 BAE 1 Table 1.4

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	6±0.2	7.1±0.14	9.12±0.25	9.55±0.19
sterptococcus faecalis	6.5±0.08	9±0.21	9.47±0.17	10.82±0.14
Aeromonas hydrophilia	8.15±0.33	9±0.3	10.25±0.5	6.1±0.14
Salmonela typhae	7.05±0.33	8±0.24	10.02±0.36	11.35±0.26
Stphylococcus cohni	11±0.25	16.05±0.73	17.02±0.68	9.87±0.15
Serratia ficaria	6±0.2	6±0.21	8.15±0.3	6.97±0.05
E.coli	9.85±0.91	9.57±0.33	10±0.25	11±0.16

Extract 5 BAE 2 Table 1.5

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	6.1±0.11	7.1±0.2	10.07±0.15	13.02±0.25
sterptococcus faecalis	7.1±0.18	6.97±0.18	9.05±0.26	10.05±0.31
Aeromonas hydrophilia	6±0.5	7.85±0.42	8±0.43	8.55±0.05
Salmonela typhae	6.15±0.23	7.95±0.47	8.47±0.18	10.25±0.5
Stphylococcus cohni	6.5±0.21	6±0.23	8.35±0.34	12.07±0.67
Serratia ficaria	6±0.06	7.07±0.09	8.52±0.12	12.02±0.37
E.coli	13.02±0.53	14.9±0.45	16.17±0.88	16.52±0.17

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	14.92±0.53	13.02±0.74	14.07±0.09	15.97±0.20
sterptococcus faecalis	14±0.08	11.82±0.55	11.35±0.12	13.02±0.28
Aeromonas hydrophilia	13.47±0.12	14.9±0.37	12.97±0.47	10.02±0.36
Salmonela typhae	12.5±0.16	14.25±0.42	16.05±0.45	14.57±0.22
Stphylococcus cohni	9.82±0.20	10.75±0.25	12.97±0.45	15.05±0.55
Serratia ficaria	10.05±0.20	11.2±0.4	12.87±0.42	11.42±0.22
E.coli	9.87±0.29	8±0.24	8.82±0.43	15±0.4

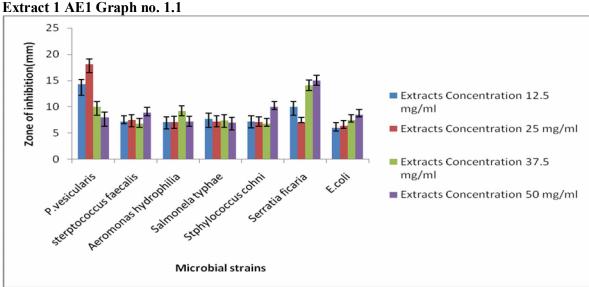
Extract 6 BCE Tables 1.6

Extract 7 ACE Tables 1.7

	Extracts Concentration			
Microbial Strains	12.5 mg/ml	25 mg/ml	37.5 mg/ml	50 mg/ml
P.vesicularis	10.77±0.33	8.92±0.35	8.1±0.08	10.1±0.11
sterptococcus faecalis	10.95±0.19	7.9±0.34	14±0.21	15.05±0.26
Aeromonas hydrophilia	7.02±0.05	13.97±0.20	10.82±0.28	11.05±0.49
Salmonela typhae	7.02±0.44	12.1±0.25	12.97±0.28	7.02±0.05
Stphylococcus cohni	9±0.16	10.3±0.6	10.97±0.25	8±0.23
Serratia ficaria	10±0.43	12±0.16	7.02±0.05	6.02±0.05
E.coli	10±0.5	13.25±0.17	13.85±0.57	14.92±0.51

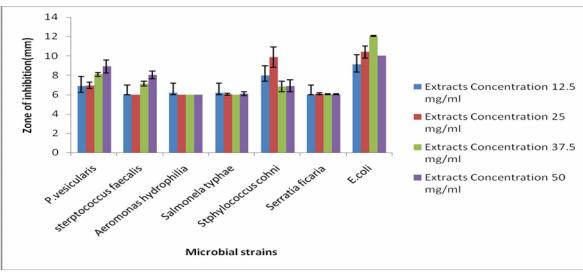
Graphs

The zone of inhibition for different extract is reported in table no. 1 and graph no. 1

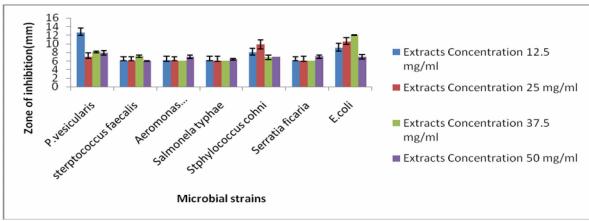


Well diffusion method

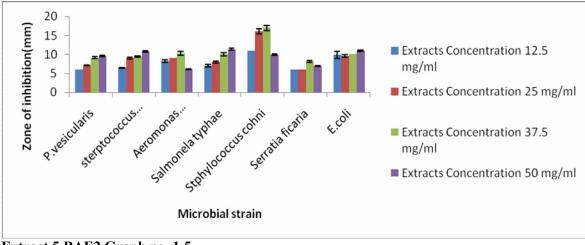
Extract 2 AE2 Graph no. 1.2



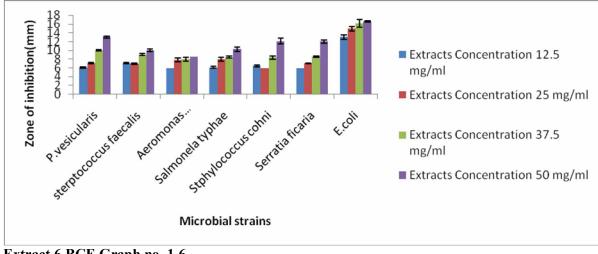
Extract 3 PEE Graph no. 1.3



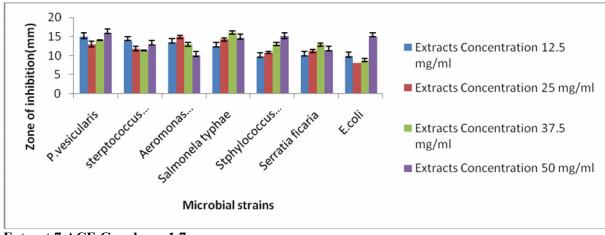
Extract 4 BAE1 Graph no. 1.4



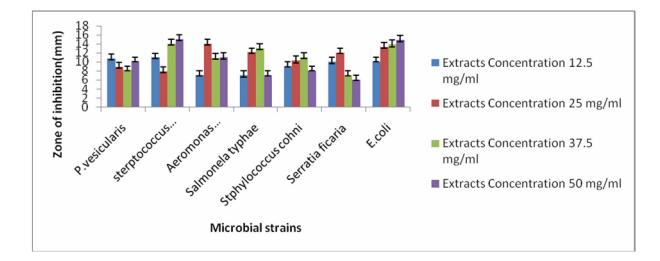
Extract 5 BAE2 Graph no. 1.5



Extract 6 BCE Graph no. 1.6



Extract 7 ACE Graph no. 1.7



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