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ANTIMICROBIAL ACTIVITY OF *CARICA PAPAYA* L.

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ABSTRACT

Antimicrobial activity of *Carica papaya* leaf extracts on opportunistic pathogenic microbes, bacteria and fungi was observed in this study. Papaya leaves were extracted by using sox let apparatus method and four kinds of solvents: acetone, aqueous, ethanol and methanol. Papaya leaf extracts were tested against human pathogenic microbes. Bacteria such as *Bacillus subtilis, Clostridium tetanus, Escherichia coli, Proteus vulgaris, Stapylococcus aureus* and fungi such as *Aspergillus conicus, Aspergillus flavus, Aspergillus niger, Aspergillus sulphureus* and *Rhizopus* by agar well diffusion method. All the leaf extracts of *Carica papaya L*. exhibited greater activity towards bacteria and fungi. The extract demonstrated higher activities against all the bacteria and fungi tested, with the highest activity (acetone extract of 13 mm zone of inhibition) demonstrated against *Saphylococcus aureus* and (ethanol extract of 18 mm zone of inhibition) demonstrated against *Aspergillus flavus*. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media, dengue fever, typhoid fever and wound infections.

KEY WORDS

Carica papaya, Antimicrobial activity and Gastroenteritis.

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INTRODUCTION

Carica papaya Linn belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit. The plant is native to tropical America and was introduced to India in 16th century. The plant is recognised by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves, is rapidly growing and can grow up to 20m tall. Traditionally leaves have been used for treatment of a wide range of ailments, like in treatment of malaria, dengue, jaundice, immunomodulatory and antiviral activity. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine,

dehydrocarpaine 1 and 2), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves. Both leaf and fruit of the *Carica papaya* Linn. possess carotenoids namely β- carotene, lycopene, anthraquinones glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities have also been established. Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc (Yogiraj *et al.*,2014)[1].

The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to synthetic drugs (Doughari *et al*., 2007)[2]. Its fruits, leaves and flowers are edible. Its roots can be used as medicine for renal and urinary bladder problem, and its seeds have anthelmintic activity (Doughari *et al*., 2007)[2]. Infectious diseases are the world's major threat to human health and account for almost 50000 deaths every day (Ahmad and Beg, 2001)[3].

The wound healing activity of the leaf extract of *Carica papaya* in experimentally induced excision and dead space wounds in diabetic rats. The wound healing processes are further worsened by the entry of pathogens. It is common traditional practice to treat the wound with the leaf-extract of papaya to accelerate the healing action. There are many reports available which demonstrate the wound-healing property of the papaya leaves. In addition, papaya leaves possess antibacterial activity which might prevent the multiplication of wound infection-causing bacteria. With this in mind, the present investigation was undertaken to predict the antibacterial properties of papaya leaves against some wound infection causing pathogens and to justify plant-based compounds could replace synthetic ones. (Aruljothi *et al*., 2014)[4].

MATERIALS AND METHODS

Collection of Leaves

Plant material were collected from in an around Thondamanoorani, Pudukkottai District. The disease free, fresh, young, and green leaves were collected from the papaya plant.

Processing of plant material

The fresh leaves were harvested and properly washed in tap water, rinsed in sterile distilled water. The leaf was dried in the hot air oven at 40º C for 3 days. The dried leaves were pulverized, using sterile laboratory mortar and pestle, to obtain a powered form. These were stored in airtight glass containers protected from sunlight until required for analysis.

Pathogen Used in the Present Study

Sample collection from open plate method in Government Hospital, Gandarvakottai, Pudukkottai District and then, they were further reconfirmed by morphological, cultural, and biochemical characteristics. Isolation and identification of bacteria from followed by Bergey's manual (1993)[5]. Isolation and identification of fungi followed by Gilman1957 (soil mycology). The cultures were emulsified in 5ml of Nutrient Broth (NB) and 5ml of Potato Dextrose Broth (PDB) incubated for 24 hrs. Fresh cultures were employed for assessing antimicrobial activity of the papaya leaf-extracts.

Preparation of leaf extract

The crude extract from the leaves of papaya was prepared according to the method proposed by Alabi *et al.* (2012)[6]. The aqueous extract was prepared by suspending 100g of powdered leaves in 200ml of distilled water. This mixture was diluted with 300ml of distilled water, and then allowed to stand for 24 hrs. The resulting extract was decanted and filtered through a Whatman filter paper. The filtrate was then concentrated with rotary evaporator at 45ºC. (this is the same procedure adopted for preparing acetone, ethanol and methanol extracts -100g of powder + 500ml of 95% ethanol, 100g of powder + 500ml of 95% acetone).

Antimicrobial Assay

The agar well diffusion method (Perez *et a*l., 1990)[7] as adopted earlier (Ahmad *et al*., 2001)[3] was used; 0.1 ml of diluted inoculum (105 CFU:ml) of test organism was spread on Mueller Hinton agar plates.

Wells of 8 mm diameter were punched into the agar medium and filled with100 ml (150 mg:ml) of plant extract, solvent blanks, antibiotic (chloramphenicol, 100 mg:ml conc.) to which the test bacteria were sensitive and antibiotic (Fluconazole , 100 mg:ml conc.) to which the test fungi were sensitive. The plates were incubated for 72 h at25°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Control test was carried out using 10mg/ml of chloramphenicol.

Phytochemical analysis

Phytochemical analysis for major Phytoconstituents of the plant extracts was under taken using standard qualitative methods as described by various authors (Vogel, 1958; *Kapoor et al.*, 1969; Odebiyi and Sofowora, 1990)[8-10]. The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

Thin layer chromatography (TLC) of plant extracts

TLC of the *carica papaya* leaf extracts with strong antimicrobial activity was carried out. Different solvent systems were used for different classes of compounds based on the polarity of the organic solvents. TLC micro slides were prepared as described by Harborne (1984)[11] with silica gel G. About 2 ml of plant extract was applied to the TLC chromatogram. Tannic acid, resorcinol, and anthrone were used as control. Solvent systems used were (1) petroleum ether and benzene, 1:1, (2) benzene and chloroform 1:1, (3) benzene and ethyl acetate 2:1, (4) acetone and alcohol, 1:1, and (5) methanol and water 1:1. Individual *R*ff or each spot was measured. TLC spots were visualized under UV light and adequate TLC reagents were used to detect the phytoconstituent.

RESULTS AND DISCUSSION

The present study ten microbes isolated and identified five human pathogenic bacteria and five fungi namely such as *Bacillus subtilis, Clostridium tetani, Escherichia coli, Proteus vulgaris, Staphylococcus aureus* and fungi such as *Aspergillus conicus, Aspergillus flavus, Aspergillus niger, Aspergillus sulphurous* and *Rhizopus* (Table No.1 and 2) and the analysis of phytochemical compounds present in various extract of acetone, aqueous, ethanol and methanol of *carica papaya* were analysed and the results were showed in (Table No. 5). The *carica papaya* showed the presence of Alkaloids, Flavonoids, phenols, Saponins, and Sterols. Emergence of human pathogenic microorganisms as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. In the present antibacterial activities of Acetone, Aqueous, Ethanol, Methanol extracts of *carica papaya* was assayed against various microbial pathogens shown in (Table No. 3 and 4) highest activity in acetone extract 13mm *Staphylococcus aureus*, lowest activity in aqueous extract 3mm *Escherichia coli* and highest activity in Acetone extract 18mm *Aspergillus flavus*, lowest activity in methanol extract 5mm *Rhizopus. Carica papaya.* Ethan botanical and phytochemical data, plant leaf used along with their acquisition code number are given in Table No.5.

Among the gram - positive and gram - negative bacteria tested, gram – negative bacteria were more susceptible to the extracts. This result, however, is at disparity with an earlier report indicating that plant extracts are more active against gram- positive bacteria than gram-negative bacteria (jigna and chanda., 2006**)**[12]. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Rahman *et al*., 2011)[13].

In our present investigation, all the extracts prepared from leaf (acetone, aqueous, ethanol and methanol) exhibited highest antimicrobial activity against pathogenic microbes bacteria and fungi. These results are in agreement with results declared by many researches. Nirosha and Mangalanayaki (2013)[14] also reported gram negative bacteria are more susceptible to the extracts of papaya leaf and stem. But the results of Suresh *et al*., (2008)[15] showed the antibacterial activity of the papaya leaf extract was more pronounced on gram positive than gram negative bacteria. According to Jigna and Sumitra (2006)[12], the plant leaf-extracts are more active against gram positive than gram negative

bacteria. Our investigation results are contrary with their findings. In our present study, it could be concluded that the papaya leaves might effectively inhibit the growth of certain wound infection-causing pathogens without any side effects. The active principle compound may be extracted and further purified, and incorporated as a base compound for the preparation of topical ointment (Aruljothi *et al*., 2014)[4] An aqueous extract of *Carica papaya* was showed antagonistic effect on the growth of various tumor cell lines and also on human lymphocytes (Otsuki *et al*., 2010)[16].

Based on the limited spectrum of activity of the other extracts compared with the ethanol extracts, it suggests that the active component is more soluble in ethanol than in the other solvents. The result further showed that the dried sample was effective against both Gram-positive and Gram-negative bacteria while the fresh sample was more effective against Gram negative bacteria. The fact that the dried sample extracts were active against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This result is very significant because of the possibility of developing therapeutic substances that may be more active against multidrug-resistant organisms. This observation is in accordance with the reports of (Doughari *et al*., 2007)[2].

Table No.1: Isolation of microbes

S.No	Organism name	Medium	Incubation	Colonies morphology
	Isolation of bacteria			
1	***Bacillus subtilis***	Nutrient agar	37ºC for 24hours	Mucoid colonies (round) colonies
2	***Clostridium tetani***	Nutrient agar	37ºC for 24 hours	Whitish colonies
3	***Escherichia coli***	EMB agar	37ºC for 24hours	Metallic sheen colonies
4	***Proteus vulgaris***	Nutrient agar	37ºC for 24hours	Milky white colonies
5	***Staphylococcus aureus***	Nutrient agar	37ºC for 24hours	Yellowish brown colonies
	Isolation of Fungi			
1	***Aspergillus conicus***	Potato dextrose agar	25ºC for 72 hours	Velvety red colonies
2	***Aspergillus flavus***	Potato dextrose agar	25ºC for 72 hours	Cottony greenish colonies
3	***Aspergillus niger***	Potato dextrose agar	25ºC for 72 hours	Cottony dark black colonies
4	***Aspergillus sulphureus***	Potato dextrose agar	25ºC for 72 hours	Velvety block colonies
5	***Rhizopus***	Potato dextrose agar	25ºC for 72 hours	White to cream

Table No.2: Biochemical test

S.No	Test name	Organism name				
		B.subtilis	***C.tetani***	***E.coli***	***P.vulgaris***	***S.aureus***
1	Morphology	rod	rod	rod	rod	Coccus
2	Arrangement	pairs, chains	single	single	single	irregular clusters
3	Gram staining	G^{+ve}	G^{+ve}	G^{-ve}	G^{+ve}	G^{+ve}
4	Motility	positive	motile	positive	positive	negative
5	Indole	positive	positive	positive	positive	negative
6	MR	negative	negative	positive	positive	negative
7	VP	positive	negative	negative	negative	positive
8	Citrate	positive	positive	negative	positive	positive
9	H_2S	negative	negative	negative	positive	negative
10	Nitrate red.	positive	negative	positive	positive	positive
11	Urease	negative	negative	negative	positive	positive
12	Catalase	positive	negative	positive	positive	positive
13	Oxidase	negative	negative	negative	negative	negative
14	Glucose	positive	negative	positive	positive	positive
15	Sucrose	positive	negative	positive	positive	positive
16	Lactose	negative	negative	positive	negative	positive

Table No.3: Antibacterial activity of *Carica papaya* L.

S.No	Test organism	Inhibition of growth (Diameter in mm)			
		Acetone extract	Aqueous extract	Ethanol extract	Methanol extract
1	*Bacillus subtilis*	9	5	7	5
2	*Clostridium tetani*	8	7	-	6
3	*Escherichia coli*	10	3	11	7
4	*Proteus vulgaris*	7	-	9	10
5	*Staphylococcus aureus*	13	-	7	6

Table No.4: Antifungal activity of *Carica papaya* L.

S.No	Test organism	Inhibition of growth (Diameter in mm)			
		Acetone extract	Aqueous extract	Ethanol extract	Methanol extract
1	*Aspergillus conicus*	7	-	12	10
2	*Aspergillus flavus*	-	-	18	15
3	*Aspergillus niger*	11	-	17	7
4	*Aspergillus sulphureus*	10	-	-	10
5	*Rhizopus*	7	-	10	5

Table No.5: Phytochemical analysis

S.No	Compounds	Result
1.	Alkaloids	+
2.	Flavonoids	+
3.	Phenols	+
4.	Saponins	+
5.	Sterols	+

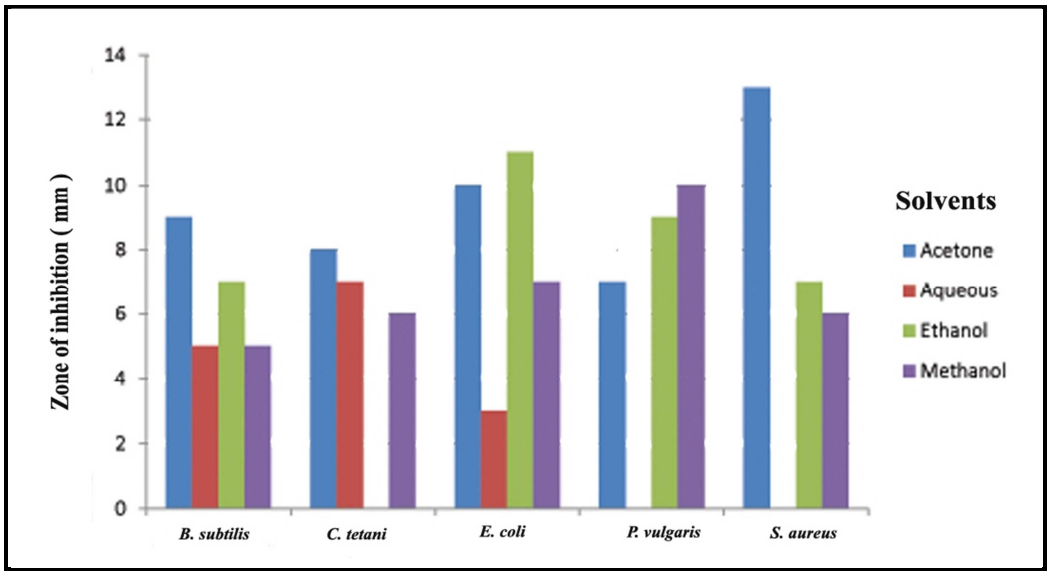

Figure No.1: Antibacterial activity of *Carica papaya* L. against bacterial pathogens as indicated by the zone of growth inhibition (mm)

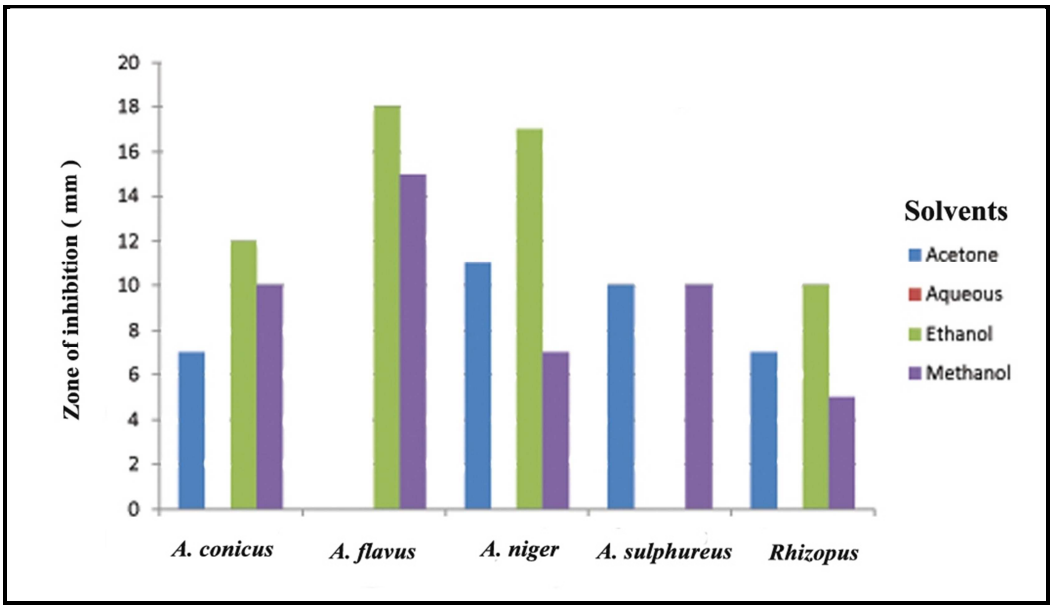

Figure No.2: Antifungal activity of *Carica papaya* L. against fungal pathogens as indicated by the zone of growth inhibition (mm)

CONCLUSION

All the leaf extracts of *Carica papaya L.* exhibited greater activity towards bacteria and fungi. The extract demonstrated higher activities against all the bacteria and fungi tested, with the highest activity (acetone extract of 13 mm zone of inhibition) demonstrated against *Saphylococcus aureus* and (ethanol extract of 18 mm zone of inhibition) demonstrated against *Aspergillus flavus*. Qualitative phytochemical tests, thin layer chromatography and TLC-bioautography of certain active extracts demonstrated the presence of common phytocompounds in the leaf extracts including alkaloids, flavonoids, phenols, saponins and sterols as major active constituents.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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