



ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *CARICA PAPAYA* AGAINST INFECTION-CAUSING BACTERIA

¹Abhilasha Shrivastava, ²Bhajan Lal Saket, ²Bharat Choudhary

¹Professor, Dept. of Botany, Govt. Model Science College, Rewa (M.P.)

²Research Scholar, Centre for Biotechnology Study, A.P.S. University, Rewa (M.P.)

*Corresponding Author: Bhajan Lal Saket

Research Scholar, Centre for Biotechnology Study, A.P.S. University, Rewa (M.P.).

Article Received on 28/04/2018

Article Revised on 18/05/2018

Article Accepted on 08/06/2018

ABSTRACT

Due to increase in the thrust for the production of plant-based antimicrobials, the present study was performed on *Carica papaya* leaves. The study revealed that papaya leaves could contain active antimicrobial compounds which may hinder the growth of wound infection-causing pathogens in invitro conditions. The leaf extract was prepared by using acetone, methanol, and water. The antimicrobial nature of the extract was studied by agar well diffusion method against wound infection-causing pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The acetone leaf extracts exerted pronounced antibacterial effect on gram negative bacteria especially *Pseudomonas sp.* The results obviously justified the importance of topical application of papaya leaf extracts to treat the wound infection as a traditional practice.

KEYWORDS: Plant-based antimicrobials, *Pseudomonas sp.*, acetone, methanol, and water.

1. INTRODUCTION

Emergence of resistant strains of pathogenic microorganism has also continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use (Timothy and Idu, 2011). 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 (Latha and Kannabiran, 2006).

The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, and Africa (Bibitha *et al.*, 2002).

Sofowora (1982) and Balandrin *et al.* (1985) defined medicinal plants as a plant in which one or more organs contain substances that can be used for therapeutic purposes or which it precursors for the manufacturing of drugs are useful for disease therapy. Since medicinal plants do not nearly save people from feeling pain but permit them to emerge unscathed, they deserve investigation.

Each part of papaya tree possess economic value when it is grown on a commercial scale (Krishna *et al.*, 2008). Even though the active components are normally extracted from all parts of the plant, the concentration of these components vary from structure to structure.

Papaya (*Carica papaya* Linn) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. However, parts known to contain the highest concentration of the principles are preferred for therapeutic purposes and it can either be the leaves, stem, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits, and the seeds (Kafaru, 1994). *C. papaya* belongs to the family Caricaceae. It is known by a variety of names, viz., pawpaw, papaya, papayer, pepol, tinti, chich put, fan kua, wan shou kuo, Kavunagaci, kepaya *etc.* Various parts of the papaya plant, which include the leaves, fruit, seed, latex, and root, are known to contain bioactive compounds. The plant parts are found to possess some properties like analgesic, amebicide, antibacterial, cardiotoxic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic, and vermifuge (Afolayan, 2003).

The leaves of the papaya plants contain chemical compounds of karpain, substance which kills microorganisms that often interfere with the digestive function (Udoh *et al.*, 2005). Chymenopapain and papain are the two important bioactive compounds present in *C. papaya*. Papaya leaves are made as tea for the treatment of malaria. Antimalarial and anti-plasmodial activity has been noted in some preparations of the plant. Papaya leaf-extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5, 7-

dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid (Romasi *et al.*, 2011; Peter *et al.*, 2014). During the last few decades, considerable progress has been achieved regarding the therapeutic properties of papaya.

The use of *C. papaya* L (Caricaceae) in traditional medicine relies on papain, the active principle which exerts an ulcer protective effect. The *C. papaya* possess antimicrobial, antioxidant, and anti-inflammatory activities. It is reported to heal chronic ulcer. Shivananda Nayak *et al.* (2007) examined the wound healing activity of the leaf extract of *C. 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.

2. MATERIALS AND METHODS

2.1. Collection of Leaves

Disease free, fresh, young, and green leaves were collected from the papaya plants. The leaves were washed thoroughly 3–10 times in sterile distilled water. Then, they were air-dried under shade at room temperature for 8 days and finely powdered using a blender.

2.5. RESULT

Investigation on Antibacterial Activity of *Carica Papaya* Leaf Extracts against Wound Infection-Causing Bacteria

Table 1: Antibacterial activity of acetone extract of *Carica papaya* leaf on wound infection causing pathogens (well diffusion method).

S.NO	Wound pathogens	Diameter of zone of inhibition (mm)			
		25	50	75	100
		mg/ml	mg/ml	mg/ml	mg/ml
1	<i>Staphylococcus aureus</i>	0	0	10	12
2	<i>Escherichia coli</i>	0	10	12	13
3	<i>Klebsiella pneumonia</i>	0	10	11	14
4	<i>Proteus vulgaris</i>	0	12	13	15
5	<i>Pseudomonas aeruginosa</i>	0	14	17	24

2.2. Pathogen Used in the Present Study

The test pathogens were procured in Centre for Biotechnology Studies, A.P.S. University and then they were further reconfirmed by morphological, cultural and Biochemical characteristics. The cultures were emulsified in 5ml of Nutrient Broth (NA) and incubated for 24 hrs. Fresh cultures were employed for assessing antibacterial activity of the papaya leaf-extracts.

2.3. Crude Extract Preparation

The crude extract from the leaves of papaya was prepared according to the method proposed by Alabi *et al.* (2012). 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 45°C. (this is the same procedure adopted for preparing acetone and methanol extracts – 100g of powder + 500ml of 95% ethanol, 100g of powder + 500ml of 95% acetone)

2.4. Test for Antibacterial Activity

Agar well diffusion method was adopted to assess the antibacterial activity of papaya leaf-extracts against wound pathogens. The crude extracts were further diluted with diluent at the concentration ranging 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml. For the test, Muller-Hinton agar plates were swabbed with test organism, and the wells (5mm) were filled with different concentration of the extracts. After incubation for 24 hrs, the antibacterial efficiency of the leaf-extract were determined by measuring the zone of inhibition formed around the well.

Table 2: Antibacterial activity of methanol extract of *Carica papaya* leaf on wound infection causing pathogens (well diffusion method).

S.NO	Wound pathogens	Diameter of zone of inhibition (mm)			
		25	50	75	100
		mg/ml	mg/ml	mg/ml	mg/ml
1	<i>Staphylococcus aureus</i>	0	0	10	14
2	<i>Escherichia coli</i>	0	12	13	17
3	<i>Klebsiella pneumonia</i>	0	10	12	15
4	<i>Proteus vulgaris</i>	0	11	14	16
5	<i>Pseudomonas aeruginosa</i>	0	13	24	26

Table 3: Antibacterial activity of aqueous extract of *Carica papaya* leaf on wound infection causing pathogens (well diffusion method).

S.NO	Wound pathogens	Diameter of zone of inhibition(mm)			
		25	50	75	100
		mg/ml	mg/ml	mg/ml	mg/ml
1	<i>Staphylococcus aureus</i>	0	0	0	10
2	<i>Escherichia coli</i>	0	0	11	11
3	<i>Klebsiella pneumonia</i>	0	0	10	11
4	<i>Proteus vulgaris</i>	0	0	12	13
5	<i>Pseudomonas aeruginosa</i>	0	0	14	15

3. RESULT AND DISCUSSION

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 properties (Adriana *et al.*, 2007). Plants are the cheaper and safer alternative sources of antimicrobials (Pretorius *et al.*, 2001; Doughari *et al.*, 2007).

Anibijuwon and Udeze (2009) extracted bioactive compounds from leaf and root of *C. papaya* using water and organic solvents, which were investigated for antibacterial activity against some human pathogenic bacteria. Both leaf and root extracts showed pronounced inhibition against gram positive bacteria than the gram negative bacteria tested, but the highest activity was seen with *Pseudomonas aeruginosa*. In our present study, the better inhibition was seen with acetone extract against *Pseudomonas aeruginosa* than the other organisms tested (Table 1).

Ocloo *et al.* (2012) studied the efficacies of crude extracts of *C. papaya* seeds against *Staphylococcus aureus*, *Escherichia coli* and *Shigella flexneri* using disc diffusion method. The crude organic (acetone, methanol) extracts inhibited the growth of all three organisms. The aqueous extract of *C. papaya* leaves were investigated for evaluation of wound healing potential in rats. These results strongly document the beneficial effects of plant extract for the acceleration of wound healing process in rats (Mahmood *et al.*, 2005).

In our present investigation, all the extracts prepared from leaf (acetone, water, and methanol) exhibited

highest antibacterial activity against gram negative organism. These results are in agreement with results declared by many researches. Nirosha and Mangalanayaki (2013) also reported gram negative bacteria are more susceptible to the extracts of papaya leaf and stem. But the results of Suresh *et al.* (2008) 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), the plant leaf-extracts are more active against gram positive than gram negative bacteria. Our investigation results are contrary with their findings.

Ogunjobi and Elizabeth (2011) reported that leaf and seed extract of *C. papaya* have inhibitory effect on *Staphylococcus aureus*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Salmonella typhi*. They also demonstrated that ethanol extracts contributed much more antibacterial activity than aqueous extract (Ezeifeke *et al.*, 2004). Our findings are correlated with their results, herein, acetone extracts demonstrated the highest antibacterial activity than aqueous and methanol extracts (Tables 1–3). The highest antibacterial activity brought about by organic solvent might be due to better solubility of the active components of leaves.

From 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.

REFERENCES

- Adriana, B., Almodóvar, A.N.M., Pereiral, C.T., and Mariângela, T.A. Antimicrobial efficacy of Curcuma zedoaria extract as assessed by linear regression compared with commercial mouthrinses. *Braz. J. Microbiol.*, 2007; 38: 440–445.
- Afolayan, A.J. Extracts from the shoots of *Aretotis arotoides* inhibit the growth of bacteria and fungi. *Pharm. Biol.*, 2003; 41(1): 22–25.
- Alabi, O.A., Haruna, M.T., Anokwuru, C.P., Jegede, T., Abia, H., Okegbe, V.U., and Babatunde, E. Esan. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of carica papaya (L) on clinical bacterial and fungal isolates. *Advances in Applied Science Research*, 2012; 3(5): 3107–3114.
- Anibijuwono, I. I. and Udeze, A.O. Antimicrobial activity of papaya on some pathogenic organisms of clinical origin from south-western Nigeria. *Ethnobotanical Leaflets*, 2009; 13: 850–864.
- Balandrin, M.F., Kjocke, A.J., Wurtele, E.S., and Bollinger, W.H. Natural plant chemicals sources of industrial and mechanical materials. *Science*, 1985; 228: 1154–1160.
- Bibitha, B., Jisha, V.K., Salitha, C.V., Mohan, S., and Valsa, A.K. Antimicrobial activity of different plant extracts. *Short Communiacion. Indian J. Microbiol.*, 2002; 42: 361–363.
- Doughari, J.H., El-mahmood, A.M., and Manzara, S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afri. J Microbiol. Res.*, 2007: 37–41.
- Ezeifeke, G.O., Orji, M.U., Mbata, T.I., and Patrick, A.O. Antimicrobial activities of *Cajanusajan*, *Garciniakol*, and *Xylophia aethiopica* on pathogenic microorganisms. *Biotechnology*, 2004; 3(1): 41–43.
- Jigna, P. and Sumitra, C. In-vitro antimicrobial activities of extracts of *Launaea procumbns* Roxb. (Labiatae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cypperaceae). *African Journal of Biomedical Research*, 2006; 9(2): 89–93.
- Kafaru, E. Immense Help From Nature's Workshop. Elika Health Services Ltd, Academic Press Plc. Lagos, Nigeria, 1994: 1–27.
- Krishna, K.L., Paridhavi, M., and Patel, J.A. Review on nutritional, medicinal and pharmacological properties of papaya (*carica papaya* Linn.) natural product radiance. *Indian Journal of Natural Products and Resources (IJNPR)*, 2008; 7(4): 364–373.
- Latha, S.P. and Kannabiran, K. Antimicrobial activity and phytochemicals of *Solanum trinobatum* Linn. *African Journal of Biotechnology*, 2006; 5(23): 2402–2404.
- Mahmood, A.A., Sidik, K., and Salmah, I. Wound healing activity of *Carica papaya* L. aqueous leaf extract in rats. *International Journal of Molecular Medicine and Advance Sciences*, 2005; 1(4): 398–401.
- Nirosha, N. and Mangalanayaki, R. Antimicrobial activity of leaves and stem extract of *Carica papaya* L. *IJAPBC*, 2013; 2(3): 2277–4688.
- Ocloo, A., Nwokolo, N.C., and Nicholas, T.K.D. Dayie. Phytochemical characterization and comparative efficacies of crude extracts of *Carica papaya*. *Int. J. Drug Res. Tech.*, 2012; 2(5): 399–406.
- Ogunjobi, A.A. and Elizabeth, O.T. Comparative study of antimicrobial activities of ethanol extracts of the Bark and seed of *Garcinia kola* and *Carica papaya*. *Afr. J. Biomed. Res.*, 2011; 14: 147–152.
- Peter, J.K., Kumar, Y., Pandey, P., and Masih, H. Antibacterial activity of seed and leaf extract of *Carica papaya* var. Pusa dwarf Linn. *IOSR – Journal of Pharmacy and Biological Sciences*, 2014; 9(2): 29–37.
- Pretorius, C.J. and Watt, E. Purification and identification of active components of *Carpobrotus edulis* L. *J. Ethnopharmacol*, 2001; 76: 87–91.
- Peter, J.K., Kumar, Y., Pandey, P., and Masih, H. Antibacterial activity of seed and leaf extract of *Carica papaya* var. Pusa dwarf Linn. *IOSR – Journal of Pharmacy and Biological Sciences*, 2014; 9(2): 29–37.
- Pretorius, C.J. and Watt, E. Purification and identification of active components of *Carpobrotus edulis* L. *J. Ethnopharmacol*, 2001; 76: 87–91.
- Romasi, E.F., Karina, J., and Parhusip, A.J.N. Antibacterial activity of papaya leaf extract against pathogenic bacteria. *Makara, Teknology*, 2011; 15(2): 173–177.
- Shivananda Nayak, B., Pereira, L.P., and Maharaj, D. Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats. *Indian Journal of Experimental Biology*, 2007; 45: 739–743.
- Sofowora, E.A. *Medicinal Plants and Traditional Medicine in Africa*. John Wiley and Sons, U.S.A., 1982; 10–40.
- Shrikant Kol et al. Identification of microbes related to crude oil components consumption and optimization of their growth parameters: *International Journal of Development Research*, November, 2014; 4(11): 2437–2441.
- "Saket, R. D., et al. "Compliment Receptor Type-1 (CD35) Gene Polymorphism and Plasmodium Falciperum Malaria." *Int J Med Biotech-nol Genetics*, 3.3 (2015): 27-33.
- Padma Shri Mishra, Shrikant Kol, Rashmi Arnold and R.M. Mishra. 2016. Pathogenicity of Dental Caries; Isolation and Antimicrobial Efficacy by Herbal Plants. *Int. J. Curr. Microbiol. App. Sci.*, 5(7): 929-935.
- Suresh, K., Deepa, P., Harisaranraj, R., and Vaira Achyadhan, V. Antimicrobial and phytochemical investigation of the leaves of *Carica papaya*, *Cynodon dactylon* (L) Pers., *Euphorbia hirta* L., *Melia azedarach* L. and *Psidium guajava* L. *Ethnobotanical Leaflets*, 2008; 12: 1184–1191.

28. Timothy, O. and Idu, M. Preliminary phytochemistry and in vitro antimicrobial properties of aqueous and methanol extracts of *Icacina trichantha*. *Oliv. Leaf.*, 2011; 1(3): 184–188.
29. Neeraj K. Charmkar, Shrikant Kol and Rishabh D. Saket, 2017. “Associational study of fto gene polymorphism with obesity in vindhyan” *International Journal of Current Research*, 9,(09): 56902-56905.region”.
30. Udoh, P., Essien, I., and Udoh, F. Effect of *Carica papaya* (Paw Paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. *Phytother Res.*, 2005; 19: 1065–1068.