IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 2, Issue 6 Part: II (Sep. – Oct. 2016), PP 01-06
www.iosrjournals.org

Antimicrobial Activity of Carica papaya, Piper nigrum and Datura stramonium Plants on Drug Resistant Pathogens Isolated from Clinical Specimens

Dr. Nagesh Malik[1*], Samreen Ahmed[2]
[1,2] *Department of Microbiology, V. E. S. College of Arts, Science and Commerce, Chembur, Mumbai 400071, India.*

Abstract: *The aim of the present study was to investigate the antimicrobial activity and phytochemical screening of Carica papaya, Piper nigrum and Datura stramonium plant extracts. Ethanol and Methanol were used as the solvents for extraction. The antimicrobial activities of these plant extracts were tested against the drug resistant Gram-positive and Gram-negative bacterial strains. The bacteria used in the study were Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter baumannii, Salmonella spp., Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecium and Neisseria spp. The ethanol and methanol extracts of leaves of Datura stramonium and Carica papaya showed promising results against the drug resistant pathogens, where as Piper nigrum was found to be inefficient in inhibiting the drug resistant pathogens. The ethanol extracts gave larger zone of inhibition than methanol extracts, suggesting that ethanol is a better solvent in terms of extraction of phytochemicals from the plants. The extracts worked better towards the inhibition of gram positive bacteria Staphylococcus aureus and Staphylococcus epidermis than the gram negative bacteria used in this study.*

Keywords: *Antibacterial activity, Drug resistant pathogens, ethanol, methanol, Phytochemical screening*

I. Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these plants are still widely used in ethnomedicine around the world.[2] Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics. New and emerging drug resistant bacterial strains, particularly methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), Mycobacterium tuberculosis (MTB), and multidrug resistance (MDR) Gram-negative bacteria, are increasing worldwide and add to the gravity of the situation. Development of resistant bacteria due to the chromosomal mutations is more commonly associated with the horizontal transfer of resistance determinants borne on mobile genetic elements [11]. Plants, particularly ethnomedicinal plants are important sources of natural products. They are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids and have been well-established to possess antimicrobial properties. Many plants have been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistant modifying agent [9]. Ethnomedicine is concerned with the study of medical systems from the native's point of view. The ethnomedical approach proves particularly useful for the study of indigenous therapeutic agents because it allows the researcher to understand treatment patterns according to native explanatory models instead of only through the lens of biomedicine [5]. Plants are rich with a range of secondary metabolites such as tannins, alkaloids, flavonoids and a few more, which have been found in vitro to have antimicrobial properties. A number of phytotherapy manuals and research journals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity on humans [9]. Thereby, plants are now being considered as a source of antimicrobials not only to treat bacterial infections but also certain parasitic and viral infections. Considering the potency of plants derived drugs requires the research to explore the varied aspects which would remain unexplored [6]. The isolation of bioactive compounds from medicinal plants, based on traditional use or ethnomedical data, is a highly promising potential approach for identifying new and effective antimalarial drug candidates. [13]

II. Materials And Methods

A.Sample Collection: The following samples of plants were collected from different sources as shown in the Table No.1

Table No.1: Samples of plants and their sources

Sr No.	Common Name	Scientific Name	Part Used	Source of Collection
1	Datura	Datura stramonium	Leaf	Kharghar, Flower Vendor
2	Papaya	Carica papaya	Leaf	Chembur, Market
3	Black Pepper	Piper Nigrum	Fruits	Chembur, Condiment Shop

B. Screening of Clinical Pathogens for the drug resistance profile

- **Media used:-** St. Mueller's Hinton Agar, St. Trypticase Soya Broth
- **Antibiotics:-** Standard Antibiotic Discs impregnated with the test antibiotics which were selected by referring standard CLSI chart.

C. Plant Tissue Homogenization and Extract Preparation

- **Plant Materials: -** Dried plant leaves of *Datura stramonium*, *Carica papaya* and fruits of *Piper nigrum*.
- **Reagents:-** 95% Ethanol ,95% Methanol

D. Antibacterial Activity of the Plant Extract and Determination of Minimum Inhibitory Concentration.

- **Media used:-** Sterile Muller Hinton Agar plates
- **Test Samples:-** Plant Extracts
- **Reagents:-** 95% Ethanol, 95% Methanol

E. Preliminary Phytochemical Analysis of the Plant Extracts

Reagents:-

Mercuric chloride, Distilled water, Potassium iodide, 2% H_2SO_4, Conc. H_2SO_4, Diluted ammonia solution, Fehling's solution A and Fehling's solution B, Chloroform, aqueous 5% $FeCl_3$, 5%w/v solution of $FeCl_3$ in 90% alcohol

Antibiotic Susceptibility Test (AST)

The clinical pathogens were subjected to AST to determine the antibiotic resistance profile for the isolates, by the Kirby Bauer's method, using Mueller-Hinton Agar plates. A set of 12 antibiotics were selected to be tested against each test culture. Test cultures which were found to be resistant to four or more than four selected antibiotics were considered as drug resistant pathogens and were selected for further studies.

Homogenization and Preparation of plant extracts

Fresh plants parts (leaves, rhizomes or seeds) were purchased from local markets or from the residential gardens. They were washed twice, once with tap water and later with distilled water. The washed samples were left to dry in incubator at 55°C for a 24 hours.

The dried samples were made into fine powder by crushing in the mortar and pestle.

50gm of the powdered sample was soaked in 50ml of the solvents methanol and ethanol separately. The extraction was carried out continuously for a period of seven days by placing flasks on the shaker at 150 rpm. The plants extracts was concentrated by reducing the extract volume to approximately 5ml by heating at 64.7 °C and 78.37 °C for methanol and ethanol extracts respectively. The homogenized plant extracts were filtered first through the muslin cloth and then through whatman filter paper. The extracts were collected in screw-cap bottles and stored in refrigerator at 4 °C.

Antibacterial Activity of the Plant Extract by Agar Cup Diffusion Method

This was carried out by using standard Agar Cup Diffusion Method in which St. Mueller's-Hinton Agar assay plates were used, growth inhibition was measured as diameters of inhibitory zone and compared with the control.

III. Results And Discussions

Antibacterial Susceptibility Test

Amongst the test cultures *E Coli* was found to be resistant to only Nitrofurantoin and sensitive to remaining eleven antibiotics, *Enterococcus faecium* was found to be resistant to Vancomycin , Erythromycin, Tetracycline and Gentamycin, and sensitive to remaining eight antibiotics, Salmonella spp was found to be resistant to only Erythromycin. The remaining test cultures *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter baumannii, Proteus mirabilis* and *Neisseria* spp. were found to be

resistant to more than four antibiotics amongst the twelve antibiotics tested. Antibiotics for Antibiotic Susceptibility Test for Neisseria were selected by referring to the work of Reza Khaltabadi Farahani.
Of the above pathogens tested for their drug resistance profile *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas*, *Proteus mirabilis*, *Neisseria, Acinetobacter baumannii* and *Enterococcus faecium* were found to be resistant to four or more antibiotics tested.

Staphylococcus aureus remains an important cause of nosocomial blood- borne infection. These organisms are more likely to cause infections on the wards than in intensive care units. In our study, the antibiotic susceptibility test results for *Enterococcus faecium* are in agreement with the study conducted by Lynette M. Johnston et al. Overall, E. faecium was found to have a higher prevalence of resistance among the panel antibiotics, particularly tetracycline, vancomycin and erythromycin. It was even found to be resistant to gentamycin. [8]

Proteus mirabilis was found to be resistant to trimethoprim, kanamycin, tetracycline, ciprofloxacin and ampicillin. The results of this study are in agreement with the study conducted by Thomas T. Yoshikawa on drug resistant strains prevalent in hospital environment. [15]
Pseudomonas spp. was found to be resistant to neticillin, piperacillin, sulphafurazone, trimethoprim and tetracycline antibiotics.

Acinetobacter baumannii was found to be resistant a wide spectrum of antibiotics which includes ticarcillin, piperacillin, oxacillin, cefalothin, ceftazidime, nallidixic acid, norfloxacin, erythromycin, clindamycin and nitrofurantoin. *Neissseria* spp. was found to be resistant to penicillin, rifampicin, tetracycline, cefazolin and cephalothin based on the Antibiotic Susceptibility Test results. From a once easily treatable infection, *Neisseria* spp. has evolved into a challenging disease.

Antibacterial Activity of the Plant Extracts

In the present study, the antibacterial activity of ethanolic and methanolic extracts of seeds or rhizomes of *Carica papaya, Piper nigrum, Datura stramonium* were tested against eight drug resistant test cultures /clinical isolates Agar well-diffusion method.

Ethanolic and Methanolic extract of *Piper nigrum*

The methanolic as well as ethanolic extracts of the *Piper nigrum* seeds showed no antibacterial activity against the drug resistant test cultures /clinical isolates.
Phytochemicals having solubility in ethanol include tannins, polyphenols, polyacetylenes, flavonol, sterols and alkaloids. Accordingly, two solvents namely ethanol and methanol were selected for the plant extraction. the black pepper extracts exhibited no antibacterial activity in the two solvents against the test cultures... This is contradictory to the findings of Ram Kumar Pundir et al., (2010) [12] who had reported antibacterial activity of water, ethanolic and methanolic black pepper extracts against *Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Escherichia. coli*.

Pepper ethanol and aqueous extracts were analyzed by Shamsuddeen et al., (2009) [14]and it was found to contain phytochemicals like Alkaloids Flavonoids, Saponins and Reducing sugars in ethanol and aqueous extracts and lacked steroids and Tanins.
According to Ram Kumar Pundir et al., (2010), the antimicrobial activity of black pepper is due to the presence of essential oil (3%), whose aroma is dominated by monoterpenes hydrocarbons: sabinene, β-pinene and limonene.. The mechanism of action of terpene is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. [12]

Ethanolic and Methanolic extracts of ***Carica papaya***

The ethanol and methanol extracts of leaves of *Carica papaya* showed profound activity against the gram positive organisms than gram negative organisms. *Staphylococcus aureus, Staphylococcus epidermidis* were found to be inhibited maximum amongst the test cultures used. Papaya leaf extracts was found to be the only one that could all the gram negative test cultures including *Pseudomonas* spp, *Neisseria* spp., *Proteus mirabilis, Acinetbacter baumanni* and *Enerococcus faecium* were found to be inhibited to some extent. These result are as shown in Table No.2.

Table No.2: Antibacterial Activity of *Carica papaya* in terms of Diameter of Zone of Inhibition

Test Cultures	Leaf extracts of ***Carica papaya***	
	Ethanol	Methanol
Staphylococcus aureus	18	11.5
Staphylococcus epidermidis	15	12
Pseudomonas spp.	13	12.5
Acinetobacter baumannii	10	10
Enterococcus faecium	13	13.5

Proteus mirabilis	12	12
Neisseria spp.	14	12.5

Ethanol and Methanol leaf extracts of *Carica papaya* was found to inhibit all the test pathogens under study to some extent. The extracts demonstrated maximum antibacterial activity against the gram positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* but the extracts also inhibited all the gram negative bacteria including *Neisseria* which only was found to be inhibited by *Carica papaya* leaf extracts. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto-constituents of extracts of leaf on the target organism.

The study conducted by C. Baskaran et al.,2012[4] revealed the same pattern of inhibition that is gram positive bacteria were found to be inhibited to a greater extent as compared to gram negative bacteria. The comparison of zone of inhibition for the two extracts shows that the ethanolic extract gives better inhibition than methanolic extract. Thus, this study also demonstrates that the ethanol is a more efficient solvent in terms of extraction of bioactive compounds from leaf as compared to methanol.

The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. The presence of saponins supports the fact that pawpaw leaves have the cytotoxic effects such as permealization of the intestine as saponins are cytotoxic. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties as per [1]

The ethanol and methanol leaf extracts of Carica papaya were analyzed for the presence of phytochemicals. The results of this study revealed that extracts contained Alkaloids, Steroids, Glycosides, Saponins, Phenols and Caretenoids and did not showed the presence of Reducing Sugars, Tanins and Terpenoids. The results of this study are in accordance with the work of C. Baskaran et al., 2012. [4]

Ethanolic and Methanolic extracts of ***Datura stramonium***

The antimicrobial activity of ethanol and methanol extracts of leaves of *Datura stramonium* showed profound activity against the gram positive organisms with significant amount of inhibition against *Staphylococcus epidermidis* with zone diameter of 25mm, 27mm and *Staphylococcus aureus* with zone diameter of 30mm, 23mm for ethanol and methanol extracts. It was found that the extracts less effective against the gram negative organisms. *Proteus mirabilis*, *Neisseria* spp. were found to be unaffected by it but *Pseudomonas* spp. *Acinetobacter baumannii* and *Enterococcus faecium* were inhibited to some extent.

Ethanol and Methanol extracts of leaves of *Datura stramonium* showed promising antibacterial activity against the test pathogens. The extracts were found to be more effective against the gram positive bacteria than the gram negative bacteria. *Staphylococcus aureus* and *Staphylococcus epidermidis* were inhibited to a greater extent with zone diameter of inhibition exceeding 20mm. Amongst the gram negative bacteria *Acinetobacter baumannii* and *Enterococcus faecium* were also inhibited by the leaf extracts of *Datura stramonium* with zone diameter in the range of 14mm to 20mm suggesting that the extracts have the potential for use as an effective antibacterial agent for treating infections, as shown in Table No 3

Table No 3: Antibacterial Activity of *Datura stramonium* in terms of Diameter of Zone of Inhibition

Test Cultures	Leaf extracts of ***Datura stramonium***	
	Ethanol	Methanol
Staphylococcus aureus	30	23.5
Staphylococcus epidermidis	26.5	27
Pseudomonas spp.	-	-
Acinetobacter baumannii	12	14.5
Enterococcus faecium	19.5	20
Proteus mirabilis	-	-
Neisseria spp.	-	-

The resistance of the Gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts. [3]

In case of leaf extracts of *Datura stramonium* the solvents used for extraction of phytochemicals were ethanol and methanol. The antibacterial activity of the two solvents did not differed much in terms of antibacterial activity and it is evident from diameter of zone of inhibition. The secondary metabolites identified in the plant materials used in this study could be responsible for antimicrobial activity exhibited by these plants.
The leaf extracts of Datura stramonium were found to contain Alkaloids, Caretenoids, Steroids, Phenols, Saponins, Glycosides and Tannins. The extracts lacked Terpenoid, and Reducing sugar. The results of this study are in agreement with work conducted by [10].

Preliminary Phytochemical Analysis of the Plant Extracts

The preliminary phytochemical analysis was carried out in this study for the plant extracts which unravel the presence of various phytochemicals. The literature on antibacterial activity of plant extracts suggest that these phytochemicals identified plays a key role in inhibiting the bacteria, thereby reflecting the importance of the bioactive phytochemicals, possessing the desired antibacterial activity.

In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. These results are as shown in Table No 4

Table No 4: Preliminary Phytochemical Analysis of the Plant Extracts

Plant under study	***Carica papaya***		***Datura stramonium***	
Phytochemical Tests / Solvents	**EtOH**	**MeOH**	**EtOH**	**MeOH**
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	-	-	-	-
Glycosides	+	+	+	+
Phenols	+	+	+	+
Tannins	-	-	+	+
Flavanoids	+	+	+	+
Saponins	+	+	+	+
Reducing Sugars	-	-	-	-
Caretenoids	+	+	+	+

This study dealt with the screening of drug resistance profile of the pathogens obtained from the hospitals and testing the antibacterial activity of the ethanol and methanol extracts of the *Piper nigrum, Datura stramonium* and *Carica papaya*. The work also revealed that of the nine bacterial pathogens tested for their drug resistance profile seven of the bacteria were found to be drug resistant. The present work demonstrates the antimicrobial potential of plant extracts by using two solvents. The results of this study reveals that ethanol and methanol extracts of *Datura stramonium* and *Carica papaya* leaves posses a considerable amount of antibacterial activity, ethanol was found to be a better solvent when compared to methanol. *Piper nigrum* fruit extracts demonstrated no antibacterial activity against the test pathogens. The results also indicate that the plant extracts have little or no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the gram negative organisms. The observed inhibition of Gram-positive bacteria, *Staphylococcus epidermidis* and *Staphylococcus aureus*, suggests that the plant extracts possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol as the solvent. Preliminary phytochemical analysis of the extracts revealed the presence of Alkaloids, Terpenoids, Glycosides, Reducing Sugars, Steroids, Phenols, Flavanoids, Caretenoids, etc to varying extent in the extracts. Past work suggest that these phytochemicals are the major contributing factor towards the antibacterial activity of the extracts.

References

[1]. Ajayi I. A., Ajibade O. and Oderinde R. A. 2011. Preliminary Phytochemical Analysis of some Plant Seeds. 1.
[2]. Behboud Jafari, Amirreza Ebadi, Babak Mohammadi Aghdam and Zarifeh Hassanzade**,** 2012**.** Antibacterial Activitiesof Lemon Grass Methanol Extract and Essence on Pathogenic Bacteria. American-Eurasian J. Agric. & Environ. Sci. **12**:1042-1046.
[3]. Bipul Biswas, Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels and Anand Yadav. 2013**.** Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria. 2013:7.
[4]. C. Baskaran, V. Ratha bai, S.Velu and Kubendiran Kumaran**.** 2012. The efficacy of Carica papaya leaf extract on some bacterial and a fungal strain by well diffusion method. Asian Pacific Journal of Tropical Disease. 658-662.

[5]. Debasmita Dubey, Rabindra N Padhy. 2012. Surveillance of multidrug resistance of two Gram-positive pathogenic bacteria in a teaching hospital and in vitro efficacy of 30 ethnomedicinal plants used by an aborigine of India. The Asian Pacific Journal of Tropical Disease.
[6]. I.I. Anibijuwon and A.O. Udeze**.** 2009**.** Antimicrobial Activity of Carica Papaya (Pawpaw Leaf) on Some Pathogenic Organisms of Clinical Origin from South-Western Nigeria. Ethnobotanical Leaflets. **13**:850-64.
[7]. Kazhila C. Chinsembuand Marius Hedimbi**.** 2010. Ethnomedicinal plants and other natural products with anti-HIV active compounds and their putative modes of action. International Journal for Biotechnology and Molecular Biology Research. **6**:74-91.
[8]. Lynette M. Johnston and Lee-Ann Jaykus**.** 2012**.** Antimicrobial Resistance of Enterococcus Species Isolated from Produce. 3133–3137.
[9]. Pinanong Na Phatthalung, SasitornChusri and Supayang P Voravuthikunchai. 2012**.** Thaiethnomedicinal plants as resistant modifying agents for combating Acinetobacter baumannii infections. BMC Complementary and Alternative Medicine. **12**:56.
[10]. Priyanka Soni, Anees Ahmad Siddiqui, Jaya Dwivedi and Vishal Soni. 2012. Pharmacological properties of Datura stramonium L. as a potential medicinal tree: An overview. Asian Pacific Journal of Tropical Biomedicine. **2**:1002-1008.
[11]. Ramar Perumal Samy, Jayapal Manikandan, and Mohammed Al Qahtani**.** 2013. Evaluation of Aromatic Plants and Compounds Used to Fight Multidrug Resistant Infections. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine. Volume 2013:17 pages.
[12]. Ram Kumar Pundir and Pranay Jain. 2010. Comparative Studies on the Antimicrobial Activity of Black Pepper (Piper Nigrum) and Turmeric (Curcuma Longa). Volume 1.
[13]. Rios JL and RecioMC. 2004. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. **100**:80-4.
[14]. Shamsuddeen, U. Ameh, J. B. Oyeyi T. I., Dantata and A. A**.** 2009. Study of the Phytochemical and Invitro Antibacterial Activity of Some Medicinal Extracts on Some Bacteria Isolated from Meat Products. **2**:101– 104.
[15]. Thomas T. Yoshikawa, Sharon A. Shibata, Anthony W. Chow and Lucien B. Guze. 1978. Outbreak of Multiply Drug-Resistant Proteus mirabilis Originating in a Surgical Intensive Care Unit: In Vitro Susceptibility Pattern. **13**:177-179.