



Anti-microbial activity of seed extract of *Cucumis sativus* L., *Carica papaya* L. and *Annona squamosa* L.

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Article History:

Received on: 22 Sep 2020

Revised on: 22 Oct 2020

Accepted on: 24 Oct 2020

Keywords:

Anti-microbial activity,
Antibiotic resistance,
Cucumis sativus L.,
Carica papaya L.,
Annona squamosa L.

ABSTRACT

In recent days, threat related to infectious and antibiotic-resistant pathogens are increasing widely, and this created an urge to search for an alternative way of producing drugs. The sources selected for the production of drugs must have some attributes like readily available, available in bulk quantities, easy processing and containing less or negligible toxic effects. Herbal medications and drugs derived from medicinal plants may serve as a smart and an alternate way. In this study, ethanolic seed extracts have been used from three readily available plants like *Cucumis sativus* L., *Carica papaya* L., *Annona squamosa* L. The antibacterial activity of ethanolic seed extracts was performed against the pathogens present in the pus sample using agar disc diffusion method. It was found that the ethanolic seed extracts of *Cucumis sativus* L. were found to have a maximum zone of inhibition of 14 mm at conc., of 900 μ g against *S. aureus*, *Carica papaya* L. was found to have maximum inhibition of 13mm at conc., of 900 μ g against *S. aureus* and *Annona squamosa* showed negligible resistance to all pus pathogens. From the various bioactive compounds present in the plants, an efficient drug can be manufactured, and it may find a great place in the pharmaceutical industry.



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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i4.3603>

Production and Hosted by

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INTRODUCTION

The skin act as a first-line defence mechanism, any incision in the skin structure leads to microbial contamination, due to penetration of pathogens into the skin. Folliculitis, Impetigo, furunculosis, abscesses, cellulitis, scarlet fever, erysipelas, necrotizing fasciitis etc. are some of the most occurring types of skin infection. These types of pyogenic infection cause severe local inflammation with pus (Shama *et al.*, 2018). Pallavali *et al.* (2017) reported that the most common isolated wound pathogens are *S. epidermidis*, *S. aureus*, *Streptococcus spp.*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and

Proteus species etc. Nowadays the development of an antibiotic-resistant organism is considered as one of the biggest threats faced by clinical sectors, due to indiscriminate use of commercial antibiotics for the treatment of infectious disease and wounds (Vikas *et al.*, 2013; Samrot *et al.*, 2019). This results in difficulties to diagnose and treat the related severe infections (Palma *et al.*, 2019). For the better and appropriate treatment, it is necessary to understand the anti-microbial susceptibility and pattern as the resistance to an antibiotic within the group of pyogenic bacteria is increasing gradually (Singh *et al.*, 2013).

Production of highly efficient drugs against microbial infection draws the attention of many researchers (Balakumar *et al.*, 2010). This resulted in an urge to find an alternative source like medicinal plants or seaweeds etc. for the production of efficient drugs because it is harmless, dependent, compatible (Samrot *et al.*, 2016a,b). In worldwide, Medicinal plants and its parts such as flowers, leaves, barks, seeds have been used for the production of drugs (Raji *et al.*, 2017). In early ages even before the existence of modern antibiotics and modern drugs and it is used in the treatment of various disorders like skin disorder, asthma, gastrointestinal symptoms, urinary problems etc. (Ali *et al.*, 2019; Phuyal *et al.*, 2019). Plants are the great source of Bioactive compounds they possess various phytochemical constituents like tannins, flavonoids, alkaloids etc. and various other secondary metabolites which can be used for the preparation of therapeutic compounds (Samrot *et al.*, 2018, 2016c).

In this study seeds of three common plants like *Cucumis sativus*, *Carica papaya L.*, *Annona squamosa L.* was used. The cucumber (*Cucumis sativus*) belong to the family of Cucurbitaceae, and it is a widely cultivated plant in the gourd family. It belongs to the family of a creeper which produces cylindrical edible fruit when ripe (Vora *et al.*, 2014). *Carica papaya L.* belongs to the family of Caricaceae. Each part of the tree consists of different pharmacological effect the seeds of the papaya are used as a substitute of pepper whereas, leaves were used in the treatment of malaria (Khan *et al.*, 2012). *Annona squamosa* is a plant widely grown in dry places and most commonly found in Egypt. It is considered to be one of the important pharmacological trees as it has antidiabetic, antibacterial and antioxidant properties and the ethanolic extract of stem and leaf poses anticancer activity (El-Chaghaby *et al.*, 2014). The aim of the present research to evaluate the antibacterial activities of ethanolic seed extracts of

L. against pus sample pathogens.

MATERIALS AND METHODS

Sample Collection

Papaya, Custard apple, Cucumber seeds were purchased from the local market of Udangudi town, Thoothukudi district, Tamil Nadu, India. The collected fruits were cut into small pieces; seeds of the fruits were separated and dried. They were washed well using clean water and dried in an oven at 60°C. The dried seeds were ground to make it as a powder for solvent extraction.

Preparation of Extracts

100ml of ethanol was added in a 250ml of conical flask along with 25g of grape seed powder and mixed well. The conical flask was kept aside for 72 hours and stirred occasionally. The dissolved extract was filtered using Whatman No. 1 filter paper and poured in a Petri dishes, and kept in the oven for drying. The extract was dried and powder and kept in the refrigerator for further use.

Pathogen Collection

The pus sample of the infected patients was collected from Thoothukudi Government Hospital, Thoothukudi, Tamil Nadu, India. Collected samples were processed immediately in our microbiology lab. The pus samples were swabbed on different selective media like EMB agar, MacConkey agar, Urea agar and Mannitol salt agar for the isolation of pathogen. The cultures were purified by streaking on Nutrient Agar plates. The biochemical test was conducted for the purified sample and stored at 4°C in the refrigerator.

Preparation of Inoculum

The stock culture was maintained in nutrient agar slants at 4°C. The cultures required for the experiments were prepared by taking loopful of colonies from the stock culture and added to the Nutrient broth (NB) and was incubated at 37°C for 24 h till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600 nm which was equivalent to 10^6 - 10^8 CFU/ml (Adedayo *et al.*, 2001).

Antibiotic sensitivity of pus pathogens

Commercial antibiotics were used to find the antibacterial activity against the pathogens present in the pus using agar disc diffusion method. The test organisms were streaked on the prepared Nutrient agar plates. Fluconazole (10mcg), Chloramphenicol (30mcg), methicillin (5mcg), tetracycline (30mcg) and Neofloxin (10mcg) were the five different commercial antibiotics placed in the plates, and the plates were incubated for 24 hours at 37°C.

Antibacterial Activity of seed extract against pus pathogens

Mueller Hinton agar medium was prepared, sterilized and aseptically poured into the sterile petriplates and was kept aside till it got solidified. The surface of Mueller Hinton agar plates was swabbed with the test organisms, and the wells of 6mm diameter and about 2cm apart from each other were cut by using a well cutter. Different concentration (500 $\mu\text{g/ml}$, 600 $\mu\text{g/ml}$, 700 $\mu\text{g/ml}$, 800 $\mu\text{g/ml}$, 900 $\mu\text{g/ml}$ and 1 mg/ml) of the extracts were added into the respected wells. The incubation of plates was done for 24 hours at 37°C. The test was repeated for three times to avoid any kind of technical errors that might have happened during a single attempt. A control well was cut, and dimethyl sulfoxide was added to the well, and the plates were kept for incubation for 24 hours at 37°C. The zone of clearance was observed after 24hrs of incubation. The clear zone around the well indicates the antibacterial activity of the ethanol extract against microorganisms.

Minimum Inhibitory Concentration (MIC)

1ml of pre-sterilized Mueller-Hinton Broth was added to 11 sterilized vials each, and another vial was considered as negative growth control. After thorough homogenization, 1 mL of the solution containing, both the extract solution and the nutrient broth was transferred to the next vial containing only 1 mL of nutrient broth. Similarly, up to the 10th vial two-fold serial dilution was maintained, and from the 10th vial, 1 mL of solution was discarded, and the 11th vial was considered as the positive control. 20 μL of bacterial suspension (turbidity equal to a 0.5 McFarland standard, supposed to have 1.5×10^6 CFU/mL) was added to each vial and mixed thoroughly. The vials were then incubated for 24 h at 37°C. The lowest concentration that inhibited the growth of bacterial culture was considered as MIC.

1ml of the culture was inoculated in the prepared Nutrient broth. Then 20 μl seed extract was added in different concentration from 0.5ml- 0.9ml. The plates were incubated at 37°C for 24 hrs. After incubation, the Optical density was measured colourimetrically at 600 nm.

Minimum Bactericidal concentration

The lowest concentration required for killing or preventing the growth of microorganism is termed as Minimum Bactericidal Concentration. The test dilutions of the extracts were subcultured in the fresh nutrient agar (NA) medium, and the MBC was determined after incubating for 24 hours at 37°C. The

minimum concentration of the extract (mg/mL) that prevented the growth of a single bacterial colony on the nutrient agar medium was considered as the MBC. 0.1 ml of the bacterial culture was inoculated to the prepared nutrient broth, and the plates were tested with various concentration of seed extract ranging from 0.5ml- 0.9ml (MIC). Further, the plates were incubated for 24 hours at 37°C (*Abu-Shanab et al., 2007*).

Anti-microbial activity of commercial antibiotics

Test organism was swabbed on to the surface of the Mueller Hinton agar plates. Then commercial antibiotic discs viz., Gentamycin, Streptomycin, Norflaxin, Tetracycline were placed on the surface of the plates. Later, the plates were incubated for 24 hrs at 37°C.

Qualitative Analysis of Phytochemical

The Phytochemical analysis for the plant extracts using ethanolic solution was accessed using the standardized protocol:

Test for Saponins

The seed extract was mixed with 5.0ml of distilled water, and few drops of olive oil were added and mixed vigorously for the generation of foam.

Test for Tannin

0.5ml of seed extract was added to the 10.0ml of bromine water, and the decolouration of Bromine water indicates the presence of tannin.

Alkaline Reagent Test

The seed extract was mixed with 2ml of 2.0% of NaOH, and it resulted in the production of yellow colour. The decolouration was observed when dilute acids were added to the mixture, which indicated the presence of flavonoid.

Salkowski's test

The colour change to reddish-brown indicated the presence of steroidal aglycone part of the glycoside when 2ml of conc. H_2SO_4 was added to the seed extract.

Test for Terpenoids

5ml of seed extract was treated with 2.0ml of chloroform and placed on the water path for evaporation and then boiled with 3 ml of conc. H_2SO_4 . The appearance of a grey colour indicated the presence of terpenoids.

Test for Steroid

5ml of seed extract was treated with 2.0ml of chloroform and conc. H_2SO_4 and the presence of steroid were indicated by the formation of red colour in the chloroform layer. The presence of steroid was indicated at all the seed extracts.

RESULTS

E. coli, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, were isolated from pus sample obtained from Thoothukudi, Government Hospital through the biochemical studies according to Bergy's manual Table 1.

Drug-resistant microbes are becoming more common in the community hence antibacterial assay for selected commercial antibiotics against pathogens present in were tested. The result revealed that the isolated pus pathogenic bacteria gained resistance to Methicillin and Neofloxin. Chloramphenicol and Tetracycline antibiotics showed negligible sensitivity against pus pathogens, but only Fluconazole revealed that antibiotic susceptibility against *P. mirabilis* and *K. pneumoniae* (Table 2).

The inhibitory effect of ethanol seed extracts of cucumber, papaya and custard apple were tested against isolated pus samples. The agar well diffusion method was used to determine the anti-microbial activity against the pathogens, and the results were determined based on the inhibition zone around the well. The anti-microbial property of cucumber ethanol seed extract was evaluated according to the zone of the inhibition against pus pathogens, and the results were tabulated in Table 3.

The cucumber ethanol seed extract of 900 μ g was the most active against all pus pathogens. The zone of inhibition against *Staphylococcus aureus* was found to be 14mm and found to be maximum. The ethanol seed extract of cucumber showed inhibition in the concentration of 600 μ g against *Staphylococcus aureus*. And remaining pathogens was inhibited at the concentration of 800 μ g

The ethanol seed extract of papaya of concentration 900 μ g was the most active against all pus pathogens (Table 4). The inhibition zone against the *Staphylococcus aureus* and *Proteus mirabilis* was found to be 13mm and maximum.

The ethanol seed extract of custard apple of concentration 900 μ g showed negligible inhibition against pathogens present in pus (Table 5).

Antibacterial activities of ethanol seed extract expressed as a minimal bactericidal concentration (MBC, mg mL⁻¹) minimal inhibitory concentration (MIC, mg mL⁻¹) and showed in Table 6.

The MIC values of the ethanol seed extract of cucumber were found to have a minimum value of 300 μ g/ml against *Staphylococcus aureus*, 500 μ g/ml against *E. coli* and 600 μ g/ml against *Proteus mirabilis* and *Klebsiella pneumoniae*. The MIC values of the ethanol seed extract of papaya found

to have a minimum value of 300 μ g/ml against *Staphylococcus aureus*, and 600 μ g/ml against all other pathogens.

The phytochemical analysis of plant extracts is tabulated (Table 7). From the phytochemical analysis, the presence of flavonoids, tannins, glycosides and terpenoids and the absences of alkaloids were found on the cucumber seed ethanol extract.

DISCUSSION

Various microorganism such as bacteria, fungus and parasite infects the skin. Among all these types of infections, the most common type of infection is a bacterial infection. In the present study, bacterial pathogens were isolated from pus samples. Shama *et al.* (2018) reported that the predominant isolate in the pyogenic infections is *Escherichia coli* (57.5%), second-most is *Proteus sp.* (31.5%) and the minimum percentage is encountered by *Streptococcus pyogenes* (10.9%). Similarly, in our present study, the bacterial pathogens isolated from the pus were found as *E. coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Antibacterial assay of selected commercial antibiotics against pus pathogens mostly showed better antibiotic resistance. It is necessary to understand the anti-microbial susceptibility and pattern of the pathogens for proper treatment as the resistance to antibiotics within the pyogenic pathogens has been gradually increasing (Singh *et al.*, 2013).

To substitute synthetic antibiotics, traditional medicine has the origin of many modern and effective drugs. There is continuous research going on to discover new bioactive compounds against the remedy of many infectious diseases (Betoni *et al.*, 2006). Immense clinical problems have been created nowadays due to the increase in the antibiotic resistance among the pathogens causing many infectious diseases. Due to this rapid growth of pathogens having resistance to antibiotics, an alternate way of Herbal treatment may serve as an effective solution for the better treatment of bacterial diseases (Sharmeen *et al.*, 2012). Plants are the great source of producing bioactive compounds for treating various infectious disease with less or negligible toxic components to the host and thereby considering as a better way for producing anti-microbial drugs (Ahmad and Beg, 2001).

This situation made the researchers think of extracting the bioactive compounds from various parts of the medicinal plants like seeds, leaves, barks and flowers. The advantage of using medicinal plants as a source of extraction of bioactive com-

Table 1: Biochemical characteristics of pus pathogens

S.No	Organisms	Biochemical characteristics					Gram's Staining
		IMVIC	Urease	Catalase	Oxidase	TSI	
1	<i>Escherichia coli</i>	++--	-	+	-	A/A, G	-
2	<i>Staphylococcus aureus</i>	++++	+	+	-	A/A	+
3	<i>Klebsiella pneumoniae</i>	---+	+	+	-	A/A, G	-
4	<i>Proteus mirabilis</i>	++-+	+	+	-	A/A, H ₂ S	-

(+ Positive; - Negative; A/A Glucose, Lactose, Sucrose fermented; G Gas production; H₂S production)

Table 2: Antibiotic sensitivity of commercial antibiotics against pus pathogens

Commercial antibiotic disc	Concentration of disc(mcg)	Antibiotic sensitivity of commercial antibiotics (mm)			
		<i>E.coli</i>	<i>S.aureus</i>	<i>P.mirabilis</i>	<i>K.pneumoniae</i>
Fluconazole	10	-	-	22	13
Neofloxin	10	-	-	-	-
Chloramphenicol	10	4	5	-	4
Methicillin	10	-	-	-	-
Tetracycline	10	-	2	-	3

Table 3: Anti-microbial activities of ethanol Cucumber seed Extract against pus pathogens

Isolated microorganisms	0.5 mg	0.6 mg	0.7 mg	0.8 mg	0.9 mg
<i>Staphylococcus aureus</i>	3mm	8mm	10mm	12mm	14mm
<i>Klebsiella pneumoniae</i>	-	-	3mm	9mm	12mm
<i>Proteus mirabilis</i>	-	4mm	8mm	11mm	13mm
<i>E.coli</i>	-	3mm	5mm	8mm	10mm

Table 4: Anti-microbial activities of ethanol papaya seed Extract against pus pathogens

Isolated microorganisms	0.5 mg	0.6 mg	0.7 mg	0.8 mg	0.9 mg
<i>Staphylococcus aureus</i>	3mm	5mm	6mm	10 mm	13mm
<i>Klebsiella pneumonia</i>	-	5mm	7mm	9mm	12mm
<i>Proteus mirabilis</i>	-	2mm	5mm	9mm	13mm
<i>E.coli</i>	-	4mm	6mm	7mm	11mm

Table 5: Anti-microbial activities of ethanol custard apple seed extract against pus pathogens

Isolated microorganisms	0.5 mg	0.6 mg	0.7 mg	0.8 mg	0.9 mg
<i>Staphylococcus aureus</i>	-	2mm	3mm	3mm	3mm
<i>Klebsiella pneumonia</i>	-	-	3mm	4mm	4mm
<i>Proteus mirabilis</i>	-	-	-	2mm	3mm
<i>E.coli</i>	-	-	-	-	2mm

Table 6: MIC and MBC of ethanol extracts of seeds

Pus pathogens	Ethanol extracts of seed powders $\mu\text{g/ml}$					
	Cucumber		Papaya		Custard apple	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>E.coli</i>	500	700	600	700	---	---
<i>Proteus mirabilis</i>	600	700	600	700	---	---
<i>Klebsiella pneumoniae</i>	600	800	600	700	----	---
<i>Staphylococcus aureus</i>	300	500	200	400	----	---

Table 7: Qualitative analysis of Phytochemicals of ethanol extract of cucumber seed

Phytochemical compounds	Results
Tannins	+ ve
Saponins	+ ve
Terpenoids	-ve
Flavonoids	+ ve
Glycosides	+ ve
Steroids	+ ve

pounds is that it is cost-efficient, non-toxic, readily available and does not affect ecological balance (Joseph et al., 2008). So, in the present study, ethanol seed extracts of cucumber, papaya and custard apple were tested against isolated pus samples. Among these, Ethanol seed extract of cucumber and papaya showed better antibacterial activity against the pus pathogens. Ukaegbu-Obi et al. (2018) supported the results, the ethanol extracts of the *Carica papaya* seed showed great antibacterial activity by effectively inhibiting all the tested pathogenic bacteria. Maximum susceptibility of concentration 100mg/ml with 11mm zone of inhibition was shown against *Staphylococcus aureus* by the ethanolic extract of *Carica papaya* seed. Similarly, in the present study, the ethanol seed extract of papaya showed maximum inhibition zone of 13mm at 900 $\mu\text{g/ml}$ concentration against the *Staphylococcus aureus* and *Proteus mirabilis*.

Also, other researchers have reported that the microbial inhibition was also caused by organic extracts of the dried seed of *C. papaya* (Dawkins et al., 2003). The other reports justify that the various parts of *Carica papaya* tree showed efficient antibacterial activities (Ifesan, 2013; Nirosha and Mangalanayaki, 2013). The plant part of papaya has records of being used as antibacterial, analgesic, amebicide, digestive, cardiotoxic, cholagogue, febrifuge, emmenagogue, hypotensive, pectoral, laxative, stomachic, vermifuge and also found to be effective in treatment against jaundice (Anibijuwon and Udeze, 2009). This finding justifies the therapeutic properties of *Carica papaya* seed. The

present study was conducted to investigate the antimicrobial properties of the seed of *Cucumis sativus*. The main aim of our study is to examine the antimicrobial property of *Cucumis sativus* seed against isolated pus bacteria.

The cucumber ethanol seed extract at the concentration range of 800 $\mu\text{g/ml}$ showed efficient antibacterial activity against all pus pathogens. The maximum inhibition zone of 14mm was exhibited at the concentration of 900 $\mu\text{g/ml}$ against the *Staphylococcus aureus*. These results were backed by Muruganatham et al. (2015) stating that a great anti-microbial activity against bacteria and fungi was shown by the compounds isolated from the ethyl acetate fractions of the *Cucumis sativus* flowers. Abiodun and Adeleke (2010) reported that the excellent source of protein, fat, minerals and calcium were present at the seeds of the plant. At the same time, ethanol extract of *Cucumis sativus* seed first reported the excellent anti-microbial activity against pus pathogenic bacteria, because at most times ripened *Cucumis sativus* fruits seeds were discarded as waste.

The presence of Flavonoids, phenols, Tannin, Terpenoids, Cardiac glycosides, and Carbohydrates was found on the preliminary phytochemical analysis. Same results were evidenced by Begum et al. (2019).

CONCLUSION

In this study, seeds of *Carica papaya*, *Cucumis sativus* and *Annona squamosa* L. were chosen as it is readily available and mostly discarded when the fruits

get ripened. The results evidenced that *Cucumis sativus* seeds are capable of inhibiting the growth of pus bacteria present in the wound with its extensive and abundant source of secondary metabolites than *Carica papaya* and *Annona squamosa* L. This study leads to the development of drugs from seed extracts for better treatment and also to overcome the side effects caused by antibiotics. These phytochemical compounds had shown a better understanding of efficacy, safety and effective antibacterial activities.

ACKNOWLEDGEMENT

No Acknowledgement.

Funding Support

This research received no external funding for this study.

Conflicts of Interest

The authors declare no conflict of interest for this study.

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