ISSN 2456-3110
Vol 8 · Issue 8
August 2023

JAiMS
INTERNATIONAL

Journal of Ayurveda and Integrated Medical Sciences

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences

Maharshi Charaka
Ayurveda

Indexed

Journal of
Ayurveda and Integrated Medical Sciences
ISSN 2456-3110
ORIGINAL ARTICLE August 2023

Study of the Antimicrobial Activity and Synergistic Inhibitory Activity of *Carica papaya* leaves extract with Antibiotics on *Escherichia coli*

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ABSTRACT

Street food is one of the major causes of diarrhoea and gastrointestinal infections. *Escherichia coli* are one of the major contributors for many gastrointestinal infections. Natural sources are used to derive drugs that play striking role in the prevention and treatment of human diseases. *Ayurveda* has described *Earandakarkati* (*Carica papaya*) in many digestive disorders. This study was carried out to show the antimicrobial activity of *Erandakarkati* (*Carica papaya)* leaves extract alone and in combination with the antibiotics including Penicillin, Erythromycin, Piperacillin and Norfloxacin using agar strip diffusion method to see whether they produce synergistic or an antagonistic effect against street food isolated *Escherichia coli* and diarrhoeal *ETEC* strain (as a control). *Carica papaya* leaves extract showed antibacterial effect. The only combination of papaya leaf extract with Piperacillin resulted into a synergistic effect while the other combinations exhibited an antagonistic effect.

Key words: *Antimicrobial activity, Agar strip diffusion method, Synergistic, Diarrhoeal treatment, Coliforms, Carica papaya, Escherichia coli.*

INTRODUCTION

Street foods are often main etiological factor of many gastrointestinal infections. Earlier Chumber et al have studied the street foods from Pune and reported the presence of coliforms.[1] A report on FAO in 1995 confirms the same.[2] Sheth M et al have reported the presence of coliforms in different samples of the street food called Bhel Puri.[3] The main pathogen causing gastrointestinal infection is *Enteropathogenic Escherichia coli* (EEC).[4] The strains of *E. coli* that has the potential to cause diarrheal illness are often seen to be associated with contaminated food and water. Earlier studies by Das M have proven the presence of *E. coli* in the Paani puri, the most popular street food in India.[5] The snack comprises of three separate constituents i.e., pani (water with spices), puri (Made from all purpose flour) and filling with moong and potato (savory filling). The food being sold roadside often is accompanied with unhygienic preparation and handling, thereby making it one of the major contributors to foodborne diseases. The primary microbiological assessment testing involves detection of Coliform bacteria thereby acting as marker for prevalence of microbial contaminants. Coliforms are indicators of faecal contamination reported by Chapin *et al.*

Ayurveda has unique fundamentals in treating gastrointestinal disorders. Several medicinal plants have potential to treat gastrointestinal infections. *Erandkarkati* (*Carica papaya*) is the plant that belongs

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Submission Date: 13/06/2023 Accepted Date: 21/07/2023

Access this article online

Quick Response Code

Website: www.jaims.in

DOI: 10.21760/jaims.8.8.5

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to the family Caricaceae. Several plant parts of the papaya, including the leaves, fruit, seed, latex, and root possess medicinal properties.[6] The leaves of this plant are known to increase platelet count and are reported for its antiviral properties and hence are used against Dengue where there is decline in the number of platelet count.[7] The leaves of this plant have anti inflammatory property.[8] The leaves of the papaya plants embrace phytoactive chemical compounds of Carpaine, which is capable of killing microorganisms that often interfere with the digestive functioning.[9] Papaya leaves also contain various types of vitamins and secondary metabolites in the form of phytochemicals like; alkaloids, tannins and saponins each may have a distinctive role as antimicrobial agent. Betoni *et al.* (2006) illustrated that plants either exhibit antimicrobial properties to operate in synergism with antibiotics or own compounds that can sensitize a pathogen to an ineffective antibiotic. Synergism is a positive interaction results when the combined activity exhibited by two antimicrobial agents is greater than the sum of their independent effects as reported by Aiyegoro and Okob, 2009). Therefore, in this study we have made an attempt to explore antibacterial property of *Erandkarkati* (*Carica papaya*) leaf extract and also synergistic activity with other antibiotics if any.

Materials and Methods

The present study was carried out in Department of Microbiology, Guru Nanak Khalsa College of Arts, Science and Commerce, Mumbai, India. All the media and reagents used for microbial culture and antibiotics for Antimicrobial Susceptibility Testing were from Hi-Media Pvt. Limited, Bombay, India. Antibiotics solutions were purchased from pharmacy, Mumbai as follows:

Pharmacy: Noble Plus, SVS Road, Near Hinduja Hospital, Mahim (W), Mumbai-400016

a) Benzedrine Penicillin injection (Pfizer India Ltd.)

The batch number of used injection is 2207016J and the expiry date of the same is Jan 2024

b) Erythromycin Estolate Oral Suspension (Alembic Pharma Industries)

The batch number of used suspension is 2108000936 and the expiry date of the same is Jun 2023

c) Piperacillin and Tazobactam injection (ScotMed Care Pvt. Ltd.)

The batch number of used injection is 21DL07E and the expiry date of the same is Nov 2023

d) Norfloxacin Eye drops (Cipla India Ltd.)

The batch number of used suspension is A020510 and the expiry date of the same is Aug 2025

The antibiotics were specifically selected to which *E.coli* is resistant (Penicillin and Erythromycin) and those antibiotics to the *E.coli* are susceptible Piperacillin and Norfloxacin). The selection was thereby made to analyze if the combination is working against Gram negative organisms.

Collection of Leaves

Disease free, fresh leaves were collected from Papaya plant from Sant Tukaram Garden, Gopi Tank Marg, Citylight, Mahim, Mumbai 400016. The plant was identified and authenticated by the Department of Botany; Khalsa college, Matunga East. The leaves were cleaned and rinsed thoroughly 5 to 7 times with sterile distilled water.

Carica Papaya Leaves Aqueous Extract Preparation

Aqueous extract (Solid Liquid Extraction Method) was used for aqueous extraction. Fifteen leaves of the collected and washed leaves were pestle with 50 ml of distilled water to make a fine paste. This was followed by two step filtrations. In the first step, the obtained paste was filtered by passing through a muslin cloth. The filtrate obtained was passed through Whatmann filter paper No.4 in the second step to obtain a pure extract. The extract then was stored in clean glass bottles under refrigerated conditions. The prepared extract was screened for microbial growth and further the microbial free extract was used for analysis within a week of preparation and for next lot fresh extract was prepared.

Sample Collection and Processing

The street food; *Panipuri* samples were collected from 5 different vendors across Mumbai. The *Panipuri* samples were collected from the following vendors;

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a) Kailash Bhelpuri House, near Citylight cinema, Mahim, Mumbai-400016

b) Damodar Stall, Keluskar Road, Shivaji Park, Dadar, Mumbai-400028

c) Gupta Bhelpuri House, Mahavir Nagar, Kandivali, Mumbai- 400067

d) Varsha Chaat Corner, Ghatkopar Khaugalli, Ghatkopar East, Mumbai-400077

e) Chaatwich, Sector 17, Vashi, Maharashtra, 400703

The *Khatta Pani (sour soup)* was initially filtered using Muslin cloth to eliminate solid particulates was used for further bacteriological analysis within 1 hour of collection.

Isolation and Identification of Coliforms from Panipuri Samples

The sample was screened for the presence of *E .coli* by isolating the samples on Nutrient Agar and Eosin Methylene Blue Agar. The media were prepared from a ready composition of HiMedia and was stored at refrigerated temperature for 24 hours. Isolation was carried out until pure isolates were obtained following the incubation conditions of 37°C for 24 hours that were periodically monitored using standard Gram Staining protocol. Selective bacterial colonies were maintained on Nutrient Agar slants at refrigerated temperatures and propagated monthly. The bacterial identification was carried out by performing Biochemical tests for Indole, Methyl Red, Vogues Proskauer and Simmons Citrate. The identification was confirmed using MALDI-TOF MS by comparing the peptide mass fingerprints with that of the database.

Control Organisms used for Antimicrobial screening of Plants

The test pathogen, *Enterotoxigenic Escherichia coli* (ETEC diarrhoeal strain) was procured from Dr. Joshi's Central Clinical Microbiology Lab Pvt Ltd, Vashi, Navi Mumbai. The organism was re-confirmed through biochemical tests: indole, Methyl Red, Vogues Proskauer and Simmons Citrate. The procured strain was stored at refrigerated temperature and was used within 5 days of purchase.

Antimicrobial Susceptibility Testing using Disc Diffusion Method

Sterile Mueller Hinton Agar plates were swabbed with isolate obtained from *Panipuri* samples and obtained *Enterotoxigenic E.coli* strain. The cultures were suspended in sterile saline and the optical density was adjusted to 0.15. Antibiotic discs of Penicillin, Erythromycin, Piperacillin and Norfloxacin were placed in four different quadrants of plates. Activity was tested against both test organisms. In order to examine the activity of papaya leaf extract, sterile filter paper discs dipped in aqueous papaya leaf extract were used and were also tested against both test organisms. Two discs denoted as PC1 and PC2 were placed on either of the plates. The plates were incubated at 37°C for 24 hours post which zones of inhibition were measured.

Antimicrobial Synergy Testing

Strip Synergy Diffusion Method was adopted to assess the synergistic activity of papaya leaf extract with selected antibiotics against the two test organisms. For the test, Mueller Hinton Agar plates were swab with respective bacterial isolate suspensions. Sterile filter paper strips were dipped in antibiotic solution and placed horizontally on plates in centre. Second strip was dipped in papaya leaf extract and placed vertically over the first strip perpendicular to the earlier strip. Each combination of antibiotic solution and papaya leaf extract was tested against both test organisms. Plates were incubated at 37°C for 24 hrs post which wider zone of inhibition was checked for combined activity exhibited by two antimicrobial solutions employed. The calculations were performed for obtained values to determine Synergistic or Antagonistic effect exhibited by each set.

The results were analyzed with the formula below:

Synergy = A + B < C

Additive = A+B=C

Antagonistic = A+B>C

Key:

A = Average zone of inhibition for papaya leaf extract

B = Average zone of inhibition for respective antibiotic solutions

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A+B = Sum of both A and B

C = Combined effect of A and B measured diagonally

Phytochemical Screening of the Extract

The prepared extract was subjected to centrifugation at 10,000 rpm for 15 minutes. The obtained supernatant was collected and used in the further analysis. Phytochemical screening was performed to test for Alkaloids, Flavonoids, Tannins and Saponins.

Test for alkaloids

Hydrochloric acid (HCl) was added to the plant extracts (3 ml) and was then placed in a water bath for 5 minutes. Then few drops of Mayer regent were added to the mixtures. Turbidity indicates the presence of alkaloids.

Test for flavonoids

A few drops of diluted sodium hydroid solution were added to the stock solution of *Carica Papaya* leaves extracts (0.5 ml). An intense yellow colour appeared in the plant crude extract, which then becomes colourless upon addition of a few drops of diluted sulphuric acid that show the presence of a flavonoid.

Test for tannins

2 ml of extracts were added in 2 ml of distilled water and stirred. To this few drops of ferric chloride solution were added. The formation of green precipitate indicate the presence tannins.

Test for saponins

A stock solution from each crude extract C. papaya leaves (0.5 ml) was diluted with distilled water (20 ml), and the test tube was shaken by hand for 15 minutes. The formation of foam layer on the top of the test tube showed the presence of saponins.

Thin Layer Chromatography

Thin Layer Chromatography was carried out for the chromatographic analysis using solvent system Chloroform: Methanol: 5% HCl in the ratio of (5:5:3) as a mobile phase. TLC runs performed were in the laboratory conditions at room temperature. The commercially available (aluminium) TLC plate coated with silica gel used as stationary phase was spotted with standard and sample and allowed to run to obtain visible spots. The TLC plate was exposed to UV light of intensity of 254 nm in UV chamber for few minutes to visualize different spot positions. The *Rf* value was calculated using the standard formula as mentioned below; for the obtained spots and compared with that of standard.

Standard Formula:

Rf value = Distance travelled by solute / Distance travelled by solvent

OBSERVATIONS

Isolation and Identification of Coliforms

In the present study total five *Panipuri* samples were procured from different public realm. All samples were examined for presence of bacteria using Agar streak method using Nutrient Agar and Eosin Methylene Blue Agar. EMB Agar is selective for typical coliforms resulting into metallic green-coloured colonies. Colonies obtained were screened until a pure isolate was obtained that was monitored using Gram Staining. The Gram nature of tested colony was found to be Gram negative coccobacilli. The isolate was subjected to biochemical test for Indole, Methyl Red, Vogues Proskauer and Simmons Citrate Test and was further confirmed using MALDI-TOF MS analysis. The isolate was confirmed to be *Escherichia coli.* The data for the isolation and identification of bacteria from Panipuri samples is presented in Table 1 and Figure 1.

Table 1: Biochemical Tests for bacterial identification.

Tests	Colony 1	Colony 2
Indole Test	+	+
Methyl Red Test	-	+
Vogues Proskauer	-	-
Citrate	+	-

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Figure 1: MALDI-TOF analysis report by SRL laboratories for Escherichia coli

DIAGNOSTIC REPORT

SRL Diagnostics
Dr. Phadke Labs

PATIENT NAME : G N KHALSA COLLEGE (SHRIYA PHADNIS) REF. DOCTOR : SELF

CODE/NAME & ADDRESS : CS00005760
WALKIN - MAHIM LAB
MAHALAXMI ENGINEERING EST TATE L.J. KJILANI HIGH SCHOOL,MAHIM WEST,
MUMBAI 400016
22-62356500

ACCESSION NO : 5047WC042174
PATIENT ID : GNKHU1803245047
CLIENT PATIENT ID:
ABHA NO :

AGE/SEX :99 Years
DRAWN :18/03/2023 16:44:35
RECEIVED :18/03/2023 16:46:35
REPORTED :19/03/2023 15:45:38

Test Report Status Final	Results	Biological Reference Interval	Units

MICROBIOLOGY

IDENTIFICATION OF BACTERIA BY MALDI-TOF

SPECIMEN	Neutrient Agar Received.
ORGANISM	Escherichia coli isolated.
REMARK	This is FINAL report.

Interpretation(s)
IDENTIFICATION OF BACTERIA BY MALDI-TOF-:

Principle: MALDI-TOF is the soft ionization technique that allows ionization and vaporization of bacterial proteins mass, which produce spectral fingerprints that are unique for each organism and specific to genus, species and subspecies level.

Comment: Identification for the above isolate has been carried out by MALDI-TOF MS [Matrix Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry]

End Of Report

Please visit www.srlworld.com for related Test Information for this accession

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing was performed for both isolated *E.coli* from Panipuri samples as well as ETEC diarrhoeal isolate using Papaya leaf extract and antibiotics like Penicillin, Erythromycin, Piperacillin and Norfloxacin by disc diffusion method. The zones of inhibition observed against standard *E. coli ETEC strain* illustrate that papaya leaf extract exhibit a zone of 25 mm while the antibiotics used as a control vehicle result into a zone of inhibition of 26 mm for Norfloxacin, 24 mm for Piperacillin, 16 mm for Erythromycin and no zone for Penicillin. Whereas against the isolated *E.coli,* papaya leaf extract demonstrates a zone of clearance of 27 mm, antibiotics like Penicillin, Erythromycin, Piperacillin and Norfloxacin result into zones of 14 mm, 10 mm, 32 mm, 32 mm respectively. The results are indicated in Figure 2 and Figure 3. Against regular *E.coli* isolated, both Piperacillin and Norfloxacin were seen to be effective with larger zone of inhibition (41mm), Whereas for ETEC strain, Norfloxacin proved to be more effective. Overall, in our study, the extract of papaya leaves was seen to be efficient against both isolated and standard bacterial diarrhoeal etiological agent; *Escherichia coli.*

Figure 2: Zone of inhibition observed for antibiotics and papaya leaf extract against E.coli and ETEC strain. Plates (left) indicates zone of inhibition using papaya leaf extract against isolated E.coli (top), ETEC strain (bottom). On (right) antibiotic discs of Penicillin, Erythromycin, Piperacillin and Norfloxacin were tested against E.coli (top), ETEC strain (bottom).

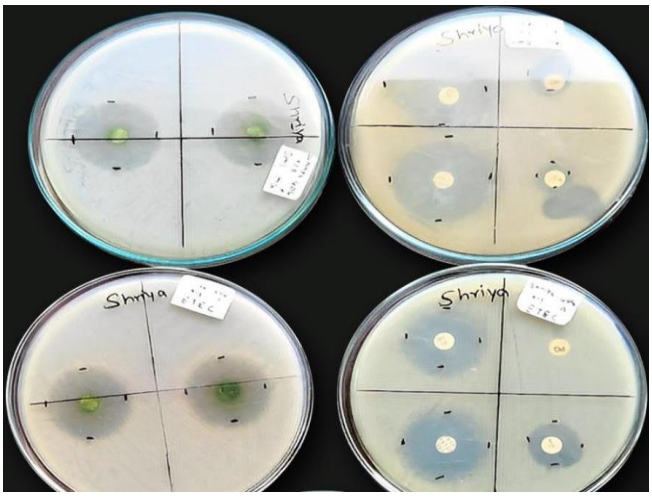
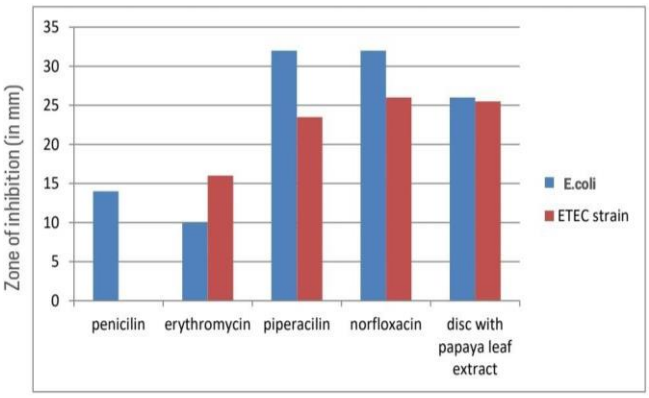


Figure 3: Average zone of inhibition of antibiotics and papaya leaf extract against E.coli and ETEC strain is represented graphically. The values indicate similar activity of papaya leaf extract against both E.coli and ETEC strain. Amongst all antibiotics, Piperacillin and Norfloxacin were seen to be equally effective.



Disc impregnated with antibiotics and papaya leaf extract respectively

Antimicrobial Synergy Testing

The results obtained for Susceptibility Testing indicates a positive antimicrobial activity of papaya leaf extract against E.coli. This thereby affirms the effectiveness and potency of extract of papaya leaves against the test organisms and its utility in combination therapy with selected antibiotics. Antimicrobial Synergy Testing was performed using Strip diffusion method employing papaya leaf extract denoted as 'A' and respective antibiotic solutions denoted as 'B'. Each combination was tested against both E.coli and

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diarrhoeal ETEC strain. The average zone obtained against isolated E.coli for papaya leaf extract (A) was seen to be 30 mm, for Piperacillin solution (B) it was also found to be 30 mm. The combined effect when measured diagonally was calculated to be 61 mm which is greater than the summation of A and B. Similarly, against the standard ETEC strain, the combined zone of inhibition was measured to be 68 mm which is greater than the summation of A and B that was calculated to be 62 mm. The combined activity of Piperacillin with papaya leaf extract resulted into a positive synergism against *E.coli* strains causing mild to severe diarrhoea. The data obtained is summarized in Figure 4 and Table 2.

Figure 4: Antimicrobial Synergy Testing using strip diffusion method employing respective antimicrobial solutions and papaya leaf extract against E.coli and ETEC strain. (Top to bottom) Set 1 involves antagonistic combination of Penicillin with papaya leaf extract, set 2 involves antagonistic combination of Erythromycin with papaya leaf extract, set 3 involves synergistic combination of Piperacillin with papaya leaf extract, set 4 involves antagonistic combination of Norfloxacin with papaya leaf extract.

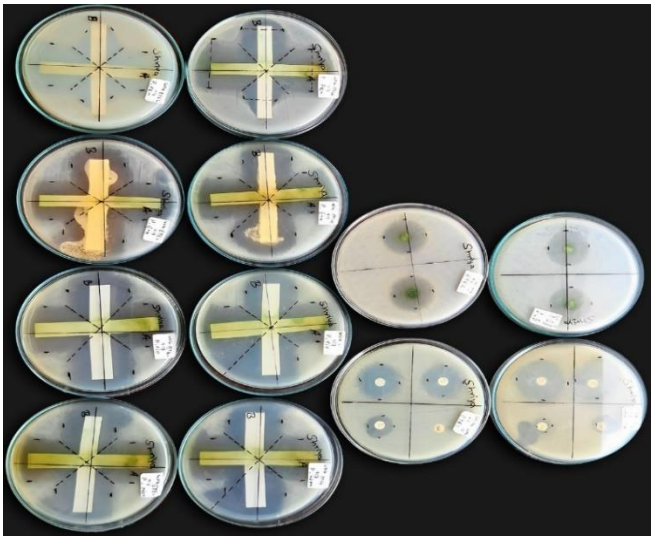

Table 2: Results of the synergistic activity of Carica papaya leaves extract with antibiotics. The zone of inhibition were measured at the tip of the strip for individual effect and diagonally from the centre for combined effect. Table represents the interpretation of the values obtained for A+B and C. Set 3 with Piperacillin and papaya leaf extract exhibit synergistic effect against both test organisms while other sets demonstrate an antagonistic effect.

Key:

A = Average zone of inhibition for papaya leaf extract

B = Average zone of inhibition for respective antibiotic solutions

A+B = Sum of both A and B

C = Combined effect of A and B measured diagonally

Synergy Antimicrobial combination	*E. coli*		ETEC strain		INTERPRETATION
	A+B	C	A+B	C	
Papaya leaf extract + Penicillin antibiotic	60 mm	44 mm	46 mm	45 mm	A+B>C **Antagonistic effect**
Papaya leaf extract + Erythromycin antibiotic	66 mm	53 mm	60 mm	49.5 mm	A+B>C **Antagonistic effect**
Papaya leaf extract + Piperacillin antibiotic	60 mm	61 mm	62 mm	68 mm	A+B < C **Synergistic effect**
Papaya leaf extract + Norfloxacin antibiotic	64.5 mm	60 mm	57 mm	47 mm	A+B >C **Antagonistic effect**

Qualitative Detection test for Phytochemicals

The pharmacological properties of *C papaya* lies in the various chemical constituents it contains. The correlation of the synergistic effect observed to the phytochemical constituents in the papaya leaf extract was determined by subjecting the extract to Qualitative Detection method. The tests indicate that the extract shows presence of secondary metabolites like Alkaloids, Tannins and Saponins each having an antimicrobial activity, while Flavonoids were seen to be absent. The results are summarized in Table 3.

Table 3: Phytochemical screening of the extract. The extract was subjected to phytochemical analysis for establishing correlation between the activity exhibited and the phytoactives present. The qualitative tests determine the presence of alkaloids, tannins and saponnins while flavonoids were seen to be absent.

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Key:

+ indicates presence of metabolite

- indicates absence of metabolite

Secondary Metabolite	Results
Alkaloids	+
Flavonoids	-
Tannins	+
Saponins	+

Thin Layer Chromatography

The chromatographic analysis for the papaya leaf extract was carried out using Thin Layer Chromatography. The mobile phase used was Chloroform: Methanol: 5% Hydrochloric acid in a ratio of 5:5:3. The spots of sample and Atropine as standard were applied to the stationary phase and was allowed to run which resulted into separation and band formation that was visualized directly and under UV chamber. The Retention factor values were calculated using the standard formula and it was observed that the values for standard and sample were in a close proximity that confirms the presence of alkaloids in the sample. The results are depicted in Figure 5 and Table 4.

Figure 5: Thin Layer Chromatography of Atropine Standard and sample papaya leaf extract. The spot (left) is for the standard, spot (center and right) are for papaya leaf extract. The image (A) was visualized directly after the run. The image (B) was visualized under UV chamber. The bands observed upon the run were used to calculate the Rf values.

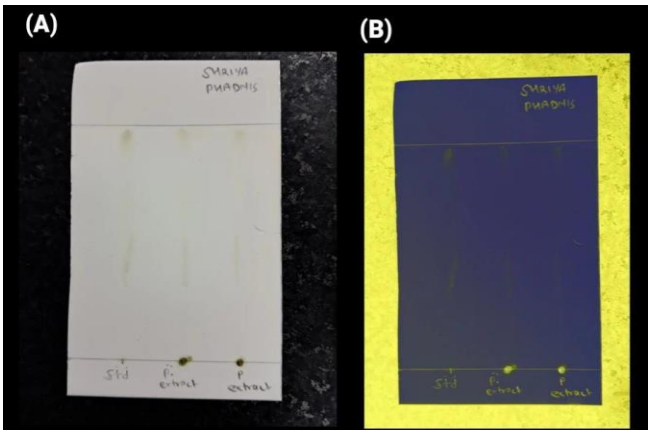

Table 4: Retention factor (Rf) values for standard and sample. The Chromatogram developed was used to calculate Rf values for sample and standard. The obtained value for standard and sample lie close indicating presence of alkaloids in the sample.

Compound Rf value	Rf value
Atropine (as standard for alkaloids)	0.97
Papaya leaf extract (spot 1)	0.95
Papaya leaf extract (spot 2)	0.95

RESULTS

A total of five samples of *Panipuri* were analyzed. All the samples were testes for the presence of Coliforms and pathogen identification and then result was interpreted. All the samples were examined for coliform presence by isolation on selective Eosin Methylene Blue Agar and Nutrient Agar. All of the *Pani* samples were seen to be contaminated with varied bacteria. The coliform isolated was identified using biochemical test and identification was confirmed using MALDI-TOF analysis and was identified to be *Escherichia coli.*

A standard ETEC diarrhoeal strain was used for a comparative analysis. Coliform and the ETEC strain were used for Antimicrobial Susceptibility and Synergy Testing. Susceptibility Testing indicates affirmative results for papaya leaf extract using disc diffusion method. The Synergy Testing using strip diffusion method against both the test organisms employed combinations of selected antibiotic solutions individually with papaya leaf extract. The results demonstrate the synergistic activity of Piperacillin with papaya leaf extract. The results emphasize the potency of papaya leaf extract in enhancing the activity of Piperacillin when used in combination.

The activity of *Carica papaya* leaf extract and the phytochemical constituents for the positive synergism was demonstrated using Qualitative detections of phytochemicals that exhibited presence of alkaloids, tannins and saponins. The alkaloid confirmation was

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demonstrated using Thin Layer Chromatography that resulted into *Rf* values for standard and sample in a close proximity indicating alkaloid presence in the sample. The results illustrate the potential for the combination therapy of Piperacillin with papaya leaf extract against mild to severe diarrhoeal etiological agents.

Discussion

Escherichia coli is a common tenant of the normal bacterial foliage of the gut.[11] As long as these bacteria don't acquire inheritable rudiments garbling for acridity factors, they remain benign commensals. Strains that acquire bacteriophage or plasmid DNA that is specifically involved in synthesizing enterotoxins or invasion factors become virulent and can beget either a plain, watery diarrhoea or an inflammatory dysentery. These conditions are most familiar to Westerners as Traveller's diarrhoea. Such scenarios are also reported in endemic countries, especially common in infants. Diarrhoeal conditions are often associated with three major groups of Escherichia coli strains, those producing enterotoxins are called *Enterotoxigenic E. coli (ETEC)*.[12] Street food preparations in India do not follow any FSSAI standard norms. Hence, there are more gastrointestinal infections found in street food consumers. Paani Puri is the most favourite street food in India. It comprises of Paani (water) with dates, jaggery and spices and the puri is filled with mashed potato and the spiced water. The water when tested for presence of contaminants often is tested positive for coliform bacteria. Yadav et al in their study also have confirmed the same.[13]

Ayurveda emphasizes the role of digestive fire and metabolism in the origin of all diseases."[14] *Erandkarkati* (*C. papaya*) is mentioned in ancient Vedic texts as *Agnideepak* meaning which enhances digestive enzymes.[15] Any infection is associated with inflammation.[16] *Carica papaya* is also known to possess anti-inflammatory properties.[17] The anti inflammatory property has been proven experientially proven by where extract of leaves of papaya showed significant reduction in the paw edema in the carrageenan test.[18] Earlier Atta F have proven antibacterial property of *Carica papaya* leaves.[19] Also Ajiboye et al in their study have found potential antimicrobial property of *Carica papaya*.[20] Earlier Unazae *et al*. have found antibacterial activity of *Carica papaya* leaf extract in *E. coli.*[21] We found phytoactives as Alkaloids, Tannins and Saponnins, (Table 3). Alkaloids are known to have antibacterial property. Thin Layer Chromatography was performed focusing on target alkaloid indicating the presence of Carpaine. (Table 4). The antimicrobial activity of *Carica papaya* leaves is probably due to the alkaloids and Carpaine.

Conclusion

Antimicrobial Strip Synergy Method resulted affirmative and a synergistic inhibition was observed for the tested coliforms using papaya leaf extract-Piperacillin combination whereas, other combinations tested although inhibited but resulted into antagonistic effect which can possibly result into resistance to organisms. The results were confirmed by synergy calculations statistically. The presence of secondary metabolites particularly that of alkaloids support the antibacterial study. Hence it can be concluded that the above stated combination of *Carica papaya* leaf extract with Piperacillin is promising to combat diarrhoea causing coliforms.

This research however, is not only restricted to the antibacterial activity but extended in exhibiting a positive synergistic effect of the papaya leaf extract with selected antibiotics against pathogenic strains of *E.coli*. The research also focuses on phytochemical analysis, the results of which are in accordance to the existing research that can thereby be correlated to the antibacterial property of papaya leaf extract.

Antimicrobial resistance is a major concern in medicine. It is a threat that is leading to deaths of millions of people across the globe.[22] There are different strategies which are planned to combat these pathogens. It has been illustrated that plants either exhibit antimicrobial properties to operate in synergism with antibiotics or own compounds that can sensitize a pathogen to an ineffective antibiotic.[23] Synergism is a positive interaction results when the combined activity exhibited by two antimicrobial

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agents is greater than the sum of their independent effects.[24] Combination therapy exhibits an expanded antimicrobial spectrum to prevent the emergence of resistant mutants, to reduce the antibiotic toxicity and to obtain a synergistic activity .

A further study can be taken up against antimicrobial resistant strains of E.coli. Though this is very limited research; it can provide a lead for further wok in this area.

Acknowledgements

The Department of Microbiology, Guru Nanak Khalsa College Arts, Science and Commerce (Autonomous), Mumbai, Maharashtra, India, is acknowledged for providing the basic infrastructure for carrying out the research work.

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ISSN: 2456-3110 **ORIGINAL ARTICLE** August 2023

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How to cite this article: Shriya S Phadnis, Krupa A Purandare, Kalpana Dhuri. Study of the Antimicrobial Activity and Synergistic Inhibitory Activity of Carica papaya leaves extract with Antibiotics on Escherichia coli. J Ayurveda Integr Med Sci 2023;08:30-39. http://dx.doi.org/10.21760/jaims.8.8.5

Source of Support: Nil, **Conflict of Interest:** None declared.

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