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Antidiarrheal Activity and Phytochemical Analysis of *Carica papaya* Fruit Extract.

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Abstract:

The current study aimed to screen the phytochemical constituents and antidiarrheal activity of *Carica papaya* fruit against selected gut pathogens. Powdered plant materials of raw and ripe fruit were extracted with diverse solvents such as petroleum ether, benzene, chloroform, acetone, ethanol and aqueous using Soxhlet apparatus. Phytochemical analysis of *Carica papaya* showed the presence of carbohydrates, tannins, saponins, proteins and amino acids, alkaloids, phenolic compounds and phytosterols. The MIC of the extracts was determined using sterile 96-well microtitre plate method. The plant extracts exhibited inhibition activity against entire tested gut pathogens. Among all the extract of raw and ripe fruit, acetone extract of ripe fruit (0.39mg/ml) and the chloroform extract of raw fruit (25.0mg/ml) showed good activity against the pathogens. The MIC and MBC values ranged between 100-0.39mg/ml. This study paves the way for the use of this common fruit extract in pharmaceuticals for their possible application in gastrointestinal disorders. Among the members of gut pathogens, the antidiarrheal activity of ripe *Carica papaya* extract was significantly seen in *Plesiomonas shigelloides* which ranged from 50mg/mL- 0.39mg/mL.

Keywords: Antidiarrheal activity, *Carica papaya*, enteric pathogens, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Phytochemical components.

INTRODUCTION

Diarrheal disease, the second common cause of death in children under five. About 1.7 billion diarrhea cases are reported every year and death is mainly due to severe dehydration and fluid loss [http://www.who.int/mediacentre/factsheets/fs330/en/].

Treating diarrheal disease is a challenge with the emerging antimicrobial-resistant pathogens. Therefore the use of traditional medicine or natural compounds is gaining importance in the antibiotic resistance era. Nature has been a source of medicinal plants for many years, a notable number of contemporary drugs have been isolated from usual sources. Currently, these plant products are gaining attraction and are the field of research because these herbal drugs are cost-effective, easily accessible and with less or no side effects. These plant products can be obtained from any part of the plants like fruits, peel, seed, leaves, flowers, roots and barks [1].

Plants possess several secondary metabolites like glycoside, saponins, alkaloids, flavonoids, tannins which give pharmacological value because they are used by the plants to fight the disease-causing pathogens. Several plant products used in traditional medical practice have been studied for the treatment of various diseases like malignancy, diabetes, arthritis and infectious diseases. Taxonomically *Carica papaya* belongs to the family *Caricaceae* having four genera. ***Carica papaya* Linn.**, (commonly called as paw-paw) is a plant with prospective medicinal significance and is seen in the tropical regions. The edible part of papaya is extensively used around the globe. The raw fruit of papaya is used as a mild laxative and abortifacient agent. Along with the fruit, the leaves are also used for the treatment of pyrexia, diabetes, gonorrhea, syphilis, inflammation and as a dressing component for wounds [2].

The present study aimed to evaluate the antidiarrheal activity of *Carica papaya* against various gut pathogens and also to analyze the phytochemical constituents present in the ripe and unripe fruit of *Carica papaya.*

MATERIALS AND METHODS:-

Collection of plant material:

The ripe and raw fruit of *Carica* papaya was obtained from the local market in Manipal, Karnataka. The fruits were rinsed and cut into small pieces and dried completely in the hot air oven at 60^0 C.

Test organisms used for the study:

Standard strains of *Escherichia coli* (ATCC 25922) , *Salmonella typhimurium* (NTCC12023), *Shigella dysentriae* (NTCC4839), *Vibrio cholerae* (NTCC4711), *Aeromonas hydrophila* (NTCC8049) and *Plesiomonas shigelloides* (NTCC9722) were obtained from the Enteric Diseases Division, Kasturba Medical College, Manipal, on sterile nutrient agar butts and subcultured onto nutrient agar plates for further testing. All the test strains were stored at 4 ^{0}C until further used.

Preparation of the extract:

The raw and ripe fruits of *Carica papaya* were dried, coarsely powdered and subjected to extraction using Soxhlet apparatus. The solvents used for the extraction were petroleum ether (60^0C-80^0C), benzene (80.1^0C), chloroform (61.2^0C), acetone (56^0C), 99.9% ethanol (78.3^0C) and distilled water. The extracts were concentrated and stored in the desiccator until further use. Dimethyl sulphoxide (DMSO) was used as the solvent for preparing the various concentrations of the extracts.

In-vitro antimicrobial assay of the extract:

The Minimum Inhibitory Concentration (MIC) of the *Carica papaya* fruit extract and the positive control - Ciprofloxacin (0.002-32mcg/mL; HiMedia Ltd, India) and Tetracycline (0.016-256mcg/mL; HiMedia Ltd, India) was determined for *Escherichia coli* (ATCC 25922) , *Salmonella typhimurium* (NTCC12023), *Shigella dysentriae* (NTCC4839) *and Vibrio cholerae* (NTCC4711), *Aeromonas hydrophila* (NTCC8049), *Plesiomonas shigelloides* (NTCC9722) respectively.

a) Minimum Inhibitory Concentration(MIC)

The MIC of the plant extracts were determined using sterile 96-well microtitre plate method. A stock solution of 200mg/ml was prepared. Plant extracts were diluted 1:2 in Muller Hinton broth (MHB) initially and diluted further in 10 dilutions to 1:1024. A quantity of 198µl from each dilution was added to the respective wells of the microtitre plate from column one to ten. A 2 µl of standardized inoculum was added to the wells from column one to eleven. 198µ l of DMSO (1ml DMSO +1ml media) was added to column eleven which served as DMSO control. The microtitre plate was incubated at 37°C for 24h [3]. [FIG1]

b) Minimum Bactericidal Activity (MBC):

5µl of inoculum from each well of MIC plate was spot inoculated onto Muller Hinton Agar (MHA) media and further incubated at 37°C for 24h. The MBC was determined as lowest dilution with no visible growth. The results of each test strain were expressed as a concentration in mg/ml. The experiment was performed in duplicates [3]. [FIG 2]

Qualitative phytochemical analysis:

The preliminary qualitative screening of the phytochemical components was carried out using standard methods for analysis of carbohydrates, tannins, saponins, proteins, amino acids, alkaloids, phenolic compounds and phytosterols [4, 5, 6].

RESULTS:

Antidiarrheal activity

The results of the present study showed the significant activity of *Carica papaya* fruit extracts against the enteric pathogens used in the study. The acetone extract of ripe fruit (MIC & MBC – 25 mg/mL) and the chloroform extract of raw fruit showed good activity against the enteric pathogens [Table 1 & 2].

Phytochemical screening

The analysis of phytochemical constituents of the raw and ripe fruit of *Carica papaya* showed the presence of carbohydrates, tannins, saponins, proteins and amino acids, alkaloids, phenolic compounds and phytosterols [Table 3].

Table 1: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of selected gut pathogens against *Carica papaya* ripe fruit.

Ripe fruit	*E.coli*	*S. typhimurium*	*S. dysentriae*	*V. cholerae*	*A. hydrophila*	*P. shigelloides*
Petroleum ether	50	50	50	50	50	50
Benzene	50	25	25	25	25	25
Chloroform	25	50	50	50	50	3.25
Acetone	50	25	25	25	25	0.39
Ethanol	25	100	50	25	50	12.5
Aqueous	**50**	**25**	**100**	**100**	**100**	**100**

Table 2: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of selected gut pathogens against *Carica papaya* raw fruit.

Raw fruit	*E.coli*	*S. typhimurium*	*S. dysentriae*	*V. cholerae*	*A. hydrophila*	*P. shigelloides*
Petroleum ether	25	50	25	25	25	50
Benzene	25	50	25	25	25	50
Chloroform	25	25	25	25	25	25
Acetone	100	100	25	50	25	25
Ethanol	100	25	50	25	100	100
Aqueous	100	100	100	100	100	100

Table 3: Phytochemical analysis of ripe and raw *Carica papaya* fruit extract.

Solvents	Alkaloids	Carbohydrates	Phenolic compounds & tannins	Proteins and amino acids	Gums	Saponins	Phytosterols
Ripe fruit							
Petroleum ether	-	-	-	-	-	-	+
Benzene	-	-	-	-	-	-	-
Chloroform	+	-	-	-	-	-	-
Acetone	-	-	+	-	-	-	+
Ethanol	+	+	+	+	+	+	-
Aqueous	+	+	+	+	+	+	-
Raw fruit							
Petroleum ether	-	-	-	-	-	-	+
Benzene	-	-	-	-	-	-	-
Chloroform	+	-	-	-	-	-	-
Acetone	-	-	+	-	-	-	+
Ethanol	+	+	+	+	+	+	-
Aqueous	+	+	+	+	+	+	-

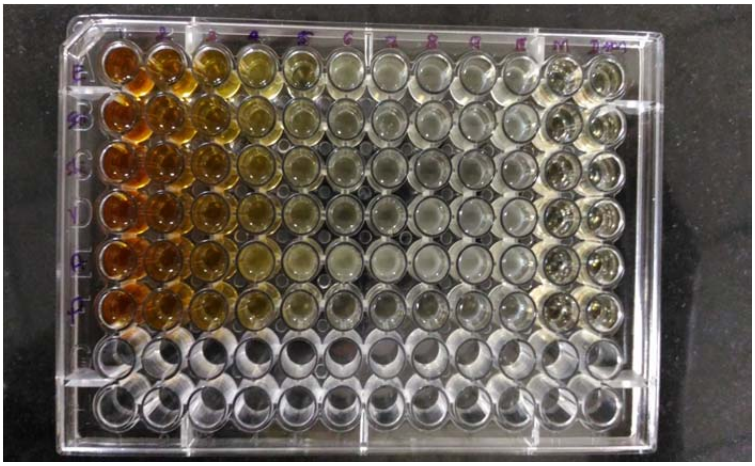

Figure 1: Minimum Inhibitory Concentration (MIC) of ripe *Carica papaya* ethanol extract.

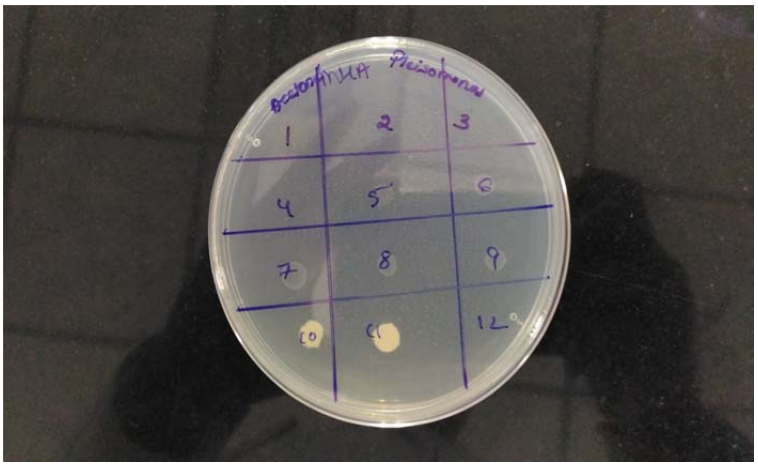

Figure 2: Minimum Bactericidal Concentration (MBC) of ripe *Carica papaya* acetone extract against *Plesiomonas shigelloides*.

Where 1= 100mg/ml, 2=50mg/ml, 3=25mg/ml,4=12.5mg/ml,5=6.25mg/ml, 6=3.125mg/ml, 7=1.56mg/ml, 8=0.78mg/ml, 9= 0.39mg/ml, 10=0.19mg/ml, 11= Organism Control, 12= Negative Control (DMSO)

DISCUSSION

The significance of medicinal plants and their application to the health of mankind is very well established. Out of 20,000 medicinal plants of the world, India possesses about 15%. About 70-80% of the people still practice traditional medicine. A variety of herbal plants is used in daily life as remedies to treat various diseases worldwide. Antibiotic resistance has substantially seen an upsurge in the recent years which poses therapeutic problem. To reduce the antibiotic resistance, we can use antibiotic resistance inhibitors which are produced from the plants that help them to defend against these pathogens. Plant extracts show target sites other than those used by antibiotics with an adequate therapeutic guide for the development of novel medications [7].

The papaya plant has been used since early times for the treatment of a diverse disease conditions. Literature review shows studies on the leaf and seed extract of *Carica papaya* against various microbial pathogens. There is no much work done on these extracts against gut pathogens. A study conducted by N Sarala et al., reported that leaf extract of *C. papaya* has beneficial properties against dengue [8].

Sudhakar et al., in their study showed that *C. papaya* acts as a versatile plant with a good source of vitamins, antioxidants, flavonoids, polyphenols, etc [2].A study in Tamilnadu by Vijay Kumar et al., reported the antimicrobial activity of leaf of Carica papaya against the opportunistic bacterial and fungal isolates The study showed that *Carica papaya* can be used for the treatment of gastroenteritis, urethritis, otitis media, dengue fever, typhoid fever and wound infections [9]. A study conducted in the aqueous seed extract of *Carica papaya* by Jyosthna et al showed activity against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *E coli* and also showed no activity against *Salmonella typhi* [10].

In a study by S. Aruljothi et al., the leaf extract was tested against wound infection-causing pathogens viz., *Escherichia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, and Pseudomonas aeruginosa* [11]. A study conducted in South Africa reported *Punica granatum* and *Indigofera daleoides exhibiting* excellent activity against diarrheal pathogens [12]. The antibacterial activity of different extracts of *Ocimum gratissimum leaves was tested* against the various pathogens like Staphylococcus aureus, Escherichia coli, Salmonella typhi and Salmonella typhimurium [13].

The results of the present study showed significant activity against the enteric pathogens. A similar study on the extract of Psidium guajava leaves showed activity against enteric pathogens [14]. The MIC method used in the present study for screening the antimicrobial activity of medicinal plants gives a quantitative value and is far superior to the disk diffusion or punch well method. The concentration of DMSO was less than 10% as *E. Coli* and *Salmonella species* were inhibited by 100% DMSO. The MIC and MBC values were in correspondence for all of the extracts. The MIC and MBC of acetone extract (25-0.39mg/mL) of ripe and chloroform extract (25mg/mL) of raw *Carica papaya* had a significant antidiarrheal activity against the gut pathogens. Among the members of gut pathogens, the antidiarrheal activity of ripe *Carica papaya* extract was significantly seen against *Plesiomonas shigelloides* which ranged from 50mg/mL- 0.39mg/mL. There are similar studies that report antimicrobial activity of medicinal plants against *Plesiomonas shigelloides* [15, 16] and in this study, we report the activity of *Carica papaya* fruit extract against *Plesiomonas shigelloides.* The MIC of positive controls - Ciprofloxacin (0.50mcg/mL) and Tetracycline (0.75mcg/mL) were sensitive for the enteric pathogens.

The phytochemical analysis indicated the presence of alkaloids, carbohydrates, proteins and amino acids, phytosterols, phenolic compounds and tannins in both the ripe and raw fruit extracts. The phytosterols, phenolic compounds, and tannins present in ripe fruit and the alkaloids in raw fruit have shown to have the antidiarrheal effect against the gut pathogens. Further exploration into the mechanism of action of these phytochemical components along with their toxicity against these pathogens is essential. Thus the present study provides a scientific basis for the use of the common fruit extracts in pharmaceutics for the preparation of drugs and their possible application in gastrointestinal disorders.

CONCLUSION:

The practice of using conventional medicinal plants play a fundamental role in basic health needs in developing countries to fight with the drug resistant bacteria. The presence of phytochemical constituents in the plants play an important role in fighting with the infectious agents. In the present study, *Carica papaya* fruit showed good antidiarrheal activity with the presence of various phytochemical constituents in it. Further studies of quantification and toxicity of phytochemical constituents are needed to bring out the therapeutic value of papaya against the gut pathogens.

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