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SCREENING OF ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF *CARICA PAPAYA* LEAF AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Nowadays, production of plant-based-drugs increases for the treatment of many diseases. The present study investigates the presences of antimicrobial activity and phytochemical compounds of *Carica papaya* leaf extracts. The leaf extract was prepared using five organic solvent (petroleum ether, chloroform, ethyl acetate, ethanol, and methanol) and water by maceration method. The phytochemical analysis of resulted extracts confirm the presences of reducing sugar, alkaloids, flavanoids, tannin, steroids and terpenoids. The antimicrobial nature of the resulted extracts were studied by agar well diffusion method against infection-causing pathogens viz., *Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Salmonella enterica, Acenetobacter and Pseudomonas aeruginosa*. Methanol leaf extract revealed the pronounced antibacterial effect with 19mm inhibition zone against on *Salmonella enterica*. Ethyl acetate extract showed their activity with inhibition zone of 17mm against *P.aeruginosa.* Maximum MIC value was found between 5-25µg/ml. The results obtained were justified that papaya leaves contain some active antimicrobial compounds which may be used as an antimicrobial agents to treat the infection as a traditional practice.

KEYWORDS: *Carica papaya*, antimicrobial activity, phytochemical constituent, MIC

INTRODUCTION

There are rich tradition of herbal medicines as evident from Ayurveda.[1][2] it was defined that medicinal plants as a plant in which one or more organs contain substances that can be used for therapeutic purposes or which it precursors for the manufacturing of drugs are useful for disease therapy. Since medicinal plants do not nearly save people from feeling pain but permit them to emerge unscathed, they deserve investigation.[3] *Carica papaya* belongs to the family Caricaceae is commonly known for its food and nutritional values throughout the world. Papaya is also called as, "The Fruit of the Angels", each part of papaya tree possess economic value when it is grown on a commercial scale.[4-8] The two important bioactive compounds present in *C. papaya* are chymnopapain and papain used in bloating and conditions of chronic indigestion. The papain, the proteolytic enzyme is a milky juice used in the treatments of arthritis. In the early 1980s it was clear by FDA that chymnopapain for intradiscal injection in patients with documented herniated lumbar inter-vertebral discs whose signs and symptoms did not responded to conservative therapy over an adequate period of time. *Carapine*, an alkaloids present in papaya, can be used as a depressant, amoebicide and diuretic.[9-12] Papaya is good source of antioxidants and nutrients such as Vitamin B, carotenes, and flavonoids, fibre and mineral like magnesium and potassium. There are many reports available which demonstrate the wound-healing property of the papaya leaves. The leaves of the papaya plants contain chemical compounds of karpain, substance which kills microorganisms that often interfere with the digestive function.[13-17] Leaves of papaya are also used for therapeutic purpose as it has ability to cure the dengue fever. Their extracts boosts as well as regenerate the platelets (thrombocytes) and white blood cells level. It has some natural ingredients including the Vitamin A, C and E which supports the immune system by regulating the T- cell.[18-20] With this in mind, the present research was done to evaluate the qualitative analysis of phytochemical constituents and their antibacterial properties against some infectious pathogens and to justify plant-based compounds could replace synthetic ones.

MATERIALS AND METHOD

Plant materials collection

The fresh leaves of *Carica papaya* were collected from the area of Balawala, Dehradun. Washed the leaves 2-3 time with running water followed by distilled water and were dried under the shed. The dry weight was measured for yield per cent.

Extraction

Different solvents were used for the extraction of different chemical constituent present in the leaves. Dried material was crushed into powder with the help of mortar and pestle. The powder material was successively extracted with the petroleum ether, chloroform, ethyl acetate, ethanol, methanol, aqueous sequentially using hot maceration method. Crude extracts were obtained by evaporating the solvent extracts using water bath at 55°C.

Phytochemical studies

The methods described by Harborne[21] were used to test for the presence of the active ingredients in the crude extracts.

Bacterial cultures- collection and maintenance

Pure cultures of *Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Salmonella enterica, Acenetobacter and Pseudomonas aeruginosa* used to assess the antibacterial properties were obtained from the Department of Microbiology, SBSPGI, Dehradun. All bacteria were maintained and grown on nutrient agar slants at 37°C. For antibacterial assays, Loop full bacteria were inoculated into nutrient broth (Himedia, India) and incubated overnight at 37°C.

Antibacterial susceptibility test

The antibacterial susceptibility test was done using some antibiotics by Kirby Bauer disc diffusion method. Autoclaved nutrient agar was poured into sterile plates. About 20 µl of fresh bacterial culture spread over the solidified agar plates. Spreaded plates were left for 10-15 minutes and then selected antibiotic discs were placed over the plate using sterile forcep. The plates were kept for incubation at 37^{o}C for 24 hours. Zone of inhibition were observed due to the diffusion of discs were measured and records were compared with the measured value of plant leaves extracts.

Screening of papaya leaf extracts for antibacterial activity

Antibacterial properties of various solvent extracts were demonstrated by well diffusion method against infection causing bacteria.

Standardization of extracts

30mg of crude extract of different solvents were dissolved in 1 ml of 100% Di methylsulfoxide (DMSO). This prepared solution of each solvent extract was used for

antibacterial test. Antibiotic disc used in susceptibility test were considered as positive and DMSO as a negative control.

Agar well diffusion method

This technique was used to determine the antibacterial activity of plant extract of leaves. Muller's Hilton agar media was used for this test. 20µl of 24 hrs fresh culture of each test organism was spreaded over sterile agar poured plates with the help of sterile glass spreader. After spreading, wells were made in plates using sterile cork borer. Each plate contain four wells in which three wells were filled with dissolved plant extract in DMSO by using micro-pippete while remaining one with negative control (DMSO). These plates were left for adequate diffusion of extracts and then incubated in the upright position at 37^{o}C for 24 hours. The plates were observed for the formation of clear zone of inhibition around the well indicating the presence of antibacterial activity and measured the inhibition zone.

Determination of minimum Inhibitory Concentration (MIC)

The MIC value of leaves extract is considered the lowest concentration of extract which exhibit maximum antibacterial activity. Various concentrations were prepared to demonstrate the minimum inhibitory concentration of antibacterial substance. It is the lowest concentration of the extract inhibiting the visible growth of microorganism. 1 ml of the concentration ranged between 5 – 30 mg/ml was injected with the help of sterile micro-tips in the culture tubes, each tube contain 9 ml of fresh culture broth of specific pathogen. No plant leaf extract was added to the control tube containing 9 ml of culture broth. After this, sealed the tube with sterile cotton plug and were incubated at 37^{o}C for overnight. Tube with no or little growth of organism after 24 hours compared with the turbidity of control tube was considered as the MIC value.

Relative percentage inhibition

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula.[24, 30]

$$\text{Relative percentage inhibition} = \frac{100 \times (a - b)}{(c - b)}$$

Where,

a: total area of inhibition of the test extract/latex

b: total area of inhibition of the solvent

c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using Area of inhibitory zone = πr^2
Where r is radius of zone of inhibition

RESUITS

Phytochemical analysis

Table 1 shows the total yield and % yield of different parts of the plant. Ethanol extract obtained the maximum total yield (4.22) and per cent (%) yield (10.26). Table 2 confirms that organic extracts have more bioactive compounds like alkaloids, flavinoids, carbohydrates, glycosides, saponins, phenolic compounds and tannins than found in the aqueous extracts. Ethyl acetate and ethanol extract contain maximum compounds.

Antibacterial activity

Evaluation of antibacterial potential of papaya leave revealed that organic extracts possess some antibacterial activity against organisms tested. Table 3 indicates that *E.coli* was resistance with no inhibition zone against all the extracts. Extract of petroleum ether, ethyl acetate, and ethanol showed moderate activity. *Salmonella enterica* found more sensitive towards methanolic extract of papaya with the highest inhibition zone of 19mm and relative percentage inhibition of 90.48. Ethanol extract of papaya showed the inhibition zone of 16 mm against *P. aeruginosa*, 11 mm against *S. aureus*, 16 mm against *S. enterica*, 11 mm against *M. luteus* and 13 mm against *Acenetobacter* with relative percentage inhibition 66.60, 68.75, 76.19, 50.00 and 59.09 respectively, whereas ethyl ether extract showed inhibition zone of 17 mm against *P. aeruginosa*, 12 mm against *S. aureus*, 16 mm against *S. enterica*, 12 mm against *M. luteus* and 15 mm against *Acenetobacter* with relative percentage of inhibition of was 70.83, 75.00, 76.19, 54.55 and 68.18 respectively. Antibiotic sensitivity test revealed that only Ofloxacin antibiotic show highest activity against *P. aeruginosa* with 24 mm zone of inhibition. *S.aureus*, *P.aeruginosa* and *E.coli* were highly resistant to Ciprofloxacin, Amoxyclav, Cephalexin antibiotics (Table 3). *Micrococcus luteus* and *Acenetobacter* found sensitive against Cephalexin with inhibition zone of 19 mm and 20mm, respectively. No activity was found in negative control (DMSO).

Minimum Inhibitory Concentration (MIC)

The results of MIC determination on the test organisms by well diffusion method. The MIC values ranged between 5 – 25 mg/ml were demonstrated and represented in graph (Fig 1).

TABLES

Table 1. Yield (%) of papaya leaf extracts		
Solvent	**Total yield(g)**	**Yield (%)**
P.ether	2.07	4.14
E.acetate	2.23	4.46
Chloroform	1.01	2.02
Ethanol	6.22	12.44
Methanol	1.83	3.66
Aqueous	2.11	4.22

Dried powder weight was 50g.

Table 2. Phytochemical evaluation of different solvent extracts of *C.papaya* leaf						
Chemical test	**P.ether**	**Chloroform**	**E.acetate**	**Ethanol**	**Methanol**	**Aqueous**
Carbohydrates	++	+	+	++	++	+
Alkaloids	+	+	++	++	+	++
Tannins	-	+	+	+	++	-
Flavonoids	+	+	++	++	+++	+
Terpenoids	-	-	-	-	+	-
Glycosides	+	-	-	+	++	-
Saponins	-	-	-	++	++	-
Proteins	+	-	+	++	+	+

Symbol indicates (-) = not present, (+) = less, (++) = good, (+++) = very good

Table 3. Antibacterial activity of *C.papaya* leaves extract of different solvent against pathogens											
Test pathogens	**Zone of Inhibition (mm)**										
	Extracts (30 mg)							**Antibiotics**			
	PE	EA	Ch	Et	Mt	Aq		Cip	Am	Cex	Ofl
P. aeruginosa	10	17	13	16	-	10		-	-	-	24
S.aureus	12	12	8	11	14	13		-	-	-	16
S.enterica	14	16	10	16	19	9		-	15	-	21
E.coli	-	7	-	5	-	-		-	-	-	14
M.luteus	13	12	16	11	11	11		15	26	19	22
Acenetobacter	11	15	-	13	10	9		-	-	20	22

PE- Petroleum ether, EA- ethyl acetate, Ch- Chloroform, Et- Ethanol, Mt-Methanol, Aq-Aqueous, Cip- Ciprofloxacin, Am- Amoxyclav, Cex- Cephalexin, Ofl- Ofloxacin.

Table 4. Relative percentage inhibition of different solvent extracts of the *Carica papaya*

Test pathogens	**Relative percentage inhibition (%)**					
	P.ether	**E.acetate**	**Chloroform**	**Ethanol**	**Methanol**	**Aqueous**
P. aeruginosa	41.66	70.83	54.16	66.60	-	41.66
S.aureus	75.00	75.00	50.00	68.75	87.50	81.25
S.enterica	66.66	76.19	47.62	76.19	90.48	42.86
E.coli	-	50.00	-	35.71	-	-
M.luteus	59.09	54.55	72.73	50.00	50.00	50.00
Acenetobacter	50.00	68.18	-	59.09	45.45	40.91

GRAPHS

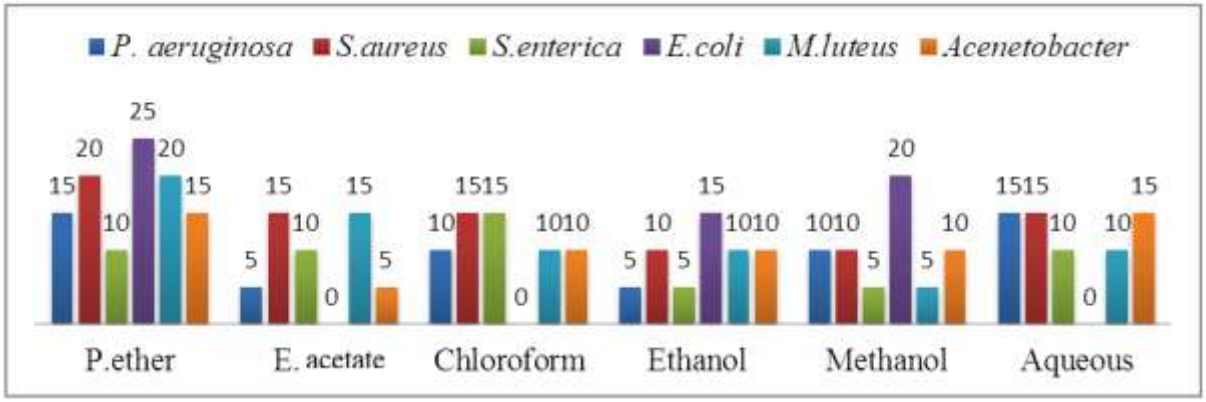

Fig 1. Graphic representation of Minimum inhibitory concentration of leaf extracts

DISCUSSION

Nature is a source of a variety of medicinal agents and an impressive number of modern drugs have been isolated from plants. There is no plant that does not have medicinal value. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.[8, 10] The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products.[13,22,23] Leaves of the *C.papaya* contain an enzyme called Papain (vegetable pepsin) which works in relieving the pain of people suffering from osteoporosis, arthritis and edema. Present study evaluates the presences of major bioactive compounds which have the ability to show anti-allergic, anti-inflammatory, antimicrobial activity. The good antibacterial activity was found in ethanol, methanol and ethyl acetate extracts of papaya leaves was believed due to the presence of flavonoids, tannins and phenolic compounds. The wound healing property of this plant can be attributed to the presence of tannins.[24, 25] Aqueous extract demonstrated sensitive activity towards pathogens. Ethyl acetate extract was effective against *P.aeruginosa* with inhibition zone 17mm followed by *S.enterica* 16mm. Organic extract shows effectiveness than aqueous extract. This may be due to the better solubility of the active components in organic solvents.[6, 26, 27] When results were compared with the inhibition zone of antibiotics, ethyl acetate, ethanol, and methanol extract found to be more effective.

Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistance bacteria, which are becoming a threat to human health.

CONCLUSION

The present study results focused on antimicrobial activity, and phytochemical screening of *C.papaya*. Qualitative analysis revealed the presence of active compounds which have the ability to inhibit the growth pathogens and showed high antimicrobial activity. These results may be useful in drug manufacture by the pharmaceutical industries.

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