JMCDD

Journal of Medicinal Chemistry and Drug Discovery

Available online at http://www.jmcdd.com
February-March, 2015, Vol. 6, pp -14-24
ISSN: 2347-9027

Research Article

EVALUATION OF ANTIMICROBIAL PROFILE OF VARIOUS EXTRACTS OF FRUITS OF *CARICA PAPAYA L*

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Abstract

The papaya is the fruit of the plant Carica papaya, the sole species in the genus Carica of the plant family Caricaceae. It consists of high content of zeaxthin, proteolytic enzymes like papain and chymo papain, vitmin K, β-carotene, other phytoconstituents like polyphenols, sugars, aromatic amino acids and sulphur containing amino acids, phytosterols, starch and nutrients e. g P, S, K, Ca, Fe, Mg etc and had good health protective effects. The main objective of the present research work was to determine various bioactive compounds and to evaluate the *in vitro* antimicrobial profile of different fruit extracts of Carica papaya against different gram positive and gram negative bacteria and different fungi and based on this a new series of constituents have been planned to extract by Ethanol (E-C_2H_5OH), Methanol (E-CH_3OH), Acetone (E-

CH_3COCH_3) and Chloroform (E-CHCl3)) from papaya fruits. The *in-vitro* antibacterial activity was carried out by Paper disc diffusion method and MIC was determined by Agar streak dilution method. The results displayed all the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-CHCl3) were found to exhibit good anti-bacterial activity and good antifungal activity and all the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) exhibited moderate to good antibacterial activity with an MIC range of 10-27 µg/ml and antifungal activity with an MIC range of 9-28 µg/ml. The MIC values of various extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-CHCl3) fruits of ***Carica papaya*** **for** different bacteria were found to be S. aureus (MIC: 10-25 µg /ml), E. coli (MIC: 11-24 µg /ml), P. aeruginosa (MIC:12-26 µg /ml) and B. subtilis (MIC: 9-27 µg /ml). The extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) of fruits of Carica papaya were active against all the tested microorganism with the range of MIC values for A. niger (MIC: 9-26 µg/ml), A. flavus (MIC:11-28 µg/ml) and C. albicans (MIC: 13-27 µg/ml) and B. dermatitis (MIC:13-25 µg/ml).

Keywords: Zeaxthin, Phytoconstituents, Antibacterial, Antifungal and MIC etc.

INTRODUCTION

The papaya is the fruit of the plant Carica papaya, the sole species in the genus Carica of the plant family Caricaceae. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 cm (20–28 in) in diameter, deeply palmately lobed, with seven lobes. Unusually for such large plants, the trees are dioecious. The tree is usually unbranched, unless lopped. The flowers are similar in shape to the flowers of the Plumeria, but are much smaller and wax-like. They appear on the axils of the leaves, maturing into large fruit - 15–45 cm (5.9–17.7 in) long and 10–30 cm (3.9–11.8 in) in diameter. The fruit is ripe when it feels soft (as soft as a ripe avocado or a bit softer) and its skin has attained an amber to orange hue. Carica papaya was the first transgenic fruit tree to have its genome deciphered **[1, 2].**

Papaya plants come in three sexes: "male," "female," and "hermaphrodite." The male produces only pollen, never fruit. The female will produce small, inedible fruits unless pollinated. The hermaphrodite can self-pollinate since its flowers contain both male stamens and female ovaries. Almost all commercial papaya orchards contain only hermaphrodites **[3].** Gaining in popularity among tropical fruits worldwide, papaya is now ranked third with 11.22 Mt, or 15.36 percent of the total tropical fruit production, behind mango with 38.6 Mt (52.86%) and pineapple with 19.41 Mt (26.58%). Global papaya production has grown significantly over the last few years, mainly as a result of increased production in India **[4].**

Global papaya production is highly concentrated, with the top ten countries averaging 86.32 percent of the total production for the period 2008–2010. India is the leading papaya producer, with a 38.61 percent share of the world production during 2008–2010, followed by Brazil (17.5%) and Indonesia (6.89%). Other important papaya producing countries and their share of global production include Nigeria (6.79%), Mexico (6.18%), Ethiopia (2.34%), Democratic Republic of the Congo (2.12%), Colombia (2.08%), Thailand (1.95%), and Guatemala (1.85%). Originally from southern Mexico (particularly Chiapas and Veracruz), Central America, and northern South America, the papaya is now cultivated in most tropical countries. In cultivation, it grows rapidly, fruiting within three years. It is, however, highly frost-sensitive, limiting its production to tropical climates. Temperatures below −2 °C (29 °F) are greatly harmful if not fatal. In Florida and California, growth is generally limited to southern parts of the states. In California, it's generally limited to private gardens in Los Angeles, Orange, and San Diego counties. It also prefers sandy, well-drained soil as standing water will kill the plant within 24 hours **[5].**

The literature survey revealed that the papaya extracts were possessed a wide range of pharmacological activities viz Age-related macular degeneration, Asthma prevention, Cancer, Bone health, Diabetes, Digestion, Heart disease, Inflammation, Skin and healing and antimicrobials etc. The objective of the present work is to evaluate the anticancer activity of fruit extracts of *Carica papaya.*

MATERIALS AND METHODS

Chemicals and drugs

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade. Standard drugs Tetracyclin (antibacterial) and Amphoterecin B (antifungal) were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade. The standard strains were procured from the American type culture collection (ATCC), USA, Rockville and the pathological strains such as bacterial cultures *Staphylococcus aureus (ATCC 9144), Bacillus subtilis (ATCC 6633) (Gram-positive organism), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922)* (Gram negative organism) and fungal cultures such as *Aspergillus niger (ATCC 9029), Aspergillus flavus (ATCC 204304), Candida albicans (ATCC 10231), Blastomycetic dermatitis (ATCC 26199)* were procured from the **department of Biotechnology of C.L. Baid Metha college of pharmacy, Chennai, India** and maintained on Nutrient agar slant and Sabouraud dextrose agar medium at 4^0C.

Methodologogy for extraction [6]

Weigh 20 g of papaya paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating and obtained the **ethanolic extract-E1 (E-C_2H_5OH)** of fruits of ***Carica papaya***. Same procedure is followed for the extraction of **methanolic extract-E2 (E-CH_3OH); acetone extracts-E3 (E-CH_3COCH_3) and chloroform extract-E4 (E-$CHCl_3$)**.

Preliminary Phytochemical Screening [7, 8, 9]

Preliminary Phytochemical Screening has to be carried out for the identification of reducing sugars, pentoses, disaccharides, polysaccharides, proteins and amino acids phytosterols,

polyphenols and carotenoids etc. The presence of all the molecules in various extracts are con confirmed by their qualitative confirmatory Tests.

Evaluation of *in vitro* antibacterial and antifungal profile by Paper disc diffusion method [10]

The sterilized (autoclaved at 120°C for 30 min) medium was inoculated (1 mL/100mL of medium) with the suspension [105 cfu m/l (colony forming unit per milliliter)] of the microorganism (matched to McFarland barium sulphate standard) and poured in Petri dish to give a depth of 3-4mm. The paper impregnated with the test extracts **(50, 100,150 µg/ml in dimethyl formamide)** was placed on the solidified medium. The plates were pre-incubated for 1hr at RT and incubated at 37° C for 24 hr for anti-bacterial and antifungal activities respectively. **Tetracyclin (100 µg/disc) and Amphotericin B (100 µg/disc**) were used as a standard drugs. The observed zone of inhibition was compared with standard drugs.

Determination of MIC by Agar streak dilution method [11]

MIC of the extract was determined by agar streak dilution method. A stock solution of the extracts (100µg/ml) in Dimethyl formamide were prepared and graded quantities of the test extracts were incorporated in specified quantities of molten nutrient agar medium. A specified quantity of the medium containing the extract was poured into a Petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately105 cfu m/l and applied to plates with serially diluted extracts in Dimethyl formamide to be tested and incubated at 37° C for 24hr. for bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria on the plate.

RESULT AND DISCUSSION

Phytochemical screening

Preliminary Phytochemical screening of various extracts **(E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) of fruits of *Carica papaya*** had shown the presence of various

bioactive compounds such as carbohydrates, aminoacids and peptides, phytosterols , carotenoids and polyphenols etc.

Antimicrobial screening

The extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) were (50, 100 and 150 µg/ml) were screened for antimicrobial activity by paper disc diffusion method. From the data shown in Table 1 and 2 the observations were made as followed: most of the extracts executed moderate to good antimicrobial activity against the tested micro-organisms. When compared to standard drugs (Tetracyclin); the extracts E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$ were found to exhibit good Anti-bacterial activity. The order of antibacterial spectrum was given as below: E-$CHCl_3$ > E-C_2H_5OH > E-CH_3OH; > E-CH_3COCH_3 etc. When compared to standard drugs (Amphotericin B), the extracts E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$ were found to exhibit good Anti-fungal activity. The order of antifungal spectrum was given as below: E-$CHCl_3$ > E-C_2H_5OH > E-CH_3OH; > E-CH_3COCH_3 etc.

The MIC of the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) was screened by agar streak dilution method. From the data shown in Table 3 and 4 observations were made as followed: all the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) exhibited moderate to good antibacterial activity with an MIC range of 10-27 µg/ml and antifungal activity with an MIC range of 9-28 µg/ml. The MIC values of various extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) of fruits of ***Carica papaya*** for different bacteria were found to be S. aureus (MIC: 10-25 µg /ml), E. coli (MIC: 11-24 µg /ml), P. aeruginosa (MIC:12-26 µg /ml) and B. subtilis (MIC: 9-27 µg /ml). The extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) of fruits of ***Carica papaya*** were active against all the tested microorganism with the range of MIC values for A. niger (MIC: 9-26 µg/ml), A. flavus (MIC:11-28 µg/ml) and C. albicans (MIC: 13-27 µg/ml) and B. dermatitis (MIC:13-25 µg/ml).

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TABLE 1: FOR ZONE OF INHIBITION (mm) OF BACTERIA BY VARIOUS EXTRACTS FRUITS of *Carica papaya*

Name of the extracts	S. auereus			B. subtilis			P. aeruginosa			E. coli		
	Concentration of E1; E2; E3 & E4 (µg/ml)											
	50	100	150	50	100	150	50	100	150	50	100	150
	Zone of inhibition(mm) at different concentration											
E1	10	15	17	9	17	19	7	16	18	8	18	20
E2	9	15	16	9	18	20	8	15	20	9	19	21
E3	8	13	15	10	16	19	9	18	21	10	20	22
E4	12	19	24	11	18	22	10	19	22	9	18	23
TCLN (100 µg/ml)	28			28			30			30		

TABLE 2: FOR ZONE OF INHIBITION (mm) OF FUNGI BY VARIOUS EXTRACTS FRUITS of *Carica papaya*

Name of the extracts	A. niger			A. flavus			C. albicans			B. dermatitis		
	Concentration of E1; E2; E3 & E4 (µg/ml)											
	50	100	150	50	100	150	50	100	150	50	100	150
	Zone of inhibition(mm) at different concentration											
E1	9	15	19	9	16	19	7	16	18	9	17	20
E2	8	16	18	7	15	20	8	17	20	10	18	21
E3	7	13	16	11	14	17	9	15	19	8	14	20
E4	10	16	21	11	18	20	9	18	21	9	18	23
Ampho. B (100 µg/ml)	30			30			30			30		

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TABLE 3: FOR MIC OF VARIOUS EXTRACTS OF FRUITS of *Carica papaya* AGAINST DIFFERENT BACTERIA

Extracts	Minimum Inhibitory Concentration (MIC) [µg/ml]			
	S. auereus	B. subtilis	P. aeruginosa	E. coli
E1	16	15	17	14
E2	19	20	19	18
E3	25	27	26	24
E4	10	9	12	11
TCLN	0.2	0.2	0.2	0.2

Extracts	Minimum Inhibitory Concentration (MIC) [µg/ml]			
	A. niger	A. flavus	C. albicans	B. dermatitis
E1	12	14	15	12
E2	16	17	20	15
E3	26	28	27	25
E4	9	11	13	13
Ampho. B	0.5	0.5	0.5	0.5

TABLE 4: FOR MIC OF VARIOUS EXTRACTS OF FRUITS of *Carica papaya* AGAINST DIFFERENT FUNGI

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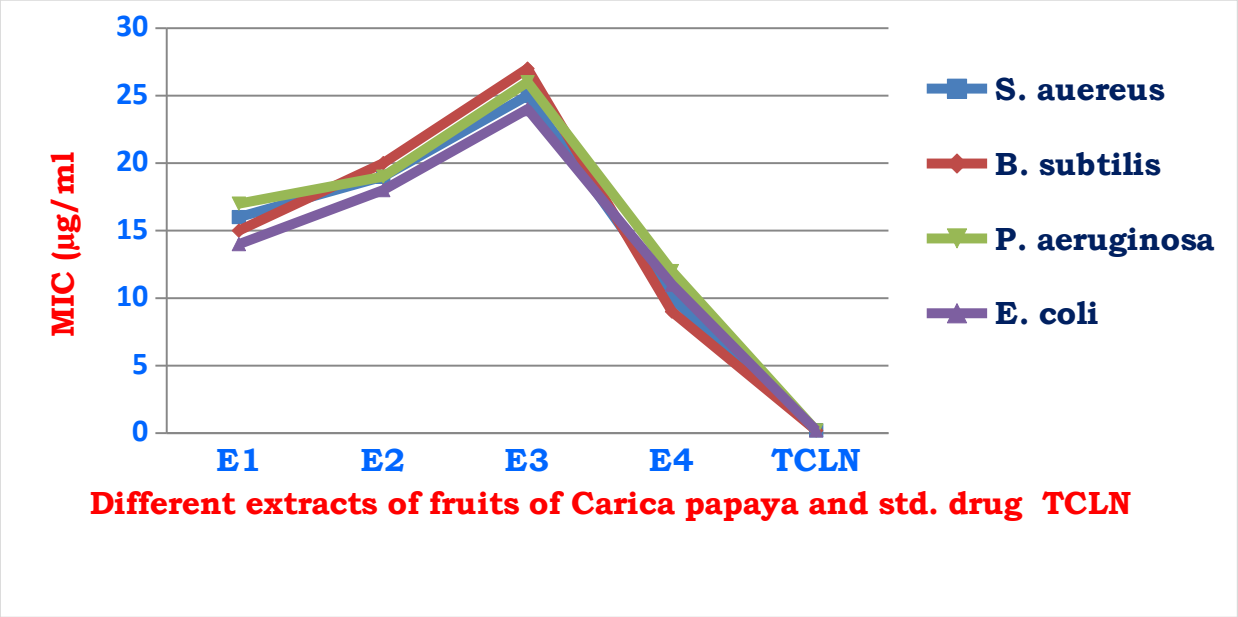

Fig 3: Graphical representation of MIC of various extracts of fruits of Carica papaya and std. drug Tetracyclin (TCLN) against different bacteria.

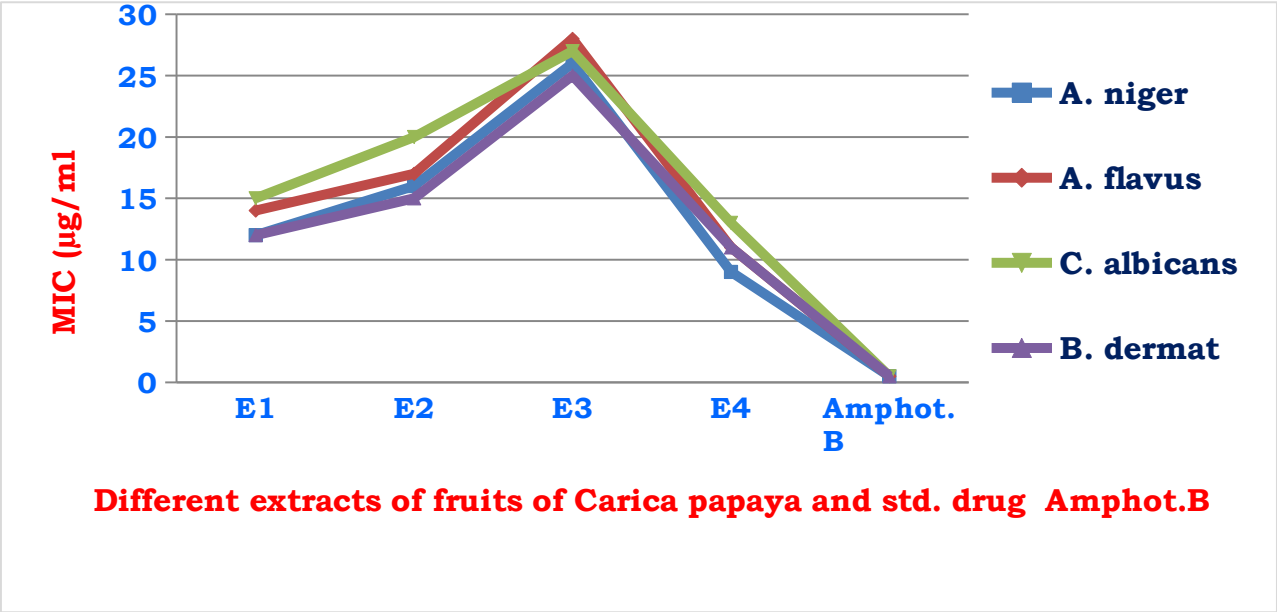

Fig 4: Graphical representation of MIC of various extracts of fruits of Carica papaya and std. drug Amphot. B against different fungi.

CONCLUSION

From the present study it can be concluded that most of the extracts executed moderate to good antimicrobial activity against tested microorganism. It can be reported that all the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-CHCl3) were found to exhibit good anti-bacterial activity and good antifungal activity and all the extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) exhibited moderate to good antibacterial activity with an MIC range of 10-27 µg/ml and antifungal activity with an MIC range of 9-28 µg/ml. The MIC values of various extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-CHCl3) of fruits of ***Carica papaya*** **for** different bacteria were found to be S. aureus (MIC: 10-25 µg /ml), E. coli (MIC: 11-24 µg /ml), P. aeruginosa (MIC:12-26 µg /ml) and B. subtilis (MIC: 9-27 µg /ml). The extracts (E-CH_3OH; E-C_2H_5OH; E-CH_3COCH_3 and E-$CHCl_3$) of fruits of Carica papaya were active against all the tested microorganism with the range of MIC values for A. niger (MIC: 9-26 µg/ml), A. flavus (MIC:11-28 µg/ml) and C. albicans (MIC: 13-27 µg/ml) and B. dermatitis (MIC:13-25 µg/ml).

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