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HPTLC and In vitro Cytotoxicity studies of Carica papaya leaf Extracts

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ABSTRACT

The use of plants in medicines has been prevalent since ancient times. Recent advances in Biotechnology have helped in the validation of their potential as drugs for the treatment of numerous diseases. Various parts of the Carica papaya, popularly grown for their fruits have also been used as ethnomedicine for a number of ailments like diabetes, indigestion, viral infections and menstrual pain. The present study focusses on preparation of extracts from the leaves of Male, Female and Hermaphrodite Carica papaya plants using solvents of varying polarities. The solvent was removed from the extract using Rotary Vacuum Evaporator. A comparative study of the HPTLC analysis of these extracts revealed that the Leaf Extracts of Hermaphrodite Carica papaya plants had the maximum number of compounds present in them. In vitro cytotoxicity studies of the shortlisted extracts done using MTT assay on BHK-21 cells. The leaf extracts of Hermaphrodite Carica papaya plants showed no sign of toxicity up to a concentration of 100 µg/ ml, hence proves them to be safe for therapeutic use.

Keywords— *Carica papaya, Hermaphrodite, MTT assay, BHK-21*

1. INTRODUCTION

Fruits, leaves, seeds and latex of *Carica* papaya a herbaceous plant, has medicinal value due to phytochemicals like carotene, vitamin C, vitamin B, flavonoids, phenol, flavonoids, proteins, tannins, folate, pantothenic acids and minerals such as potassium and magnesium. Extracts of ripe fruits have been used in the treatment of ringworm, malaria and hypertension (Shubham S., 2019, Singh P. *et al*., 2018) *Carica* papaya leaves have antioxidant and antimicrobial activity against gram-negative and positive microbes. (Alorkpa *et al*., 2016) A study conducted on rats has shown potential beneficial action of chloroform extract of *Carica* papaya leaves to treat the symptoms of diabetic patients (Juárez-Rojop *et al*., 2014). Aqueous extract of *Carica papaya* leaves exhibits anti-tumour activity and immunomodulatory effects (Otsuki *et al*, 2010). The aqueous extract of the fruit has been proved to reduce wound area in experimentally induced diabetic rats (Nayak *et al*., 2007). The ethanolic leaf extract showed anti-inflammatory activity in swiss albino rats experimentally induced with multiple infections from *Salmonella Typhi* and *Staphylococcus aureus* (Oladunmoye and Osho, 2007). Alcopyne extracted from leaves showed cytotoxicity on breast cancer cell lines (Rahmat *et al*, 2002). *Carica papaya* seed extract showed nephroprotective activity in Carbon Tetrachloride induced renal injured Wistar rats (Olagunju *et al*., 2009).

Cytotoxic studies are important in the initial phase of screening and development of any antiviral drug. It gives an understanding of the possible dosage that can be used without causing damage to healthy cells (Sharif *et al*., 2016). The MTT Assay is the most commonly used Assay for assessing the *in vitro* cytotoxicity of drugs on various cell lines. The present study aims to prepare Leaf Extracts of Male, Female and Hermaphrodite *Carica papaya* plants in solvents with varying polarities in order to extract out the maximum number of secondary metabolites present in them. A comparative chemical analysis of these extracts was done using HPTLC in order to select the best plant material that can be used for therapeutic purposes. *In vitro* cytotoxicity analysis of the extracts selected from HPTLC results was done using MTT Assay.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

Leaves of Male, Female and Hermaphrodite *Carica papaya* plants were collected from Thrissur District of Kerala. The differentiation of the Male, Female and Hermaphrodite plants was done by analysing the flower anatomy. The leaves were cleaned with water and shade dried. The dried leaves were powdered using an electronic blender and stored in airtight containers at room temperature until further use.

2.2 EXTRACTION

Three solvents of varying polarities were selected for the extraction. The solvents used were Methanol, Chloroform and Petroleum Ether. Soxhlet Extraction of all the 3 plant materials was done using these solvents for 24 hours. The extracts were then concentrated using Rotary Vacuum Evaporator and further dried in an oven at 60 °C to remove the traces of solvent. The nine dried extracts were stored in airtight glass bottles at 4°C until further use.

2.3 HPTLC ANALYSIS

HPTLC was done on CAMAG HPTLC instrument with WinCATS Software (V 1.4.6 2002). 10x10 cm of Silica Gel Pre-coated plates made of Aluminium base with 60 F254 was used for HPTLC. 5µl (2mg/ml) of each extract was loaded with a syringe by CAMAG Linomat 5 and Nitrogen as an inert Gas. The mobile phase used was Hexane: Acetone (17:6). The plate was derivatised using Anisaldehyde-Sulfuric Acid Reagent and dried for 5 minutes in the oven. The plates were then observed under UV. (Anjum Varisha, Ansari Shahis Husain, Naquivi Kamran Javed, Arora Poonam, 2013)

2.4 IN VITRO CYTOTOXICITY ANALYSIS USING MTT ASSAY:

2.4.1 Cell Line used: BHK-21 cells, Baby Hamster Kidney Cell Line (ATCC CCL-10), were grown in DMEM supplemented with 10% heat-inactivated Foetal Bovine Serum (FBS) (GIBCO) and 1X Antibiotic–Antimycotic mixture (Sigma-Aldrich) at 37 °C in a humidified atmosphere containing 5% CO_2.

2.4.2 MTT Assay: BHK-21 cells at a cell density of 6×10^3 cells/well were seeded onto 96-well tissue culture plates and incubated at 37 °C in a 5% CO_2 incubator. After overnight incubation, the cells were treated with different concentrations of the compounds and incubated at 37 °C in a 5% CO_2 incubator for 7 days. After 7 days of treatment, the media was removed and 50 µl of MTT reagent (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.5 mg/ml in DMEM serum-free media) was added to each well. After 2 h of incubation at 37 °C, the medium was removed and 50 µl dimethyl sulphoxide (DMSO) was added per well and incubated further for 4 h. The optical density (OD) was measured at a wavelength of 570 nm using iMark Microplate Reader (Biorad, USA) and the percentage of viability was calculated with respect to the absorbance of untreated cells. The results were expressed as mean values, which were measured in two independent experiments with six technical replicates each.

3. RESULTS:

3.1 HPTLC ANALYSIS

The HPTLC analysis data of the extracts showed the presence of 12 different compounds in varying combinations and concentrations (Table 1 and Figure 1).

Table 1: HPTLC Analysis of Methanolic, Chloroform and Petroleum Ether Extracts of Leaves of Male, Female and Hermaphrodite *Carica papaya* plants

	M Methanol	M Chloroform	M Pet Ether	F Methanol	F Chloroform	F Pet Ether	H Methanol	H Chloroform	H Pet ether
COMPOUND 1	75.42	1.06	8.15	62.33	0.82	7.9	40.96	-	6.41
COMPOUND 2	3.15	-	19.62	12.17	-	-	13.17	38.27	-
COMPOUND 3	1.01	30.75	35.29	-	16.73	20.62	-	12.2	34.2
COMPOUND 4	2.88	24.64	19.14	8.83	23.91	17.52	8.3	26.15	18.17
COMPOUND 5	-	-	-	-	-	11.46	-	2.83	-
COMPOUND 6	17.54	0.73	-	19.7	1.03	-	12.35	1.24	-
COMPOUND 7	-		-	-	37.42	8.07	2.54	-	25.14
COMPOUND 8	-	2.18	4.97	6.97	5.7	4.17	6.6	7.9	4.63
COMPOUND 9	-	4.39	5.06	-	6.27	5.8	-	6.54	3.72
COMPOUND 10	-	33.6	-	-	-	4.42	-	-	-
COMPOUND 11	-		5.13	-	4.41	-	-	-	5.66
COMPOUND 12	-	1.54	2.63	-	2.82	3.93	9.2	0.24	2.07

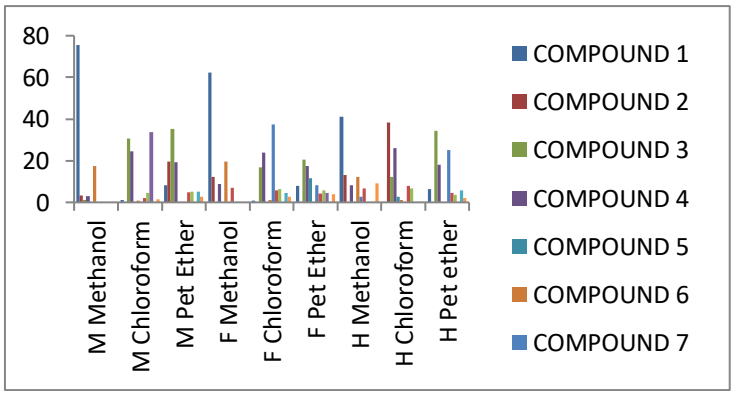

Fig. 1: HPTLC Analysis Results of *Carica papaya* Leaf Extracts

3.2 IN VITRO CYTOTOXICITY ASSAY

The results of *in vitro* cytotoxicity studies done using MTT Assay showed that all three Leaf Extracts of Hermaphrodite Papaya plants were non-toxic up to a concentration of 100 µg/ ml (Table and Fig 2).

Table 2: Percentage Viability of BHK-21 cells after 7 days of Incubation with the extracts

	Average % Cell Viability		
Concentration of Extract (µg/ ml)	**H Methanol**	**H Chloroform**	**H Pet Ether**
0	100	100	100
0.1	88.94	92.99	98.37
1	89.61	93.34	96.23
10	95.37	94.74	95.54
100	105.73	92.86	103.15

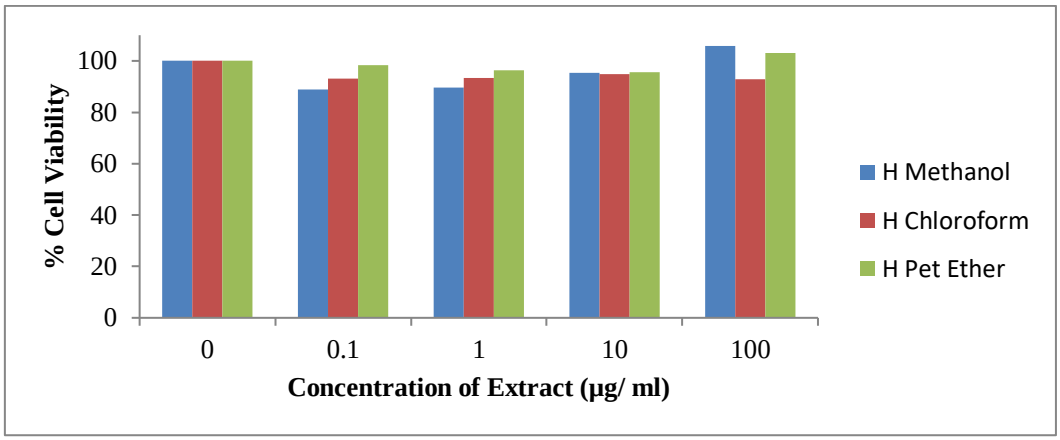

Fig. 2: Percentage Viability of BHK-21 cells after 7 days of Incubation with the extracts

4. DISCUSSION:

Carica papaya leaf extracts have shown to possess immense therapeutic properties and have been traditionally used as ethnomedicine for many disorders including Dengue. The present study is a maiden effort carried out to extract out the phytochemicals present in leaves of Male, Female and Hermaphrodite Papaya plants in solvents of varying polarities and a comparative study of the phytochemicals present in them using HPTLC Analysis. The HPTLC results revealed that the Leaf Extracts of Hermaphrodite Papaya plants showed the presence of a maximum number of compounds. In addition to this, the fact that availability of Hermaphrodite *Carica papaya* plants was more due to their self-dependent nature with respect to propagation, unlike the male and female plants, made Hermaphrodite Papaya plants best suited for the source of plant material for any therapeutic purpose. Hence the leaf extracts of Hermaphrodite *Carica papaya* plants were further studied for their *in vitro* cytotoxicity on BHK-21 Cell lines, which is a very commonly used laboratory standard for the study of many infectious diseases and Biological processes diseases (Hernandez R & Brown DT., 2010). The results of the *in vitro* studies revealed that the Methanolic, Chloroform and Petroleum Ether Extracts of leaves of Hermaphrodite Papaya plants showed no signs of toxicity up to a concentration as high as 100 µg /ml and therefore can be safely used for therapeutic purposes. This study, therefore, opens up the possibilities of investigating each of these extracts individually for their specific therapeutic properties and further separation and characterization of the bioactive compound present in them.

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