EVALUATION OF BIOMEDICAL IMPORTANCE OF PAPAYA PULP

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Abstract:

The bioactive compounds of the peel and pulp of Carica papaya was extracted using Petroleum ether, ethyl acetate, and Methanol and investigated for the presence of secondary metabolites. All the three solvent extracts revealed the presence of alkaloids; Flavonoids, glycosides, and terpenoids were present in the peel and pulp. The bioactivities of the peel and pulp extracts were attributed to their phytochemical constituents. Antimicrobial activity of the extracts was determined using agar well diffusion method. In this present study, the petroleum ether extract has shown high zone of inhibition in Aspergillus Niger, Bacillus Subtitles, Candida albicans, Escherichia Coli, Enterococcus feacalis, Klebsiella phenomena, Pseudomonas aurogenosa Staphylococcus aureus, Staphylococcus zoopiepidermis, Vibro cholera, S, mutant. Ethyl acetate extract has shown a high zone of inhibition in Aspergillus Niger, C.albicans, E.auroges, P.auroges, E.feacalis, s.zoopiepidermis, Vibro cholera. Methanol extract has shown a high zone of inhibition in Aspergillus Niger, Candida albicans, E.coli, E. feacalis, E.aurogesS.aures, S.zoopiepidermis, S.mutant. When compared the zone of inhibition with the standard drugs like protoxin. Antioxidant activity was determined using the DPPH assay method and the absorbance measured using UV- visible spectrophotometer with ascorbic acid as control. The antioxidant activities of ethyl acetate showed more activity than the other two extracts. The ethyl acetate extract showed the antioxidant activity of 61.6% in DPPH assay. This study demonstrates the efficacy of petroleum ether peel and pulp extracts of Carica Papaya as an alternative antibiotic for the development of newer antibacterial agents.The anti-cancer activity was also determined. Breast cancer cell lines (MCF7) were used and cytotoxicity is studied using all three extracts. The result showed that there was a maximum 28% reduction in cell viability at the highest concentration tested. Ethyl acetate extract showed more toxicity to breast cancer cells than the other two extracts. The lowest cell viability against normal cells was 80 percent (ethyl acetate extract) while the cell viability.

I. INTRODUCTION

The papaya is one of the native plants of Central America; however, it has been planted widely in most tropical and subtropical countries. Generally, the name of Carica papaya is various in different countries, for instance, papaya in Malaysia and Thailand, papaw/paw paw in Australia; in Europe, papaya is also named “tree melon”. It is a good fruit having commercial importance because of its high nutritive and medicinal value. The fruit is not delicious and healthy but whole plants parts, fruit, roots, bark, peel, seeds, and pulp are also known to have medicinal properties. The many benefits of papaya to be paid due to the high content of vitamins A, B, and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal, and antimicrobial properties[1].

Papaya (Carica papaya Linn.) is commonly known for its food and dietary values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. Since each part of the papaya tree possesses economic value; it is grown on a commercial scale. During the last few decades, considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable nutraceutical fruit plant. It can be chosen as a source of papain for the development of various industrial and pharmaceutical products for various diseases [2].

There are many useful compounds are available in papayas like proteolytic enzymes, campaign, lycopene, and fibrin. It has antibacterial, antifungal, antioxidant properties and also used to treat many diseases like colon cancer, Prevent prostate cancer, promote lung health and anti-inflammatory effects. In this present study, papaya peel was taken to study medical importance.

II. MATERIAL AND METHODS

Sample collection

The papaya was purchased from market Cuddalore, Tamilnadu. Care was taken to select the frim and mature fruit without any damage to the fruit.

Processing of Plant material

The papaya was washed under tap water and the pulp and peel are separated. The pulp and peel were cut into small pieces and then dried under sunlight. After drying the pulp and peel were ground well using mechanical blender into a fine powder and transferred into an airtight container with proper labeling for further use. The samples are labeled as Sample A (Pulp) and sample B (Peel).

Preparation of sample extracts

There are three solvents were taken to extract the samples.one is low polar solvent (Petroleum ether), another one is Middle polar (Ethyl Acetate) and the third one is high polar solvent (Methanol). Each 25g of papaya pulp and papaya peel was mixed with 100ml of petroleum ether, 100ml of Ethyl Acetate and 100 ml of Methanol respectively and kept in a shaker overnight at ambient temperature. Filter the samples by using Whatman No.4 filter paper and transfer into Petri plate and kept in an incubator for drying. After drying the samples and wash the Petri plate by using petroleum ether, Ethyl Acetate, and Methanol individually and transfer each sample into separate Eppendorf to dry.

Antioxidant Assay

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as a reference. Principle of this 1, 1 Diphenyl 2- PicrylHydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

The free radical scavenging activity was measured using DPPH by the method of [3]. A 0.1-mM solution of DPPH in ethanol was prepared and 2.96 ml of this solution was added to 0.4ml of various quantities and the reference compound, after 30 min, absorbance was measured at 517nm. BHA was used as reference material. Percent inhibition was calculated by comparing the absorbance values of control and samples.

% inhibition = A_Control - A_Test□A_Control□ 100

Anti-Microbial Assay

Agar well diffusion method was used to evaluate the anti-microbial activity of plants or microbial extracts. The agar plate (Muller Hinton agar) surface was inoculated by spreading a volume of the microbial inoculums (12 different strains) over the entire agar surface. Then, five holes are punched aseptically with a sterile tip and a volume of 100□l of the three extract solution at 10mg/ml concentration is introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganisms. The anti-microbial agent (extract) diffuses in the agar medium and inhibits the growth of the microbial strain tested. The diameter of the zone of inhibition was measured.

Phytochemical Analysis

Each dry extract was used for screening the following bioactive compounds: alkaloids, terpenoids, phenol and tannins, sugar, saponins, flavonoids, quinones, and proteins, according to the standard procedure described by [4].

Test for alkaloids

1 of plant sample extract, 2ml of concentrated hydrochloric acid and two drops of Mayer's reagent were added along the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids.

Test for coumarin

2ml of the test solution was mixed with a few drops of alcoholic sodium hydroxide. The Appearance of the yellow color indicates the presence of coumarin.

Test for phenol

2ml of the test solution was added to a few drops of ferric chloride. The formation of Bluish green or red color indicates the presence of phenol.

Test for sugars

2ml of the test solution was mixed with a very small quantity of anthrone and few drops of conc. Sulphuric acid was added and heated. The formation of Green to purple coloration indicates the presence of sugars.

Test for terpenoids

5ml of each extract was mixed within 2ml of chloroform 5ml of conc.sulphuric acid was then added to form a layer reddish brown color precipitation.

Test for tannins

Ferric chloride test: Three drops diluted solution of ferric chloride was added to the test tube 1 production of a blue or greenish black color that changes to olive green as many ferric chlorides were added indicates the presence of tannins.

Test for flavonoids

Sodium hydroxide (1ml of 2N) was mixed with 1ml of each plant extract the yellow color indicates the presence of flavonoids.

Test for protein

The extracts were treated with a few drops of conc. nitric acid, the formation of yellow color indicates the presence of protein.

Test for fat

The extract was dissolved in ethanol and a few drops of CuSO_4 and NaOH was added clear blue solution indicates the presence of fat.

Test for glycosides

Keller – killing test – To 2ml of extract, glacial acetic acid, one drop of 5□ ferric chloride and concentrated sulphuric acid were added. The appearance of reddish brown color at the junction of the two liquid layers indicates the presence of cardiac glycosides.

Test for steroids (Salkiwoski)

About 100 mg of dried extract was dissolved in 2m l of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface was indicative of the presence of the steroidal ring.

Test for carboxylic acid

One ml of the various extracts were separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to the liberation of carbon dioxide) indicates the presence of carboxylic acid.

Anticancer potential of papaya extract

The Breast cancer cells (MCF7) were plated separately using 96 well plates with the concentration of 1×105cells/well in DMEM media with 1X Antibiotic-Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in a CO2 incubator at 37˚C with 5% CO2. The cells were washed with 200 µL of 1X PBS, then the cells were treated with various test

concentration (25, 50, 100, 200, and 250 µg/mL) of the compound in serum-free media and incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37˚C for 4 h using CO2 incubator. After the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µL of PBS. The formed crystals were dissolved with 100 µL of DMSO and thoroughly mixed. The development of color intensity was evaluated at 570nm. The formazan dye turns to purple-blue color. The absorbance was measured at 570 nm using the microplate reader.

RESULTS AND DISCUSSION

Antioxidant Assay

The percentage of scavenging was calculated for all three extracts (petroleum ether, ethyl acetate, and methanol) of C.papaya pulp and peel at five different concentrations (25ug, 50ug, 100ug, 250ug, 500ug) and plotted a graph. The positive control's (ascorbic acid) percentage of scavenging was also plotted. From the result obtained, all three extracts have anti-oxidant property. The percentage of scavenging increases with increase in extract's concentration. Therefore, the anti-oxidant property of this pulp and peel was great only at higher concentrations. On comparing with petroleum ether, ethyl acetate, and methanol extract shows more percentage of scavenging. Thus, the results obtained show that the pulp and peel exhibit antioxidant property in all three extracts.

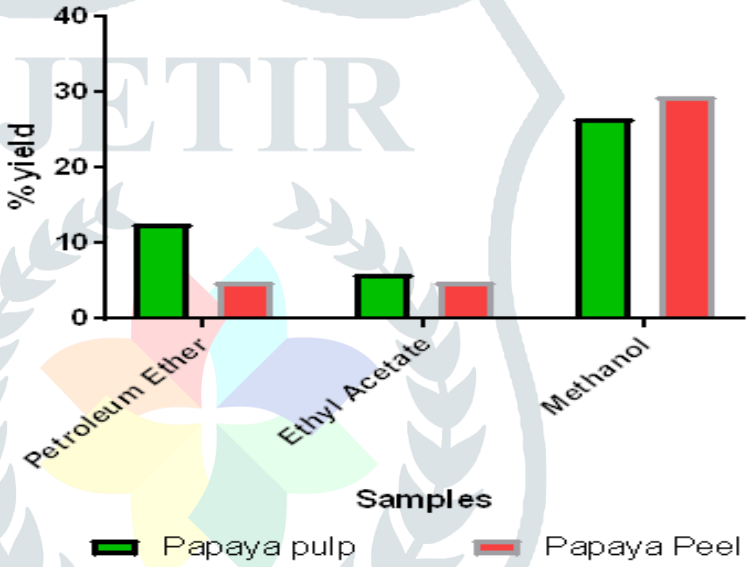


Figure 1. Total yield of papya pulp and peel

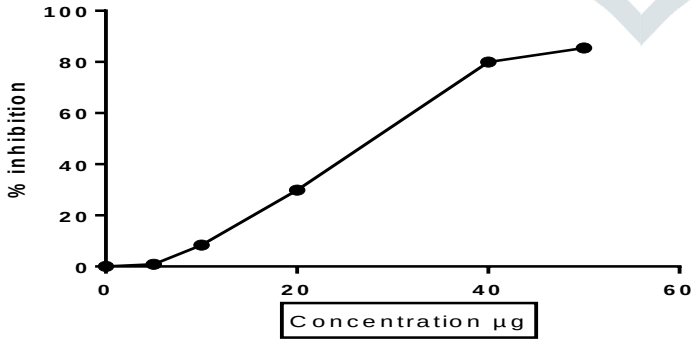


Figure 2. Percentage of scavenging for ascorbic acid (control) in five different concentrations.

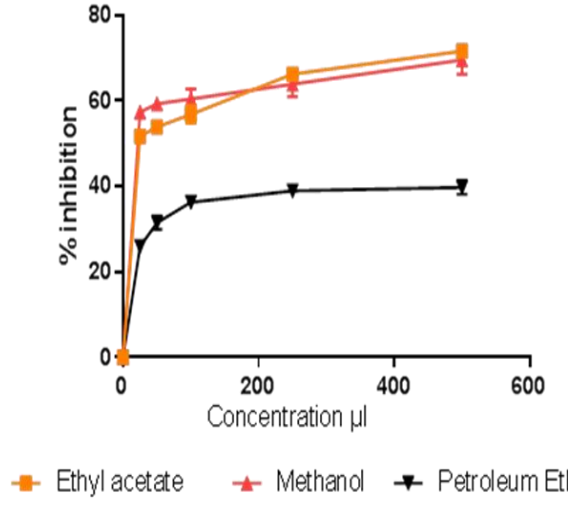


Figure 3. Percentage of scavenging for three extract sin five different concentrations of papaya pulp.

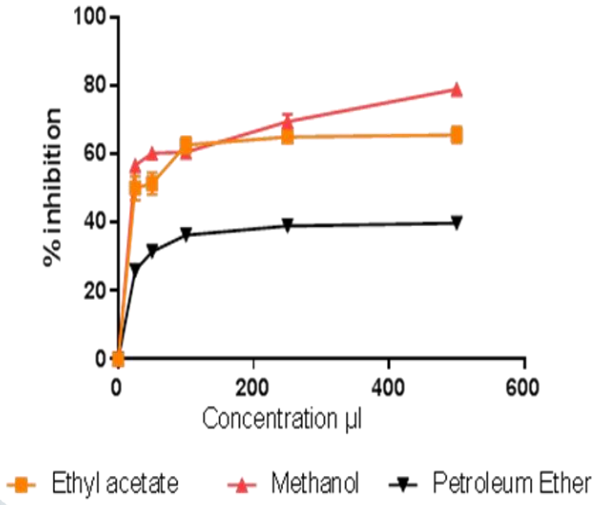


Figure 4. Percentage of scavenging for three extracts in five different concentrations of papaya peel.

Anti – Microbial assay

The agar plate was incubated overnight at 37° C and zone of inhibition's diameter is measured for all ten plates. The zone of inhibition was measured in millimeter, the results are tabulated. All three extracts possess anti-microbial activity and their strength of inhibition is unique to different strains tested. Petroleum ether extract shows more anti-microbial activity against *Aspergillus Niger, Bacillus Substillus, C.Albicans, E. Coli, E.feacalis, K. phenomena, P.auroges, S.aures, S.zoopiepidermis, Vibro cholera, S.mutant* is than other ethyl actetate and methanol extract. Ethyl acetate extract shows more anti-microbial activity against *Aspergillus Niger, C.albicansE.auroges, P.auroges, E.feacalis, s.zoopiepidermis, Vibro cholera*. Methanol extracts shows anti -microbial activity against *Aspergillus Niger, Candida albicans, E.coli, E. feacalis, E.aurogesS.aures, S.zoopiepidermis, S.mutant.*

Table 1.Anti-Microbial efficacy of Papaya Pulp and Peel Petroleum Ether Extract

STRAINS	ANTIBIOTIC (mm)	PAPAYA PULP (mm)	PAPAYA PEEL (mm)
Aspergillus Niger	34	23	12
Bacillus substilus	22	10	11
C.albicans	21	13	11
E.coli	26	12	_
E.feacalis	31	21	13
E.auroges	20	10	_
K. phenomena	24	12	12
P.auroges	30	11	10
S. aures	29	11	10
S. zoopiepidermis	25	10	15
Vibro cholera	21	20	12
S.mutant	32	14	17

Table 2.Anti-Microbial efficacy of Papaya Pulp and Peel ethyl acetate extract

STRAINS	ANTIBIOTIC (mm)	PAPAYA PULP (mm)	PAPAYA PEEL (mm)
Aspergillus Niger	35	11	10
Bacillus substilus	21	_	_
C.albicans	21	10	_
E.coli	26	_	_
E.feacalis	28	10	11
E.auroges	19	14	12
K. phenomena	26	_	_
P.auroges	25	11	_
S.aures	30	_	_
S.zoopiepidermis	22	19	10
Vibro cholera	31	13	14
S. mutant	29	_	_

Table 3.Anti-Microbial efficacy of Papaya Pulp and Peel methanol extract

STRAINS	ANTIBIOTIC (mm)	PAPAYA PULP (mm)	PAPAYA PEEL (mm)
Aspergillus Niger	32	10	_
Bacillus sustilus	18	_	
C.albicans	21	_	10
E.coli	29	_	11
E.feacalis	24	_	_
E.auroges	22	_	10
K. phenomena	27	_	_
P.auroges	_	_	_
S.aures	30	10	16
S.zoopiepidermis	20	10	_
Vibro cholera	_	_	_
S. mutant	33	12	_

Phytochemical Assay

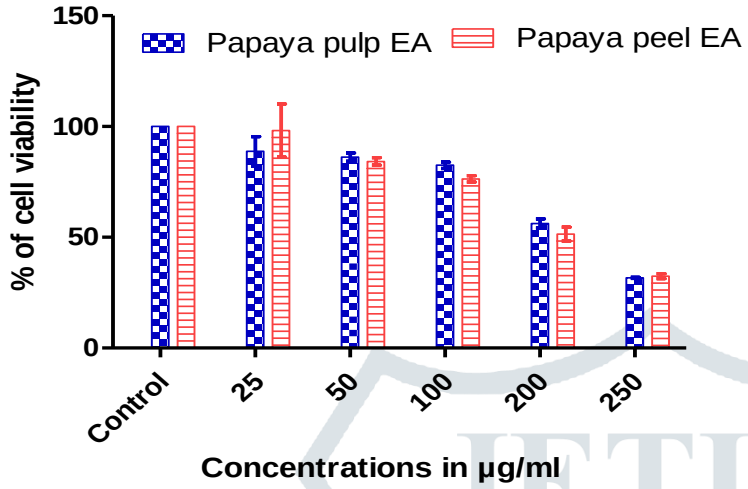
The chemical constituents of this plant were analyzed through many biochemical tests. The petroleum ether, ethyl acetate, and methanol, extracts were tested individually for twelve chemical tests to determine the presence of alkaloids, coumarin, phenol, sugars, terpenoids, tannins, flavonoids, protein, fat, glycosidase, salkwoski, and carboxylic acid. The results are tabulated below.

Table 4. Phytochemical Result of papaya pulp and papaya peel of petroleum ether, Ethyl Acetate and Methanol extract

Phytochemical Tests	Petroleum Ether Extract		Ethyl Acetate Extract		Methanol Exatract	
	Positive	Negative	Positive	Negative	Positive	Negative
Alkaloids	Peel	Pulp	Peel	Pulp	Peel	Pulp
Coumarin	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Phenol		Peel, Pulp	Peel	Pulp		Pulp
Sugars	Peel, Pulp		Peel, Pulp		Peel	Pulp
Terpenoids	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Tannins		Peel, Pulp		Peel, Pulp		Peel, Pulp
Flavonoids	Peel, Pulp		Peel, Pulp		Peel, Pulp	
Protein	Peel	Peel		Peel, Pulp	Peel, Pulp	
Fat		Peel, Pulp		Peel, Pulp		Peel, Pulp
Glycosides	Peel, Pulp		Peel	Pulp	Peel	Pulp
Salkwoski		Peel, Pulp		Peel, Pulp		Peel, Pulp
Carboxylic acid		Peel, Pulp		Peel, Pulp	Peel, Pulp	

Anticancer potential of ethyl acetate extract over breast cancer cells:

The result of MTT assays revealed that the ethyl acetate and methanol extract of *Carica papya* decreased the percent viability of all the cells but to different extent. Ethyl acetate and Methanol extract was found to induce more cytotoxicity towards breast cancer cell line (MCF7) (Figure 5 and 6).



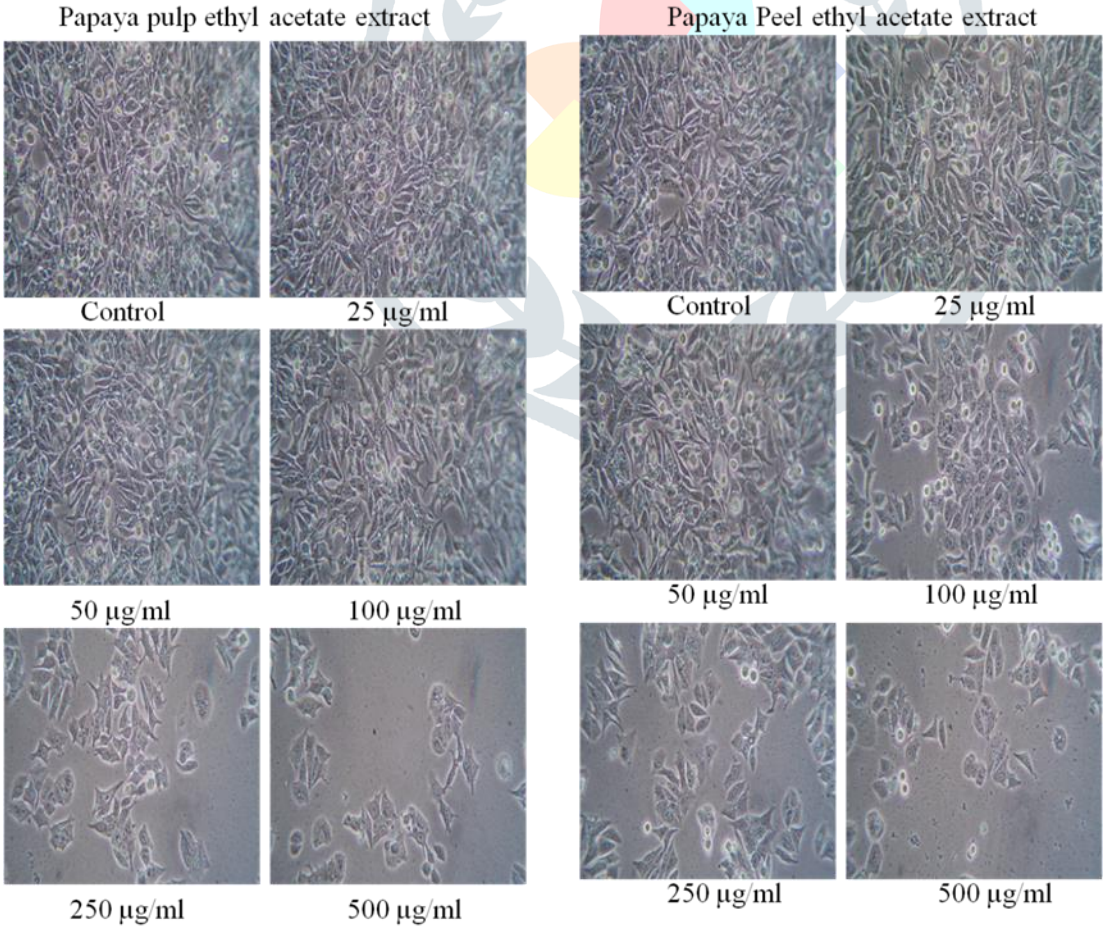


Figure 5: Estimation of anticancer potential of ethyl acetate extract on breast cancer cells in five different concentrations

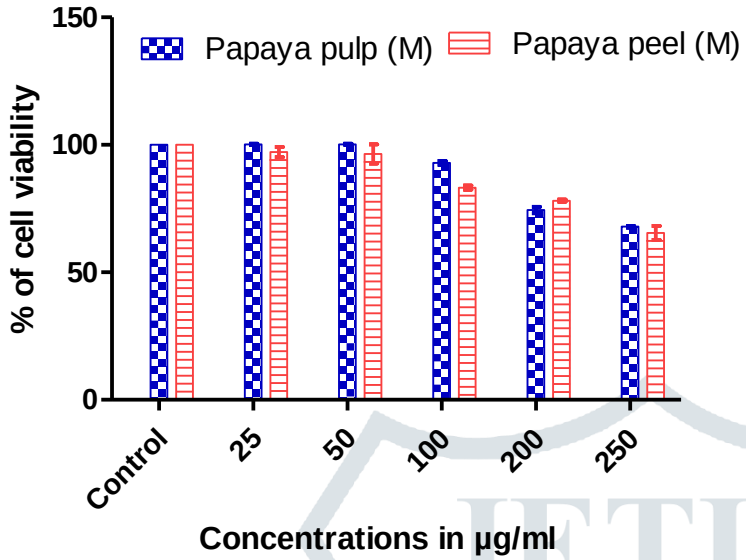
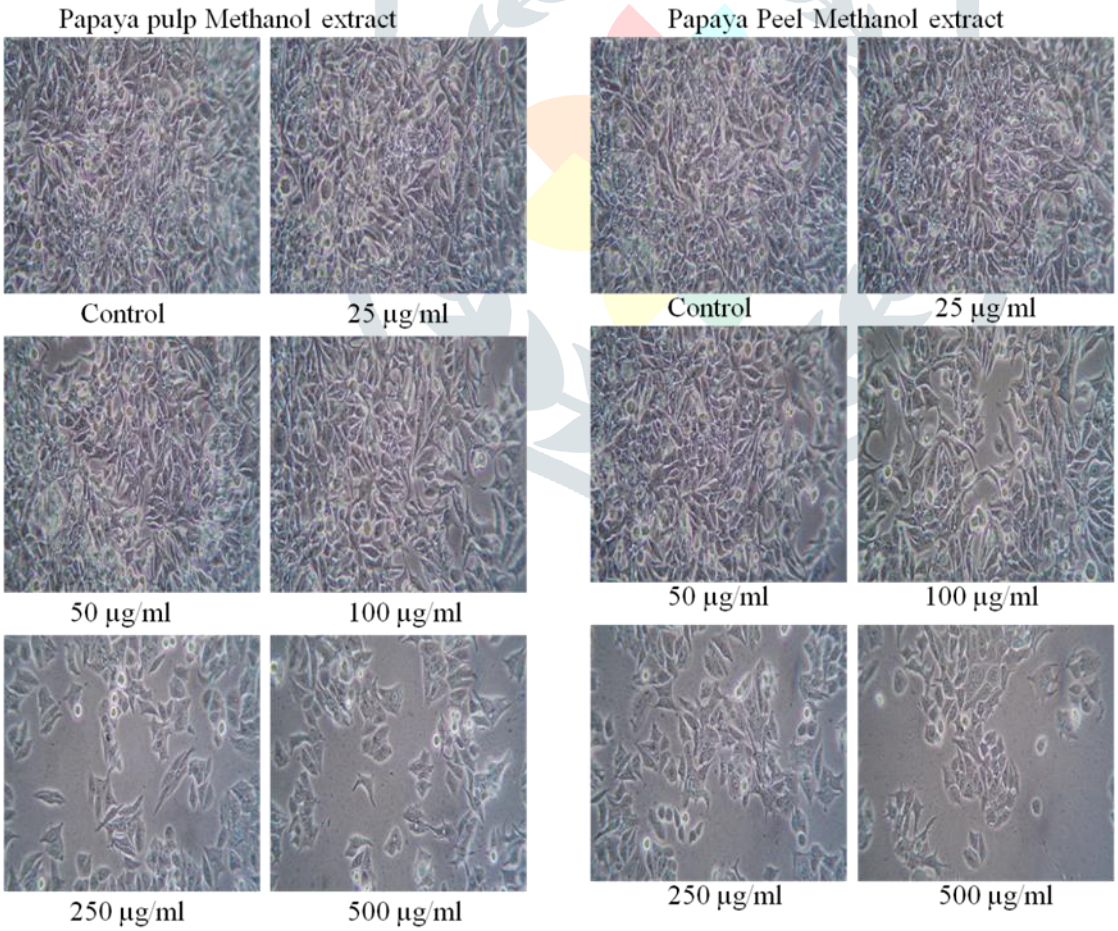


Figure 6. Anti - cancer potential of methanol extract over breast cancer cells



IV. CONCLUSION

The antioxidant activity was determined using DPPH assay. Different concentrations of the three extracts were estimated for antioxidant property and all three extracts showed antioxidant activity as their percentage of scavenging was significant especially at higher concentration. The DPPH acts as free radical and extracts quenching of DPPH was directly seen as a reduction in a color change. Ethyl acetate extract showed more antioxidizing activity than the other two extracts. The extracts can be used to treat diseases resulting from the presence of many free radicals like aging and wrinkles. That the ethyl acetate extract of this plant showed high anti-oxidant activity than methanol extract and petroleum ether extract. They used □-carotene linoleic acid model and 1, 1- diphenyl -2- picrylhydrazyl model. The ethyl acetate extract showed the antioxidant activity of 64.8% in □-carotene linoleic acid model and 61.6% in 1, 1 – diphenyl -2- picrylhydrazyl model. The three extracts were checked for anti-microbial activity using well diffusion method.

In this present study, the petroleum ether extract has shown high zone of inhibition in Aspergillus Niger, Bacillus Subtitles, C.Albicans, E. Coli, E.feacalis, K. phenomena, P.auroges, S.aures, S.zoopiepidermis, Vibro cholera, S, mutant. Ethyl acetate extract has shown a high zone of inhibition in Aspergillus Niger, C.albicans, E.auroges, P.auroges, E.feacalis, s.zoopiepidermis, Vibro cholera. Methanol extract has shown a high zone of inhibition in Aspergillus Niger, Candida albicans, E.coli, E. feacalis, E.aurogesS.aures, S.zoopiepidermis, S.mutant. When compared the zone of inhibition with the standard drugs like protoxin. The plant extracts have shown almost equal to the standard drug. The above parameter supports the strong scientific basis for the use of these plants in traditional treatment of microbial diseases. The phytochemical constitutes of the three extracts (petroleum ether, ethyl acetate, methanol) of Carica papaya was found individually. The tests indicate the presence of alkaloids, coumarin, sugars, terpenoids, flavonoids, protein, glycosides and carboxylic acid. Flavonoids and alkaloids are secondary metabolites and find application in various biological fields. The presence of phytochemicals is a more concentrated form of extract. So diluted extract should not be used while extracting phytochemicals. Other phytochemicals like fat, phenols, tannins, steroids were absent according to the result.

The anti-cancer activity was also determined using the papaya pulp and peel extracts. Breast cancer cell lines (MCF7) were used and cytotoxicity was studied using all three extracts. The result showed that there was a maximum 28% reduction in cell viability at the highest concentration tested. Ethyl acetate extract showed more toxicity to breast cancer cells than the other two extracts. When compared to [5] aerial parts of the Carica papaya showed anti-cancer activity in renal cancer, Breast Cancer cell lines. The lowest cell viability against normal cells was 80 percent (ethyl acetate extract) while the cell viability.

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V.REFERENCES

1. Ezekiel Amri and Florence Mamboya, “Papain, a Plant Enzyme of Biological Importance: A Review”. American Journal of Biochemistry and Biotechnology. 8 (2), 2012, 99-104.

2. Krishna, Paridhavi, “Review on nutritional, medicinal and pharmacological properties of Papaya (Carica papaya Linn.)”.Natural Product Radiance, Vol. 7(4), 7 April 2008.

3. Blois, M.S., “Antioxidant determinations by the use of a stable free radical”. Nature. 29:1199 1200, 1958.

4. Ayoola1 GA, HAB Coker, SA Adesegun, AA Adepoju-Bello, K Obaweya, EC Ezennia, TO Atangbayila, “Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria therapy in Southwestern Nigeria”. Tropical Journal of Pharmaceutical Research.7 (3) 2008: 1019-1024.

5. Khaled N. Rashed1, Gerda Fouche, Anticancer Activity of Carica papaya Extracts in vitro and Phytochemical Analysis, Greener Journal of Pharmacy and Pharmacology Vol. 1 (1) 2013.pp. 001-005.