

# ANTI MICROBIAL AND INVITRO ANTI LIPID PEROXIDATION ACTIVITIES OF THE LEAF EXTRACTS OF CARICA PAPAYA

(FAMILY: CARICACEAE)

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*Abstract:* The fruits, seeds, roots, latex and leaves of 'Carica papaya' (family Caricaceae) are having nutritive values and therapeutic efficacy. Papaya leaf has been used to treat the following ailments in humans: Abortifacient, Amoebicidal, Arthritis, Asthma, respiration, Bactericide, Cancer, Cardiotonic, Diarrhoea, Dysentery, Digestive, Diuretic, Constipation, laxative, Flu, Hypertension, Intestinal disorders, Scorpion bites, Tuberculosis, and Tumour (Uterus), etc. Hence the leaf part of this plant was taken for the project work to prove the ethnomedical information.

## I. INTRODUCTION

In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant materials traded across the countries. Therefore, the use and history of herbs dates back to the time of early man, who had the crudest tools as his implements and use stones to start his fire. They used herbs in their raw and cooked forms to keep fit. Since that time, the use of herbs has been known and accepted by all nations and has been known as the first art of treatment available to man.

The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.

There is no plant that does not have medicinal value. The active components are normally extracted from all plant structures, but the concentrations of these components vary from structure to structure. However, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds. The active principles singly or in combination inhibit greatly the life processes of microbes, especially the disease-causing ones. They do this by binding their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available interests to the microorganisms, other causes inflammation analysis of microbial cells.

Medicinal plants are defined as plants in which one or more of the organs contains substances that can be used for therapeutic purposes or which its precursors for the manufacturing of drugs are useful for disease therapy. The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent.

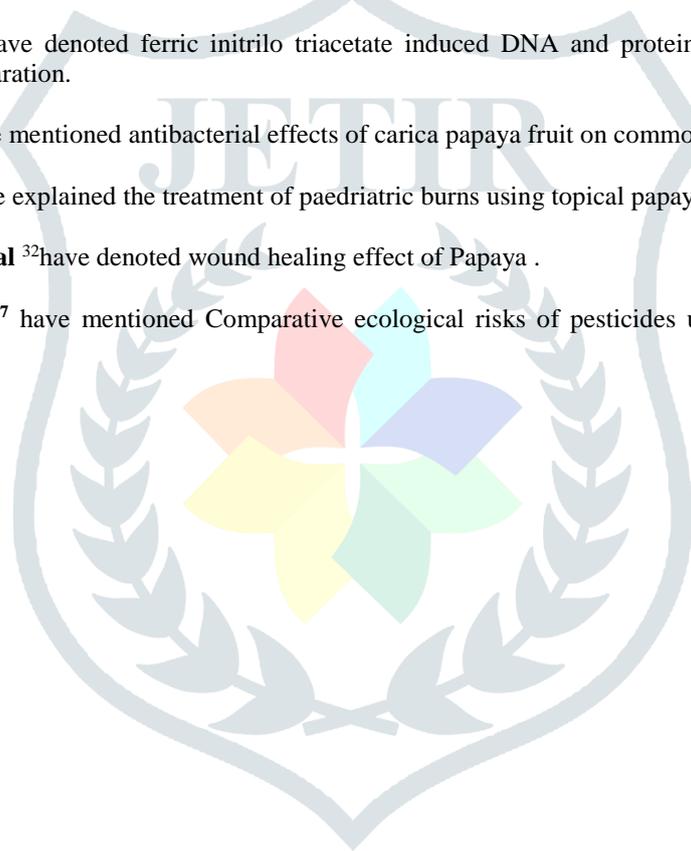
### Plan of work:

1. Collection and authentication of *Carica papaya* leaf
2. Pharmacognostical studies
  - Macroscopical Evaluation
    - Microscopical Evaluation
    - Physical Evaluation
    - Chemical Evaluation
3. Preparation of leaf Extracts with alcohol, chloroform and Petroleum ether. These extracts were used for the following activity
4. Anti-microbial activity
5. Anti-lipid per oxidation activity

### LITERATURE REVIEW

1. **Chinoy, et al<sup>3</sup>** have explained reversible effects of aqueous extract of papaya on microenvironment and sperm metabolism of cauda epididymis of rat.
2. **Akah, P.A., et al<sup>4</sup>** have denoted preliminary studies on purgative effect of *Carica papaya*.
3. **Brocklehurst, K., et al<sup>5</sup>** have mentioned that *Carica papaya* contains papain, multiple forms of chymopapain A and papaya proteinase .
4. **Joshi Harsha, et al<sup>6</sup>** have explained toxicity related response of female albino rats treated with benzene and alcoholic papaya seed extracts.
5. **K.G. Patel, et al<sup>7</sup>** have mentioned reversible effects of aqueous extract of papaya on microenvironment and sperm metabolism of cauda epididymis of rat.
6. **P.Padman. et al<sup>8</sup>** have denoted antifertility investigations on the benzene extract of *Carica papaya* in male albino rats.
7. **N.J., Dilip Trived et al<sup>9</sup>** have explained effect of *Carica papaya* extract on female rat ovaries and uteri.
8. **Ghosh, N.K., et al<sup>10</sup>** have mentioned antifilarial effect of the plant *Carica papaya*
9. **Harsha, et al<sup>11</sup>** have denoted the reversible antifertility effects of benzene extract of papaya on female rats.
10. **Redina, E.F, et al<sup>12</sup>** have explained determination of lysozyme in papaya preparations.
11. **Sandhya, et al<sup>13</sup>** have denoted studies on the biochemical constituents of exudates of papaya.
12. **Schwab, W et al<sup>14</sup>** have mentioned aryl beta -D-glucosides from *Carica papaya*.
13. **Giordani, R et al<sup>15</sup>** have mentioned fungicidal activity of *Carica papaya* and antifungal effect of D(+)-glucosamine *Candida albicans* growth.
14. **Adebayo, A. C., et al<sup>16</sup>** have denoted antimicrobial activities of the of carica papaya.
15. **Hernandez CN et al<sup>17</sup>** have mentioned Comparative ecological risks of pesticides used in plantation production of papaya.
16. **Rahmat A, et al<sup>18</sup>**, have denoted the effect of consumption of *Carica papaya* on total antioxidant and lipid profile in normal male youth.

17. **Bennett, R.N., et al<sup>23</sup>** have mentioned biosynthesis of benzylglucosinolate, cyanogenic glucosides and phenylpropanoids in *Carica papaya*.
18. **M.R.B., Amaya et al<sup>25</sup>** have denoted Volatile components and flavour of pawpaw (*Carica papaya*)
19. **Franco, M.R.B. et al<sup>26</sup>** have mentioned Volatile components of two pawpaw cultivars.
20. **Giordani, R., et al<sup>20</sup>** have denoted tributyrolylglycerol hydrolase activity in *Carica papaya*.
21. **Giordani, R., et al<sup>24</sup>** have mentioned antifungal action of *Carica papaya* isolation of fungal cell wall hydrolysing enzymes.
22. **Osato, J.A et al<sup>25</sup>** have denoted antimicrobial and antioxidant activities of papaya.
23. **Pousset, J.L., et al<sup>26</sup>** have explained. antihemolytic action of xylitol isolated from *Carica papaya* bark.
24. **Chen C F, et al<sup>27</sup>** have mentioned protective effect of *Carica papaya* on the exogenous gastric ulcer in rats.
25. **Emeruwa A<sup>28</sup>** a denoted antibacterial substance from *Carica papaya*.
26. **Rimbach G, et al<sup>29</sup>** have denoted ferric initrilo triacetate induced DNA and protein damage; inhibitory effect of a fermented papaya preparation.
27. **Dawkins G et al<sup>30</sup>** have mentioned antibacterial effects of carica papaya fruit on common wound organisms.
28. **starley I F., et al<sup>31</sup>** have explained the treatment of paediatric burns using topical papaya.
29. **Mikhalchick E .V., et al<sup>32</sup>** have denoted wound healing effect of Papaya .
30. **Hernandez CN, et al<sup>17</sup>** have mentioned Comparative ecological risks of pesticides used in plantation production of papaya.



**DESCRIPTION OF THE PLANT<sup>33</sup>****CLASSIFICATION:**

<b>Botanical Name</b>	:	<i>CARICA PAPAYA</i>
<b>Kingdo</b>	:	Plantae
<b>Division</b>	:	Magnoliophyta
<b>Class</b>	:	Magnoliopsida
<b>Order</b>	:	Violales
<b>Family</b>	:	Caricaceae
<b>Genus</b>	:	Carica L.
<b>Species</b>	:	Carica papaya
<b>Popular Name(s)</b>	:	Paw Paw, Kates, Papaw

**VERNACULAR NAMES:**

<b>Arabic</b>	:	fafay, babaya
<b>Bengali</b>	:	pappaiya, papeya
<b>English</b>	:	pawpaw papaya
<b>Hindi</b>	:	papaya, papeeta
<b>Malay</b>	:	papaya, betek, ketalah, kepaya
<b>Tamil</b>	:	pappali, pappaya

**CARICA PAPAYA ENTIRE PLANT****FIGURE NO:1**

**Habitat:**

Commonly and erroneously referred to as a "tree", the plant is properly a large herb growing at the rate of 6 to 10 ft (1.8-3 m) the first year and reaching 20 or even 30 ft (6-9 m) in height, with a hollow green or deep-purple stem becoming 12 to 16 in (30-40 cm) or more thick at the base and roughened by leaf scars. The leaves emerge directly from the upper part of the stem in a spiral on nearly horizontal petioles 1 to 3 1/2 ft (30-105 cm) long, hollow, succulent, green or more or less dark purple. The blade, deeply divided into 5 to 9 main segments, each irregularly subdivided, varies from 1 to 2 ft (30-60 cm) in width and has prominent yellowish ribs and veins. The life of a leaf is 4 to 6 months. Both the stem and leaves contain copious white milky latex.

**MACROSCOPIAL CHARACTERS<sup>33</sup>**

<b>Leaves arrangement</b>	:	spiral
<b>Apex</b>	:	crowded
<b>Petiole</b>	:	1m long, hollow
<b>Colour</b>	:	green
<b>Diameter</b>	:	25-75cm
<b>Nature</b>	:	palmate
<b>Venation</b>	:	reticulate
<b>Lobes</b>	:	deeply and broadly toothed
<b>Odour</b>	:	characteristic
<b>Taste</b>	:	Bitter

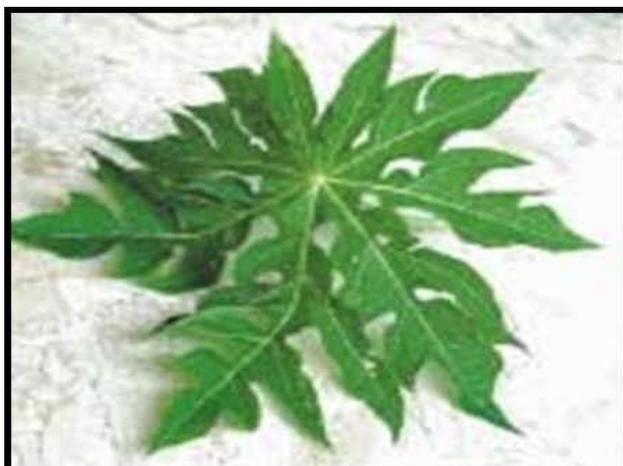
**MACROSCOPY OF CARICA PAPAYA LEAF**Figure : 2 **DORSAL LEAF**

Figure : 3 VENTRAL LEAF

### MICROSCOPICAL EVALUATION<sup>33</sup>

The transverse section of leaf of *Carica papaya* shows the following characters:

#### Cuticle:

Single layered thick cuticle was found above the upper and lower epidermal layer.

#### Upper Epidermis & Lower Epidermis:

A single layer parenchyma cells covered on the outer side by a thick warty cuticle.

#### Palisade cells:

Dorsi ventral leaf have a single layered compactly arranged narrow cells were seen on the mesophyll region.

#### Collenchyma cells:

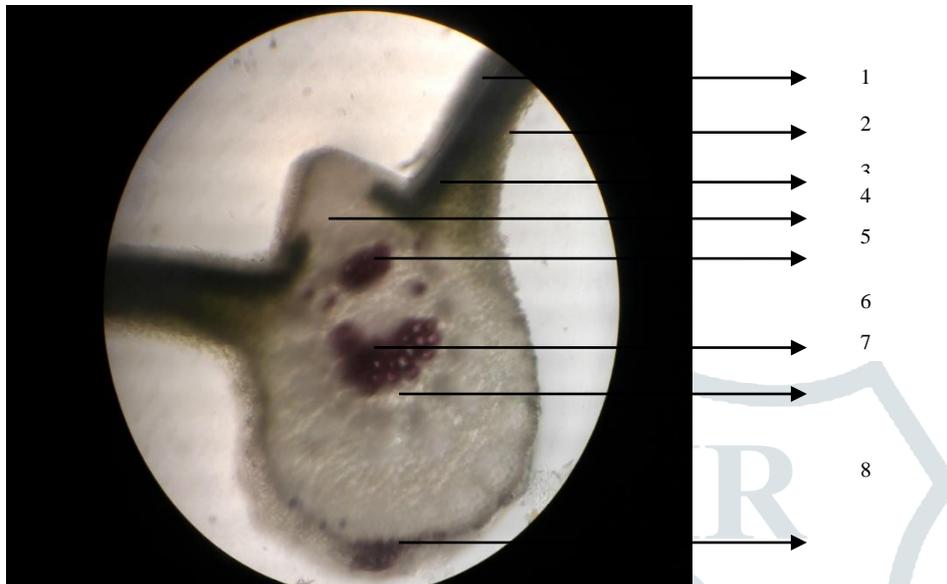
2-5 layered collenchymatous cells with cell wall at the corners get thickened with cellulose. It provides mechanical strength. Trichomes, stomata and lower palisade cells were absent.

#### Vascular bundles:

Concentric vascular bundles were seen. It consists of vessels and xylem parenchyma and phloem fibers were present. These were highly lignified.

**T.S OF LEAF**

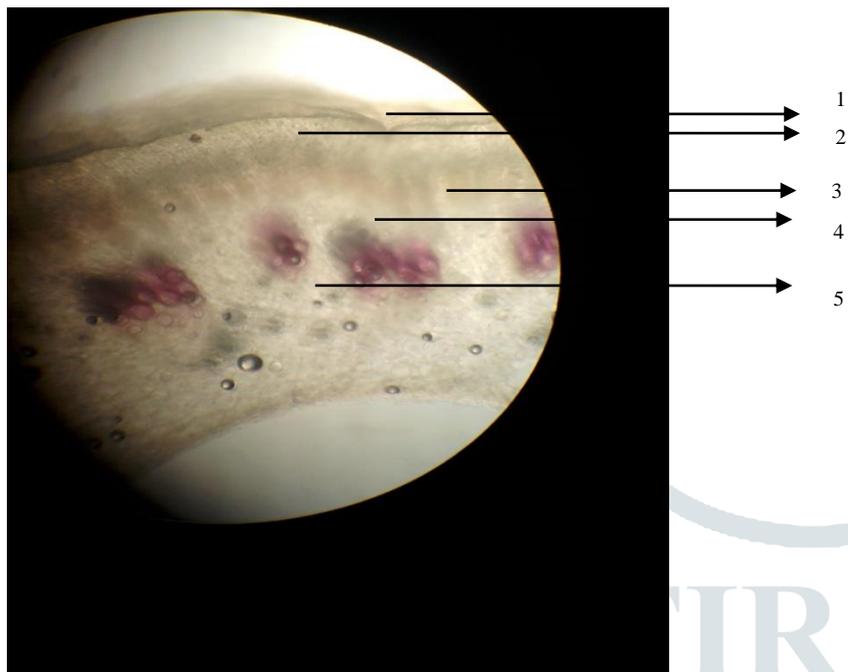
Figure No:4



- 1.Upper epidermis
- 2.Lower epidermis
- 3.Lamina
- 4.Midrib
- 5.Lignified Fiber
- 6.Xylemvessel
7. Phloem
- 8.Collenchyma

**T.S of Petiole:**

Figure No:5



- 1. Epidermis with cuticle
- 2. Palisade layer
- 3. Phloem paranchyma
- 4. Xylem
- 5. Cortical region

**VEIN ISLET OF CARICA PAPAYA LEAF**

Figure No:6



**CHARACTERS:**

<b>Nature</b> :	Coarse
<b>Colour</b> :	Green
<b>Odour</b> :	Characteristic
<b>Taste</b> :	Bitter

Powder microscopy of the leaf of “**Carica papaya**” reveals the following characters:

- Group of palisade cells.
- Double layered parenchyma
- Collenchyma cells
- Spindle shaped lignified fibers
- **Stomata and trichomes are not found.**

**PHYSICAL EVALUATION:****DETERMINATION OF ASH VALUE****PROCEDURE:**

The ash values were determined by using air dried powder of the leaf as per the Indian pharmacopoeia 1995.

**TOTAL ASH:**

Two grams of the air-dried leaf powder was accurately weighed in a platinum crucible. The powder was scattered into a fine even layer on this bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450°C, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air-dried powder was calculated and the results were tabulated in table no.1

**WATER SOLUBLE ASH:**

The ash obtained from the total ash procedure was boiled with 25 ml of water for 5 mins and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 mins at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powder and mentioned in table no. 1

**ACID INSOLUBLE ASH:**

The ash obtained from the total ash was boiled for five mins with 25 ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air-dried drug was calculated and the results were tabulated in table no.1

**Table No.1****ASH VALUES FOR THE POWDERED LEAF OF CARICA PAPAYA**

S.NO	TOTAL ASH (%w/w)	ACID INSOLUBLE ASH (%w/w)	WATER SOLUBLE ASH (%w/w)
1.	9.9	57.4	84.8
2.	9.7	58.6	85.2
3.	8.9	56.6	79.8
4.	9.2	59.6	80.5
5.	9.7	58.7	79.9

**DETERMINATION OF LOSS ON DRYING****PROCEDURE:**

For the determination of loss on drying the method described by Wallis was followed. One gram of the powdered leaf was accurately weighed in a tarred Petri dish, previously dried. The powder was distributed as evenly as possible, by gentle side wise shaking. The dish was dried in an oven at 100-105°C for 1 hour. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the air dried powder.

**Table No. 2****LOSS ON DRYING FOR THE POWDERED LEAF OF CARICA PAPAYA**

S.NO	LOSS ON DRYING % W/W
1.	29.7
2.	30.3
3.	31.3
4.	30.4
5.	30.0

**DETERMINATION OF EXTRACTIVE VALUES**

**PROCEDURE:**

Five grams of the coarsely powdered leaf was macerated separately with 100 ml of solvents in a closed flask for 24 hrs. It was frequently shaken for the first 6 hrs and allowed to stand for 18 hrs. Then it was filtered and the 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air-dried powder.

The same procedure was carried out using various solvents like, Petroleum ether, benzene, chloroform, ethanol and water.

**Table No. 3****EXTRACTIVE VALUES FOR THE POWDERED LEAF OF CARICA PAPAYA**

S.NO	SOLVENTS	EXTRACTIVE VALUE % W/W
1.	Petroleum ether	4.6
2.	Benzene	5.4
3.	Chloroform	8.8
4.	Ethanol	19.6
5.	Water	1.4

**PHYTOCHEMICAL STUDIES****TEST FOR ALKALOIDS**

Powdered drug was mixed thoroughly with 1 ml of 10% ammonia solution and then extracted for 10 mins with 5 ml methanol, under reflux. The filtrate was then concentrated. The above extract was tested with various alkaloidal reagents and the results were as follows.

1. Mayer's reagent - green color precipitate
2. Dragendorff's reagent - reddish brown precipitate
3. Hager's reagent - yellow precipitate
4. Wagner's reagent - reddish brown precipitate

The above tests indicate the **presence** of alkaloids.

**TEST FOR CARBOHYDRATES**

## 1. Molisch's Test:

The aqueous extract of the powdered material was treated with alcoholic solution of  $\alpha$  naphthol in sulphuric acid.

Purple color was obtained indicating the **presence** of carbohydrate

## 2. Fehling's Test:

The aqueous extract of the powdered material was treated with Fehling's I and II solution and heated on boiling water bath.

Reddish brown precipitate was obtained indicating the **presence** of free reducing sugar.

## 3. Benedict's Test:

The aqueous extract of the powdered drug was treated with Benedict's reagent and heated on a water bath.

Reddish brown precipitate was obtained indicating the **presence** of reducing sugar.

## TEST FOR GLYCOSIDES

### ANTHRAQUINONES

#### 1. Borntrager's Test:

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. Then inorganic layer was separated and ammonia solution was added slowly.

No color reaction was observed in ammoniacal layer indicating the **absence** of anthracene derived glycosides.

#### 2. Modified Borntrager's Test:

About 0.1 gm of powdered leaf was boiled for 2 mins with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

No color was observed in ammonical layer indicating the **absence** of anthracene derived glycosides.

## TEST FOR CARDIAC GLYCOSIDES

#### Keller Killiani Test:

About 1 gm of the powdered leaf was boiled with 10 ml of 70% alcohol for two mins, cooled and filtered. To the filtrate 10 ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid containing a trace of ferric chloride. To this 3 ml of concentrated sulphuric acid was added to the side of the test tube carefully.

No reddish-brown layer acquiring bluish green color after standing was observed indicating the **absence** of deoxy sugars of cardiac glycosides.

## TEST FOR PHYTOSTEROLS

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

#### Salkowski Test:

Few drops of concentrated sulphuric acid were added to the above solution shaken well and set aside.

The chloroform layer of the solution turned red in color indicating the presence of sterols.

#### Liebermann – Burchard's Test:

To the chloroform solution few drops of acetic anhydride was added and mixed well 1 ml of concentrated sulphuric acid through the sides of the test tube and set aside for a while.

A brown ring was formed at the junction of two layers and the upper layer turned green indicating the **presence** of sterols.

## TEST FOR SAPONINS

About 0.5 gm of the powdered leaf was boiled gently for 2 mins with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously.

Frothing was not occurred indicating the **absence** of saponins.

#### TEST FOR TANNINS

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

Bluish black color was produced, indicating the **presence** of tannins.

#### TEST FOR PROTEINS AND AMINOACIDS

##### 1. Millon's Test:

The aciduous alcoholic extract of the powdered leaf was heated with Millon's reagent.

The color was not changed to red on heating indicating the **absence** of proteins.

##### 2. Biuret Test:

To the alcoholic extract of the powdered leaf 1 ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

Violet color was not obtained indicating the **absence** of proteins.

##### 3. Ninhydrin Test:

To the aqueous extract of the powdered drug, Ninhydrin solution was added, and boiled.

Formation of no violet color indicating the **absence** of amino acids.

#### TEST FOR MUCILAGE

To the aqueous extract of the powdered leaf, ruthenium red solution was added.

No reddish pink color was produced indicating the **absence** of mucilage.

#### TEST FOR FLAVANOIDS

##### Shinoda Test:

A little amount of powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 mins.

Purple color was not obtained indicating the **absence** of flavonoids.

#### TEST FOR TERPENOIDS

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the solution tin and thionyl chloride were added.

Pink color was not obtained indicating the **absence** of terpenoids.

#### TEST FOR VOLATILE OIL

About 100 gm of fresh leaves were taken in a volatile oil estimation apparatus (Cocking Middletor Apparatus) and subjected to hydro distillation for 4 hours.

Golden yellow color volatile oil was not obtained indicating the **absence** of volatile oil.

#### TEST FOR FIXED OIL

A small amount of the powdered leaf was pressed in between the filter paper and the paper was heated in an oven at 105°C for 10 mins.

No translucent greasy spot occurred indicating the absence of fixed oil.

**PHYTOCHEMICAL STUDIES**  
**TABLE NO:4**

S.NO	TEST	POWDERED DRUG	ALCOHOLIC EXTRACT	PET ETHER EXTRACT	CHLOROFORM EXTRACT
<b>1.</b>	<b>ALKALOIDS</b>				
	MAYER'S REAGENT	+	+	+	+
	DRAGENDORFF'S REAGENT	+	+	+	+
	HAGER'S REAGENT	+	+	+	+
	WAGNER'S REAGENT	+	+	+	+
<b>2.</b>	<b>CARBOHYDRATES</b>				
	MOLISCH'S TEST	+	+	+	+
	FEHLING'S TEST	+	+	+	+
	BENEDICT'S TEST	+	+	+	+
<b>3.</b>	<b>GLYCOSIDES</b>				
	ANTHRAQUINONE	-	-	-	-
	CARDIAC	-	-	-	-
<b>4.</b>	<b>PHYTOSTEROLS</b>				
	SALKOWSKI TEST	+	+	+	+
	LIEBERMANN BURCHARD'S TEST	+	+	+	+
<b>5.</b>	<b>SAPONINS</b>	-	-	-	-
<b>6.</b>	<b>TANNINS</b>	+	+	+	+
<b>7.</b>	<b>PROTEINS AND FREE AMINO ACIDS</b>	-	-	-	-

S.N O	TEST	POWDERED DRUG	ALCOHOLIC EXTRACT	PET ETHER EXTRACT	CHLOROFORM EXTRACT
	MILLON'S TEST	-	-	-	-
	BIURET TEST	-	-	-	-
<b>8.</b>	<b>GUMS AND MUCILAGE</b>	-	-	-	-
<b>9.</b>	<b>FLAVANOIDS</b>				

	<b>SHINODA TEST</b>	-	-	-	-
<b>10.</b>	<b>TERPENOIDS</b>	-	-	-	-
<b>11.</b>	<b>VOLATILE OIL</b>	-	-	-	-
<b>12.</b>	<b>FIXED OIL</b>	-	-	-	-

(+)Indicates Positive reaction

(-) Indicates Negative reaction

### **ANTI MICROBIAL ACTIVITY** <sup>36,37,38</sup>

Microbiology is the study of living organisms of microscopic size which includes bacteria, fungi, algae, protozoa and viruses etc. Microorganisms of our study are bacteria and fungi. Bacterial infections as well as fungal infections are most commonly experienced in human beings.

#### **ANTI MICROBIAL AGENTS:**

Antimicrobial drugs have greatest contribution of the 20<sup>th</sup> century therapeutics. Their importance is magnified in the developing countries, where infective diseases predominate. As a class they are one of the most frequently used as well as misused drugs. Drugs in the class differ from all other in that they are designed to inhibit or kill the infecting organism and to have no or minimal effect on the recipient.

Antibiotics are substances produced by microorganism, which suppress the growth of or kill other microorganism at very low concentrations.

Antimicrobials act against organisms in a number of ways:

1. Inhibition of cell wall synthesis
2. Damage to cytoplasmic membrane
3. Inhibition of nucleic acid and protein synthesis
4. Inhibition of specific enzymes.

#### **MICROBIAL ASSAYS:**

The inhibition of microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of antibiotics. Microbiological assay is based upon a comparison of inhibition of growth of bacterial by measuring the concentration of the antibiotics to be examined with that produced by known concentrations of standard preparation of antibiotics having a known activity.

#### **EVALUATION:**

Conditions must be adopted

1. There should be intimate relationship between test organisms and substrate to be evaluated.
2. Microorganisms should be provided with required growth conditions.
3. Measurement of activity should be performed correctly.
4. Aseptic conditions should be maintained.

#### **METHODS:**

There are different methods available for evaluation of anti-microbial activities.

1. Agar streak dilution method
2. Serial dilution method
3. Agar diffusion method

4. Cup plate method
5. Paper disc method
6. Turbidimetric method

## CUP AND PLATE METHOD

### PROCEDURE

#### Preparation of Muller-Hinton Agar medium (M.H. medium)

Beef extract	-	300 gm
Peptone	-	17.5gm
Starch	-	1.5 gm
Agar	-	17gm
distilled water	-	up to 1000 ml.

#### METHOD:

All the ingredients were weighed and suspended in 1000ml of distilled water and heated to boiling. The PH of the media was adjusted to 7.4 with 5m Sodium hydroxide solution. Then 20ml of M.H. agar medium was transferred into boiling tube and plugged with non-absorbent cotton and sterilized in an autoclave at 15lbs at 121°C for 20min.

#### Inoculation of Microorganism:

Then M.H. agar medium was transferred into two different test tubes and inoculated with microorganism (gram +ve → *Bacillus megaterium*, gram -ve → *E.coli*)

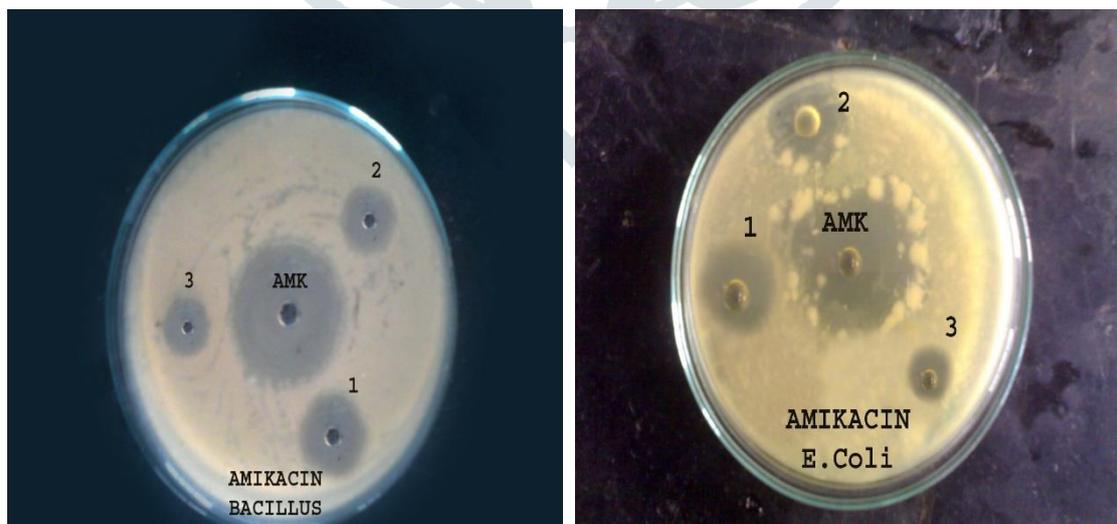
After inoculation the media was immediately poured into the previously sterilized Petri dishes with uniform thickness and allowed to solidify cups were made in Petri dishes using sterile cork borer (6mm)

#### Inoculation of test samples and standard Antibiotics:

Then the cups were filled with different extracts of alcoholic (Test – 1), chloroform (Test – 2), Pet. Ether (Test – 3) and standards Amikacin (Std – 1), Gentamycin (Std – 2), Cefotaxime (Std – 3), and incubated at 37°C for 24 hours. The diameter of zone of inhibition were measured and tabulated in table no: 5

### ANTI MICROBIAL ACTIVITY OF *CARICA PAPAYA* LEAF EXTRACTS

Figure No:8



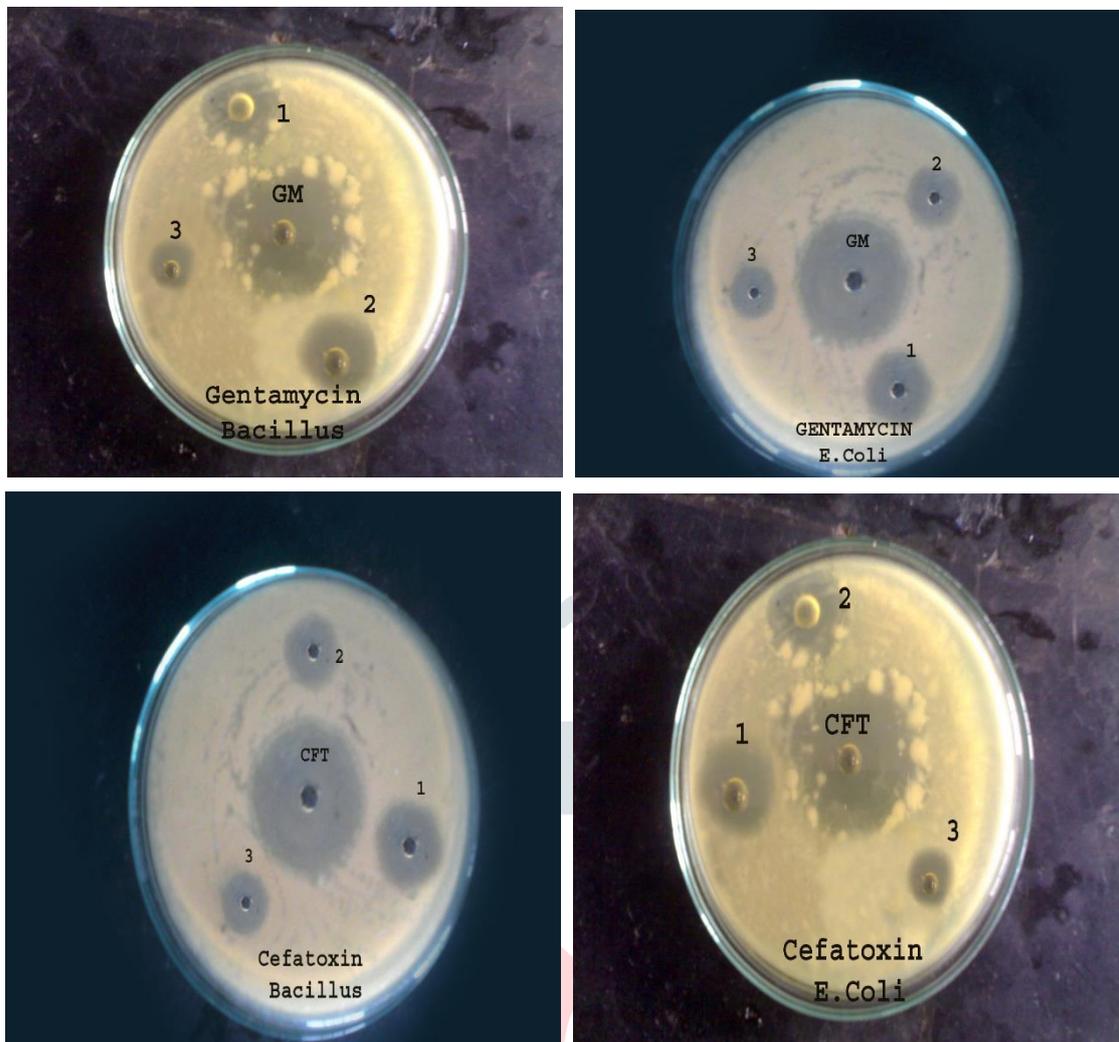


TABLE NO: 5

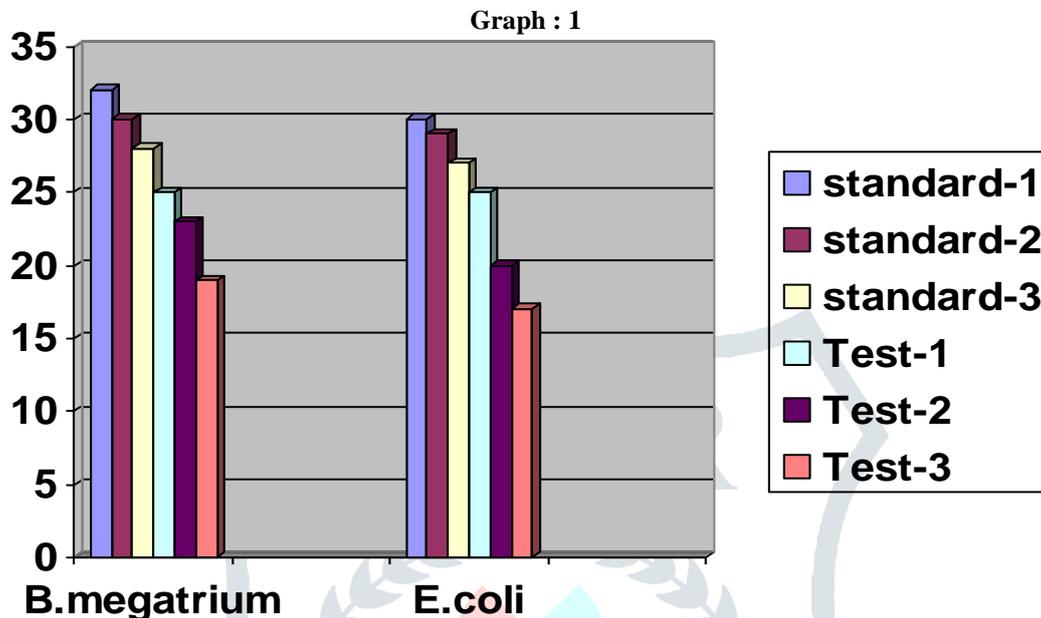
Anti microbial activity of alcoholic, pet.ether, chloroform extract of *Carica papaya* leaf

S.NO	FORMULATION	ZONE OF INHIBITION(mm)	
		GRAM POSITIVE	GRAM NEGATIVE
		BACILLUS MEGATRIUM	ESCHERCHIA COLI
1	Standard-1	32	30
2	Standard-2	30	29
3	Standard-3	28	27
4	Test-1	25	25
5	Test-2	23	20
6	Test-3	19	17

Standard-1 = AMIKACIN

Standard-2 = GENTAMYCIN  
 Standard-3 = CEFOTAXIM  
 Test 1 = ALCOHOLIC EXTRACT  
 Test 2 = CHLOROFORM EXTRACT  
 Test 3 = PETROLIUM EXTRACT

**ANTI MICROBIAL ACTIVITY OF *CARICA PAPAYA* LEAF EXTRACTS**



**Determination of Minimum Inhibitory Concentration (MIC)**

For determination of Minimum Inhibitory Concentration cup and plate method was adopted. The various extracts made into different concentrations (100,200,400,800,1000 mcg/ml) and were inoculated over the medium containing micro organism Bacillus megatrium and E.coli the Minimum Inhibitory Concentration for each extract was determine and tabulated in table no: 6

**TABLE NO:6**

**DETERMINATION OF MIC FOR DIFFERENT EXTRACTS OF *CARICA PAPAYA* LEAF**

Conc	BACILLUS MEGATRIUM			ESCHERCHIA COLI		
	ALCOHOLIC EXTRACT	CHLORO-FORM EXTRACT	PETRO-LIUM ETHER EXTRACT	ALCOHOLI C EXTRACT	CHLORO-FORM EXTRACT	PETROLIUM ETHER EXTRACT
1.	-	-	-	-	-	-
2.	10	-	09	03	-	-
3.	18	14	15	06	05	03
4.	21	20	17	13	11	15
5.	25	23	19	25	20	17

1=100mcg/ml

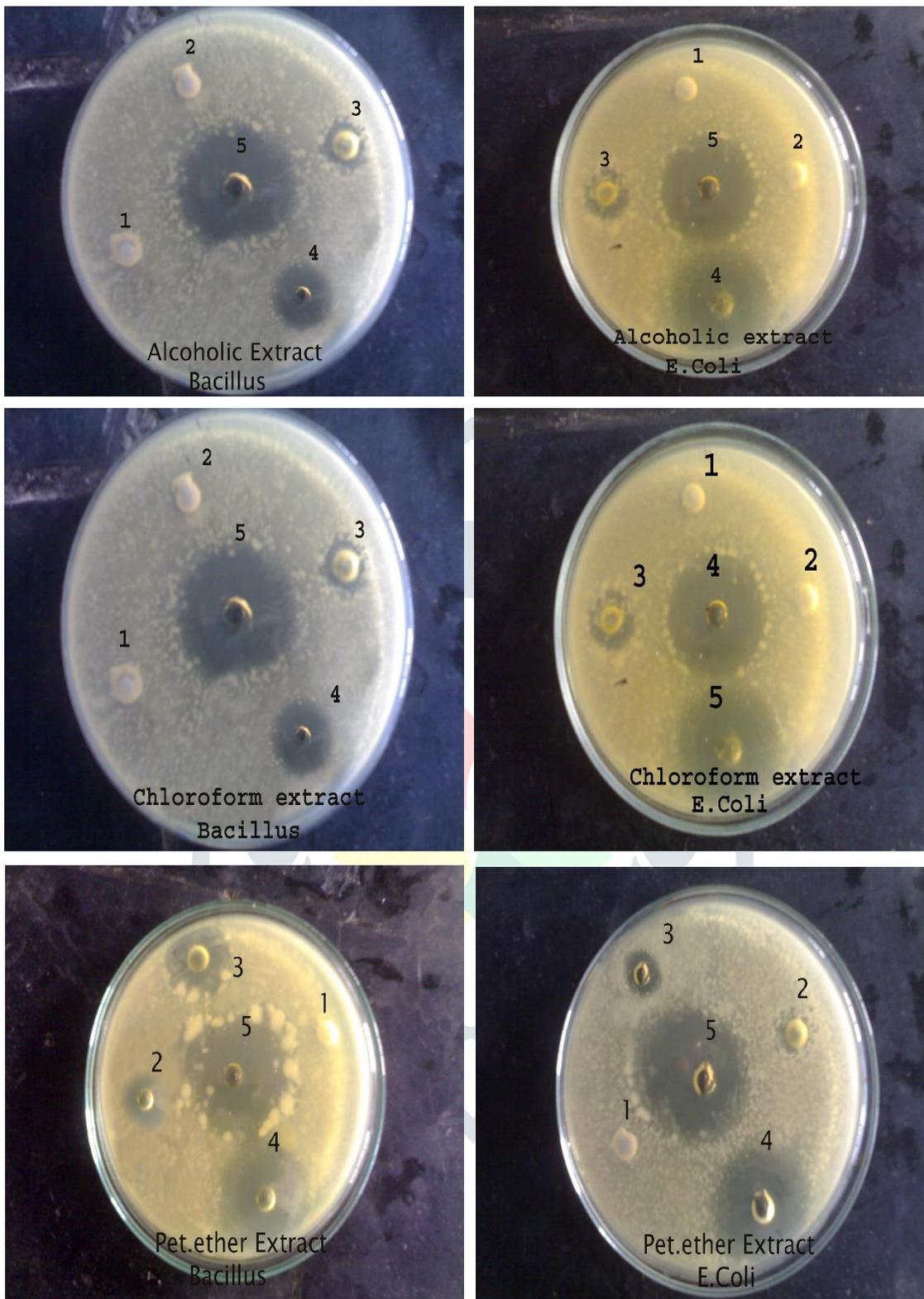
2=200mcg/ml

3=400mcg/ml 4=800mcg/ml

5=1000mcg/ml

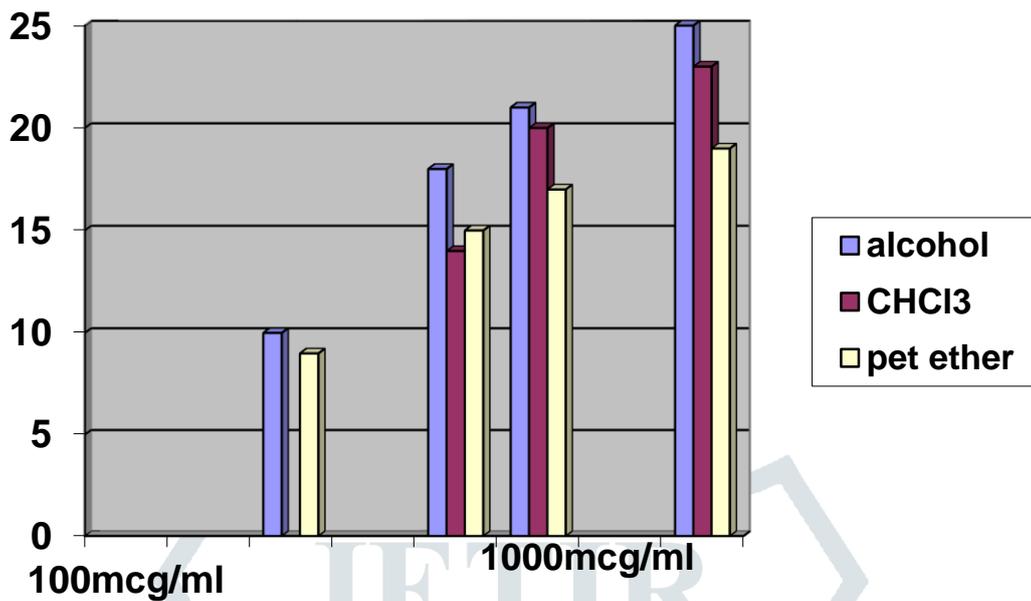
DETERMINATION OF MIC FOR DIFFERENT EXTRACTS OF CARICA PAPAYA LEAF

Figure No:9



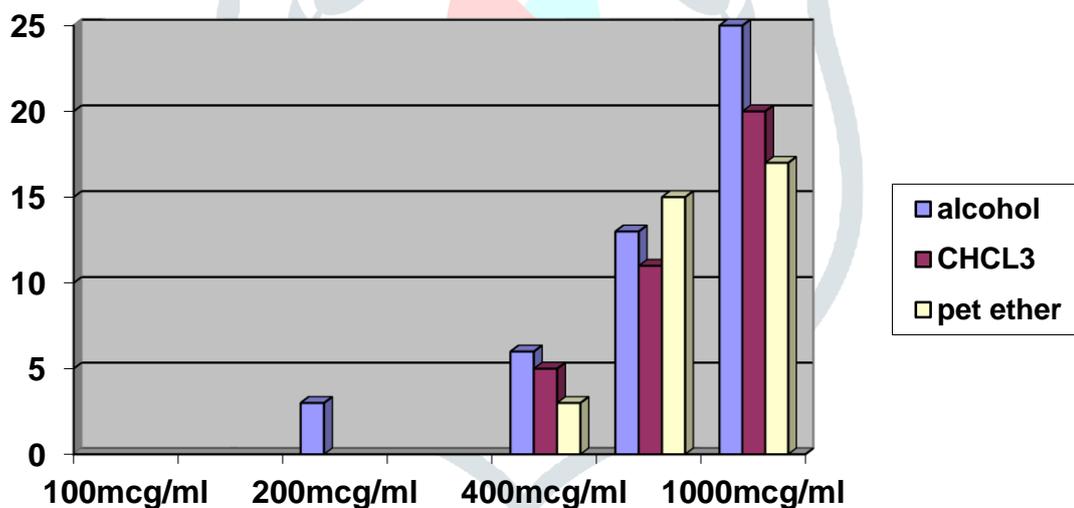
**MINIMUM INHIBITORY CONCENTRATION**  
**Concentration vs various extracts on Bacillus megatrium**

**Graph: 2**



**Concentration vs various extracts on E.coli**

**Graph : 3**



**DETERMINATION OF SYNERGISTIC /ANTAGONISTIC ACTIVITY OF DIFFERENT EXTRACTS OF CARICA PAPAYA LEAF**

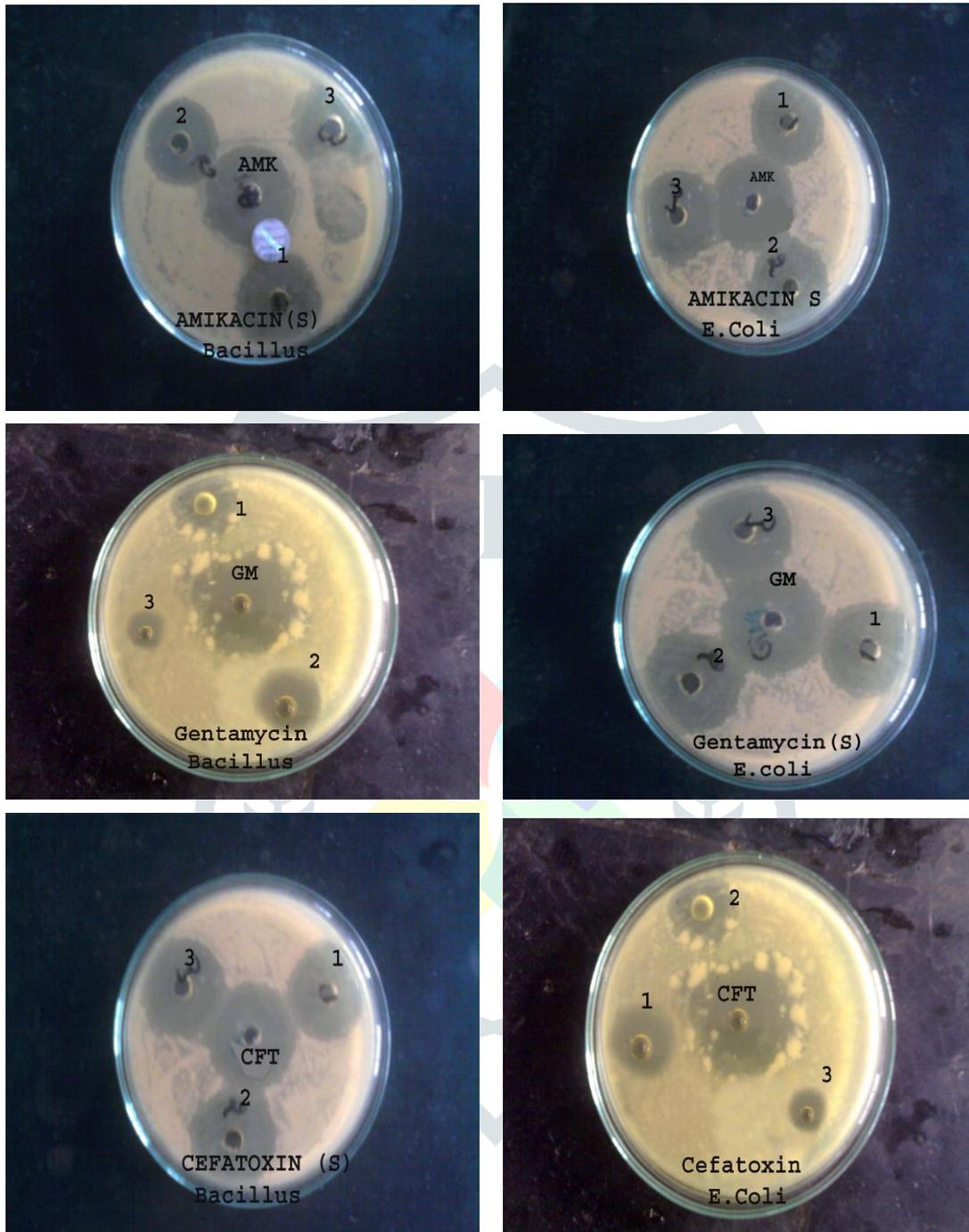
For observing the synergetic or Antagonistic Activity of different extracts of carica papaya leaf on three different antibiotics namely gentamycin, Amikacin and Cefatoxim. The cup and plate method was adopted.

**PROCEDURE:**

The three different extracts and antibiotics were mixed individually with each other at 1000mcg/ml and poured into the cups of petridishes. The antimicrobial effect was studied for the Bacillus megatrium and E.coli micro organism. The zone of inhibition was measured and tabulated in table no:7

DETERMINATION OF SYNARGITIC /ANTAGONISTIC ACTIVITY OF DIFFERENT EXTRACTS OF CARICA PAPAYA LEAF

Figure No:9



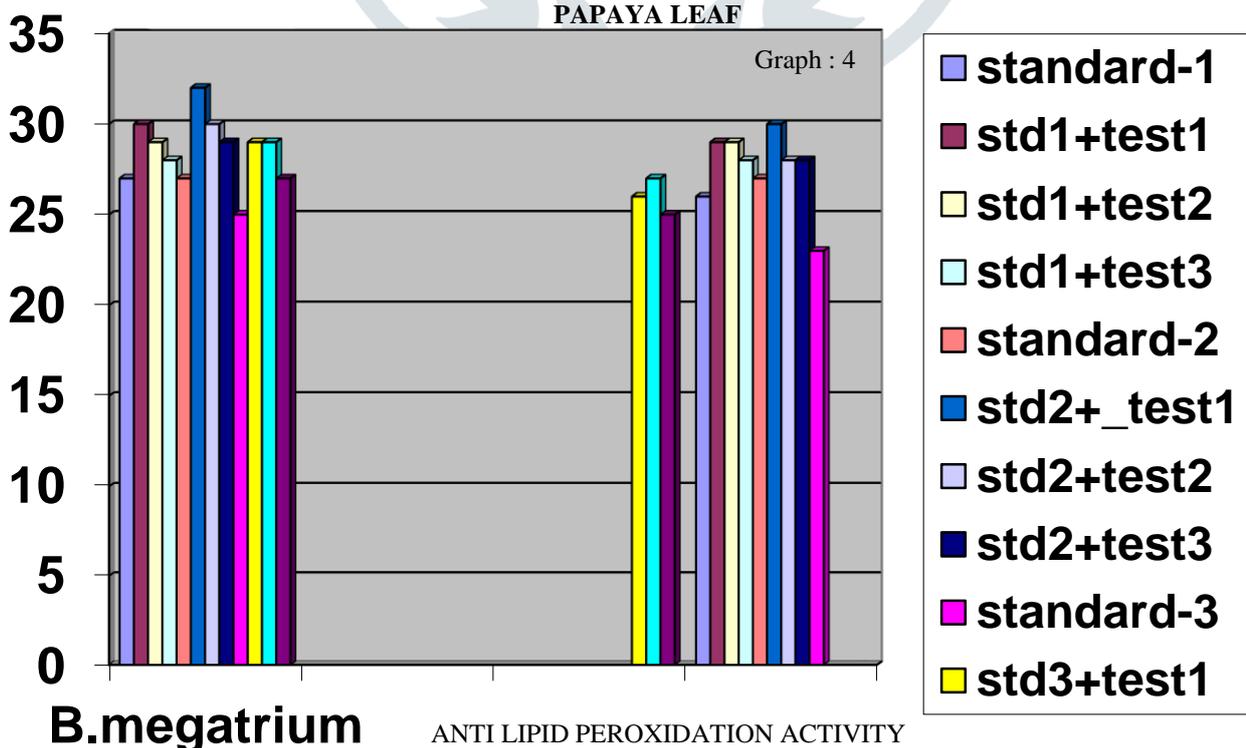
**SYNERGISTIC /ANTAGONISTIC ACTIVITY OF DIFFERENT EXTRACTS OF CARICA PAPAYA LEAF**

**TABLE NO: 7**

S.NO	FORMULATION	ZONE OF INHIBITION(mm)	
		GRAM POSITIVE	GRAM NEGATIVE
		BACILLUS MEGATRIUM	ESCHERCHIA COLI
1.	<b>STANDARD-1</b>	<b>27</b>	<b>26</b>
2.	STD-1+TEST-1	30	29
3.	STD-1+TEST-2	29	29
4.	STD-1+TEST-3	28	28
5.	<b>STANDARD-2</b>	<b>27</b>	<b>27</b>
6.	STD-2+TEST-1	32	30
7.	STD-2+TEST-2	30	28
8.	STD-2+TEST-3	29	28
9.	<b>STANDARD-3</b>	<b>25</b>	<b>23</b>
10.	STD-3+TEST-1	29	26
11.	STD-3+TEST-2	29	27
12.	STD-3+TEST-3	27	25

Standard-1 = AMIKACIN  
 Standard-2 = GENTAMYCIN  
 Standard-3 = CEFOTAXIM  
 Test 1 = ALCOHOLIC EXTRACT  
 Test 2 = CHLOROFORM EXTRACT  
 Test 3 = PETROLEUM EXTRACT

**DETERMINATION OF SYNERGISTIC /ANTAGONISTIC ACTIVITY OF DIFFERENT EXTRACTS OF CARICA PAPAYA LEAF**



Anti oxidants are compounds which even at relatively small concentrations act as inhibitions of the process of oxidation. The present study is an attempt to explore the antioxidant activity of carica papaya plant and tested by measuring the levels of Malonaldehyde (MDA) using goat liver homogenate.

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing.

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

Cell damage caused by free radicals appears to be a major contributor of aging and to degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants.

Antioxidants are capable of stabilizing, or deactivating the free radicals before they attack cells. They are absolutely critical for maintaining optimal cellular and systemic health and well-being.

### ANTI LIPID PEROXIDASE SCREENING METHODS

1. The role of lipid peroxidation and neutrophil accumulation in the gastric mucosal injury induced by aspirin-Hcl in rats; Effect of roxatidine, a histamine H2 receptor antagonist with antioxidative properties is screened by Yuji naito, toshikazu yoshikawa et al.<sup>39</sup>
2. The effects of paracetamol and propacetamol on gastric mucosal damage and gastric lipid peroxidation caused by acetylsalicylic acid (ASA) in rats is evaluated by pharmacological research.<sup>40</sup>
3. Neutrophils, lipid peroxidation, and nitric oxide in gastric reperfusion injury in rats is evaluated by yuji naito et al.<sup>41</sup>
4. Free radical, antioxidant enzymes activities and ameliorated the liver damage by an increase in antioxidant enzymes activities is screened by Jainu, et al<sup>42</sup>
5. In- vitro anti lipid peroxidation activity of papaya by measuring the level of malonaldehyde using goat liver homogenate was carried out by Nayak et al.<sup>43</sup>

### SCREENING FOR ANTI LIPID PEROXIDATION ACTIVITY PLANT EXTRACT:

#### MATERIALS REQUIRED:

- Centrifuge
- Spectrophotometer
- Ascorbic acid
- Thiopentone sodium
- Trichloroacetic acid
- Ferric chloride
- Fresh goat liver

#### PREPARATION OF LIVER EXTRACT:<sup>43</sup>

The freshly collected goat liver was purchased from the local market. After washing, the liver was homogenized in phosphate buffer (PH-7.4) to get a 10% liver homogenate.

**PROCEDURE:**

Fresh liver homogenate was mixed with phosphate buffer. The test agents (Alcoholic, chloroform and petroleum ether extract of leaf of *carica papaya*) were added in various concentrations. In vitro anti lipid peroxidation was initiated by addition of ferric chloride solution. Then different concentration (1mg, 2mg, 3mg, 4mg, 5mg) of Alcoholic, chloroform and petroleum ether extract were added to liver homogenate. After incubation for 4 hours, Trichloro acetic acid (TCA) was added to all tubes containing liver extract in 1:1 ratio and centrifuge for 30 min. The supernatant liquid was collected and Thiopentone sodium was added in 1:1 ratio and heated for 1 hour in water bath, cool it then absorbance was measured at 530nm. These absorbance was measured and tabulated in table no:8

**TABLE NO:****STANDARD: 0.562**

{S.E.M.: Standard Error Mean of 3 readings}

CONCENTRATION OF EXTRACT (mcg/mg)	ALCOHOL EXTRACT Conc. of extracts (mcg/ml)±S.E.M.	CHLOROFORM EXTRACT Conc. of extracts (mcg/ml)±S.E.M.	PET.ETHER EXTRACT Conc. of extracts (mcg/ml)±S.E.M.
S1	0.783	0.462	0.655
S2	0.802	0.555	0.761
S3	0.892	0.720	0.821
S4	0.914	0.783	0.895
S5	1.023	0.814	0.92

Statistically significant at 5% level ( $p < 0.05$ )

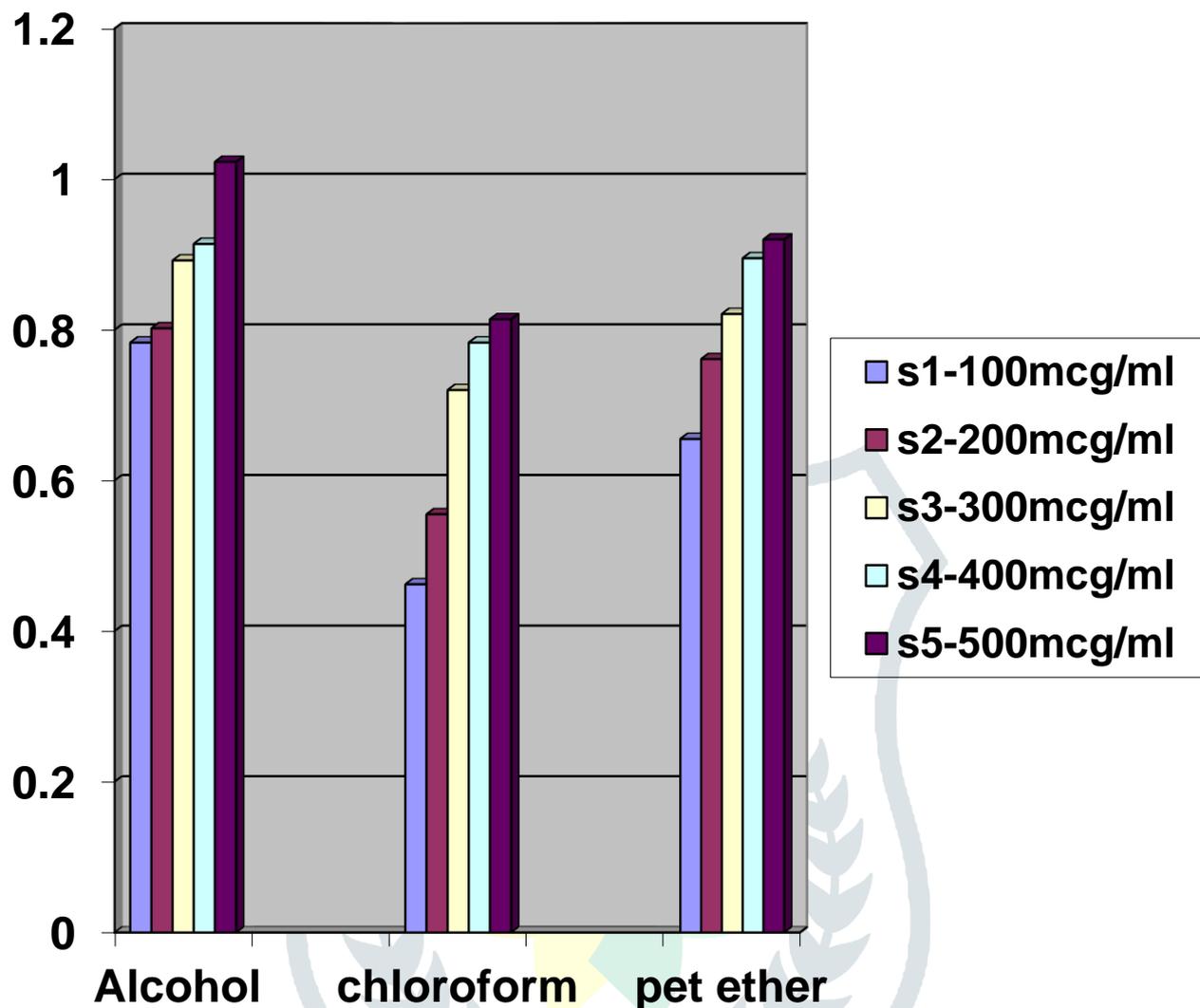
S1=100mcg/ml S2=200mcg/ml S3=300mcg/ml

S4=400mcg/ml S5=500mcg/ml

**STATISTICAL ANALYSIS:**

The results were analyzed statistically by using students "t" test and it was found significant at the level of 5% ( $p < 0.05$ ) compared to the standard ascorbic acid.

## IN-VITRO ANTI LIPID PEROXIDATION ACTIVITY

RESULTS AND DISCUSSION

This dissertation covers the works on pharmacognostic, preliminary phytochemical, anti microbial activity and anti lipid peroxidation activity screening for the leaf part of the plant *carica papaya*. (family *caricaceae*)

- **Pharmacognostic studies:**

In macro scopy the morphological characters like palmate nature, reticulate venation, deeply lobed lamina were found in carica papaya leaf.

In micro scopy of the leaf parenchyma, arenchyma, double layered palisades, xylem vessels, phloem cells, vein islets were observed and **specific characters like trichomes, stomata and calcium oxalate crystal were not found.**

- **Physical evaluation:**

The following physical parameters were determined by using powdered leaf. Maximum total ash value - **9.9**, Maximum Acid insoluble ash value - **59.6**, Maximum Water soluble ash value - **85.2**, Maximum value of loss on drying - **31.3**, **Maximum Extractive values were found in Ethanolic extract of leaf - 19.6**

- **Phytochemical studies:**

In this the preliminary phytochemical screening of the powdered leaf was performed

The powdered drug and the prepared extracts were taken for phyto chemical studies .In powdered drug contains **carbohydrates, phytosterols.**

The alcoholic extract having **alkaloids**, Chloroform extract contains alkaloids, **phytosterols**. Pet. ether extract contains alkaloids, **phytosterols**.

- **Pharmacological studies:**

In this the antimicrobial and anti lipid peroxidase properties of leaf extracts were prepared by using alcohol, chloroform and pet. ether organic solutions and determined.

The Carica papaya leaf extracts have been evaluated for the

1. Anti microbial
2. Anti lipid peroxidant

**Alcoholic extract** Carica papaya leaf at the dose of **1000mcg** showed the **maximum Zone of inhibition at 25mm** on Bacillus megatrium and **25mm** on Escherchia coli and the minimum Zone of inhibition at 10mm on Bacillus megatrium and 03mm on Escherichia coli at the dose of 200mcg.

**Chloroform extract** Carica papaya leaf at the dose of **1000mcg** showed **the maximum Zone of inhibition at 23mm** on Bacillus megatrium and **20mm** on Escherchia coli and the minimum Zone of inhibition at 14mm on Bacillus megatrium and 05mm on Escherichia coli at the dose of 400mcg.

**Petroleum ether extract** Carica papaya leaf at the dose of **1000mcg** showed **the maximum Zone of inhibition at 19mm** on Bacillus megatrium and **17mm** on Escherichia coli and the minimum Zone of inhibition at 15mm on Bacillus megatrium and 03mm on Escherichia coli at the dose of 400mcg.

From the all above investigation the MIC and Antimicrobial and synergism effect is **higher in the alcoholic extract** and low in the Pet ether extract, it is mentioned in the table no (5,6,7)

The results of current investigation reveals that the alcohol, chloroform and petroleum ether extracts showed anti-lipid peroxidation activity. **In that alcoholic extract showed maximum anti lipid peroxidation activity.** It was comparable to the standard drug ascorbic acid. The anti lipid peroxidation activity of the alcohol, chloroform and petroleum ether extracts were concentration dependent.

Hence it was concluded that Carica papaya leaf extracts possess maximum effect based on their dose. In future further studies may be carried out in our laboratory to find out the lead molecule having safety and efficacy profile.

## CONCLUSION

The ethnomedical information of plant *Carica papaya* belongs to family *caricaceae* was taken for our project work and as per the pharmacognostical work the macroscopical characters and microscopical characters and powder microscopy reveals the identification features of *Carica papaya* leaf which is easy to identify the adulterants either in the entire form or in the powder form

The anti microbial studies reveals that alcohol extract showed maximum potency of inhibiting *Bacillus megaterium* and *E.coli*

There by the extracts were found for that synergistic and antagonistic effect by using standard micro organisms. It helps to synergise the alcoholic extract.

The minimum inhibitory concentration of the different extracts were studied to findout their efficacy.

Anti lipid peroxidation activity of these extracts were proved that this plant posses the property of inhibiting the inflammation, ulcer, asthma, Cardiotonic, Digestive and diuretic activity.

Hence by the above research work the ethnomedical properties were found to be proved

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