European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Valorization Of Punica Granatum Peel Waste: A Biomedicine-To-Textile Approach for Antimicrobial, Anticancer, Antiinflammatory, And Antidiabetic Applications

Article History:

Received: 13-01-2026
Revised: 05-02-2026
Accepted: 23-02-2026
Published: 25-03-2026

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Abstract: The increasing demand for eco-friendly and multifunctional products has led to the exploration of natural resources with both dyeing and therapeutic properties. The present study focuses on the dyeing of cotton fabric using biodegradable pomegranate (Punica granatum) peel powder, along with the evaluation of its antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticancer properties, particularly targeting skin-associated pathogens and health benefits. Initially, pomegranate peels were shade-dried, powdered, and used as a natural dye for cotton cloth. The dyeing efficiency, color fastness, and stability were evaluated to establish its potential as a sustainable textile dye. Simultaneously, skin flora and pathogens were isolated from human skin swabs and identified through cultural, morphological, and biochemical characterization. The crude, isopropanol, ethyl acetate, and chloroform extracts of the pomegranate peel powder were prepared and screened for antimicrobial activity against the isolated skin microorganisms using the agar well diffusion method. Further, the extracts were assessed for antioxidant potential using DPPH radical scavenging assay, anti-inflammatory activity by protein denaturation inhibition assay, and antidiabetic property through α-amylase inhibition assay. In addition, anticancer activity on skin cells was analyzed to explore the therapeutic efficacy of the extracts. The findings demonstrated that the pomegranate peel extracts not only imparted a stable, natural color to cotton fabric but also exhibited significant antimicrobial activity against common skin pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa. The extracts also showed potent antioxidant, anti-inflammatory, antidiabetic, and anticancer activities, highlighting their multifunctional potential. Overall, this study establishes pomegranate peel powder as a promising biodegradable dye source with broad-spectrum biological properties, supporting its application in sustainable textile processing and natural skincare formulations.

Keywords: Pomegranate peel, natural dye, cotton fabric, skin pathogens, antimicrobial activity, antioxidant, anti-inflammatory, antidiabetic, anticancer, biodegradable.

INTRODUCTION

The global textile industry is a major contributor to water pollution and chemical waste due to its reliance on synthetic dyes derived from petrochemical sources. These synthetic colorants, though cost-effective and stable, often pose serious environmental and health concerns, including carcinogenicity, allergenicity, and toxicity to aquatic organisms (Ali et al., 2019).. Among natural dye sources, pomegranate (Punica granatum L.) peel, which constitutes nearly 40–50% of the fruit weight and is typically discarded despite being a rich reservoir of bioactive compounds (Viuda-Martos et al., 2010).The dye derived from pomegranate peel has been shown to produce fast and

durable colors on cotton, silk, and wool fabrics, with good resistance to light, washing, and rubbing (Bouaziz et al., 2021). In addition, the peel extract possesses inherent antimicrobial and antioxidant activities, adding functional properties to dyed fabrics (Gracy et al., 2023).

Pomegranate peel extracts have demonstrated potent antibacterial effects against Gram-positive and Gram-negative bacteria due to their rich polyphenolic content (Al-Zoreky, 2009). Pomegranate peel contains abundant antioxidants, particularly polyphenols and tannins, which not only stabilizes the dye on fabric but also provides protective benefits to skin when textiles are used in close contact (Zahin et al., 2010).Pomegranate peel extracts have shown significant anti-inflammatory effects by inhibiting pro-inflammatory mediators like cyclooxygenase (COX) and lipoxygenase (LOX) enzymes (Shukla et al., 2008). Incorporating these compounds into dyed fabrics could enhance skin health and provide soothing effects for sensitive skin users.Several studies have reported that pomegranate peel extracts possess α-amylase and α-glucosidase inhibitory activities, indicating strong antidiabetic potential (Singh et al., 2013). Textiles impregnated with such extracts may have potential in diabetic wound care applications, though further in vivo validation is needed. Also pomegranate peel extract has attracted attention for its chemopreventive and anticancer properties.

Using pomegranate peel as both a natural dye and biofunctional agent is a sustainable strategy for developing eco-friendly textiles. Such fabrics could find applications in **medical clothing, wound dressings, diabetic textiles**, and **cosmeceutical fabrics**. In addition to being biodegradable and safe for skin contact, the use of pomegranate peel aligns with green chemistry principles and waste valorization.

METHODOLOGY

SAMPLE COLLECTION

Pomegranate peels were collected from ripe fruits, thoroughly washed to remove impurities, and manually separated from the seeds. The peels were cut into pieces and dried under the sun for 2 days and shade dried until completely dehydrated. The dried peels were then ground into a fine powder and stored in airtight containers at room temperature until further use.(Fig 1)

ISOLATION OF SKIN FLORA:

Nutrient agar medium was prepared and sterilized at 121°Cin 15 lbs pressure for 15 minutes. The medium was poured into the sterile Petri plates and allow to solidify. Samples of dermatologically affected skin samples were collected using the swab technique, and they were inoculated onto the agar surfaces. The plates were incubated at 37°C for 24 hours. After incubation the skin normal flora was observed, specific colonies were picked and purified using subculture method and maintained in nutrition agar medium for further investigation as RM 01 , RM 02 , RM 03 , RM 04 . (Fig 2-5)

PREPARATION OF PURE CULTURE OF PATHOGENS

The isolated colonies obtained in the Petri plates were taken carefully without contamination (leaving out mixed culture colonies) and streaked in the freshly prepared nutrients agar medium by quadrant streak using the inoculation loop. The plates were incubated at 25°C for 24 hours.

COLONY MORPHOLOGY

Colony morphology of the pure skin isolates on Nutrient agar medium were recorded with respect to colour, mass, size and nature of the colonies. (TABLE 1)

GRAM STAINING

Thin smears of the skin isolates were made on a clean glass slide and heat fixed. Then the smear was stained by crystal violet for 1 minute, and then washed with distilled water followed by stained with Gram's idoine. After 1 minute, the slide was washed and decolourized with 95% of ethyl alcohol. After decolourization, the smear was counter strained with saffrain for 1 minute. Then, the slide was washed, air dried and examined microscopically.

BIOCHEMICAL CHARACTERIZATION

The isolates were subjected to biochemical characterization and the results were tabulated. (TABLE 3) (Fig 6-33)

Preparation of Pomegranate Peel Extracts

The extraction process was carried out using different solvents to evaluate solvent-dependent bioactivity. The crude aqueous extract was prepared as the Fifty grams of powdered pomegranate peel was soaked in 500 mL of distilled water and incubated at 60°C for 48 hours under continuous stirring. The mixture was filtered through muslin cloth followed by Whatman No.1 filter paper and stored at 4°C. For isopropanol extract, chloroform extract and ethyl acetate extract fifty grams of powdered peel was soaked in 500 mL of solvent and kept for 48 hours at room temperature with intermittent shaking. Filtration and solvent removal were performed similarly.

Antimicrobial Activity

The antimicrobial potential of pomegranate peel extracts against skin pathogens was evaluated using the agar well diffusion method. Mueller Hinton agar plates were inoculated with standardized bacterial suspensions (0.5 McFarland standard). Wells of 6 mm diameter were made, and 20µL, 40µL, 60µL, 80µL of

each extract (crude, isopropanol, ethyl acetate, chloroform) was added. Plates were incubated at 37°C for 24 hours. The zones of inhibition were measured in millimeters. (TABLE 4) (Fig 34-37)

ANTIOXIDANT ACTIVITY

Total Antioxidant activity by phosphomolybdenum method

Procedure

Determination of Total antioxidant activity:

The total antioxidant activity was evaluated by phosphomolybdenum method described by Prieto et al. 1.0 ml of the extract was mixed with 1.0 ml of the standard reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate). The tubes were cappedand incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank.(TABLE 5) (Fig 38-43)

% Antioxidant activity = Sample Absorbance/Standard Absorbance × 100

ANTI-INFLAMMATORY ACTIVITY

Protein denaturation inhibition assay

Protein denaturation inhibition assay was done according to the method described by Gambhire et al. The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 200 -1000 µg/ml of sample, and the mixture was mixed, and was incubated in a water bath (37 ◦C) for 15 min, and then the reaction mixture was heated at 70 ◦C for 5 min. After cooling, the lowry reagent was added and absorbance was measured at 660 nm using a UV/VIS spectrometer . Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula: (TABLE 6) (Fig 44-49)

% Inhibition =[Absorbance sample/Absorbance standard]×100

ANTI-DIABETIC ACTIVITY

Alpha Amylase Inhibition Assay

Procedure:

In this experiment, 1 ml of 1% starch solution was added to both the control and test sample tubes. To each of these tubes, 0.5 ml of the amylase enzyme solution was then introduced. The tubes were incubated at 37°C for 30 minutes to allow enzymatic hydrolysis of starch. Following incubation, 1 ml of DNSA (3,5-dinitrosalicylic acid) reagent was added to each tube to stop the reaction and develop color, which indicates the presence of reducing sugars. The tubes were then heated in a boiling water bath at 95°C for 15 minutes to facilitate color development. After cooling to room temperature, the absorbance of each sample was measured at 510 nm using a spectrophotometer to determine the amount of reducing sugar produced by amylase activity. (TABLE 7) (Fig 50-55)

% Inhibition = Abs Control- Abs sample/Abs Control * 100

ANTI-CANCER ACTIVITY

MTT ASSAY:

The cytotoxicity of the sample on SKMEL3 cells was determined by the method of Mosmann, (1983).

Principle

The yellow 3-4,5dimethylthiozol-2-Yl)-2,5diphenyltetrazoliumbromide (MTT) is reduced by mitochondrial dehydrogenase of viable cells yielding a measurable purple formation product. Viable cells contain NAD(P) H-dependent reductase, which reduces the MTT reagent to formazon, with a deep purple colour. Formazon crystals are then dissolved using solubilizing solution, and the plate reader measures absorbance at 500-600 nm.

Reagents

MTT stock solution:

MTT (50mg) dye was dissolved in 10 ml of PBS. After vortexing for 1 minute , it was filtered through 0.45 micro filters .The bottle was wrapped with aluminium foil to prevent light ,as MTT was light sensitive. The preparation was stored at 4 °c .

Procedure

Cell viability assay ,lung cancer (A 549) viable cells were harvested and counted using hemocytometer diluted in DMEM medium to a density Density of 1×10^4 cells /ml seeded in 96 well plates for each cell and incubated for 24 hours to allow attachment .Then the cells were treated with the sample of different concentration (20-100 ug/ml) applied to each well.All the treated cells were incubated at 37°C in a humidified 95% arirand 5% CO2 incubation for 24 hour.After incubation, the drug -containing were washed with fresh culture medium and MDT (5 mg /ml in PBS) dye was added to each well ,followed by incubation and for another 4 hour at 37°C. the purple precipitated forms from the most served in 100 ulof concentration of DMSO and the cell viability was abosorbanceand measured 540 nm using a multi -well plate reader. The result were expressed at the percentage of stable cells containing the control.The half -maximal inhibitory concentration (IC50) values were calculated. (TABLE 8,9) (Fig 56-60)

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbance of the control} - \text{Mean absorbance of the sample} \times 100}{\text{Mean absorbance of the control}}$$

APOPTOSIS ASSAY:

Measurement of apoptotic induction using acridine orange/ethidium bromide (AO/EB) dual staining method

The fluorescence microscopic analysis of apoptotic cell death was carried out according to the method of Baskic et al. (2006).

Principle

AO is permeable and it stains all the SKMEL3 cells viable/nonviable cells. It emits green fluorescence if intercalated into double-stranded nucleic acid (DNA) or red fluorescence if bound to single-stranded nucleic acid (RNA). EB is taken up only by nonviable cells that have lost membrane integrity and emits red fluorescence by intercalation into DNA. They distinguished four types of cells according to the fluorescence emission and the morphological aspect of chromatin condensation in the stained nuclei. Viable cells have uniform bright green nuclei with an organized structure. Early apoptotic cells (which still have intact membranes but have started to undergo DNA cleavage) have green nuclei, but perinuclear chromatin condensation is visible as bright green patches or fragments. Late apoptotic cells have orange to red nuclei with condensed or fragmented chromatin. Necrotic cells have uniformly orange to red nuclei with no condensed chromatin.

Reagents

1. Acridine Orange (AO)
2. Ethidium Bromide (EB)
3. PBS (1%)

Procedure

SKMEL3 cells were seeded at 5 x 10^4 cells / well in a 6-well plate and incubated for 24 hours. After treatment with Sample Powder (400 and 600 µg/ml) for 24 h, the cells were detached, washed with cold PBS, and then stained with a mixture of AO (100µg ml−1)/ EB (100µg ml−1) ratio (1:1) at room temperature for 5 min and examined immediately under a fluorescent microscope with 20x magnification.

DYEING OF COTTON CLOTH

Pretreatment (Scouring and Mordanting):

Cotton cloth samples were pretreated with a 5% sodium carbonate solution at 90°C for 30 minutes to remove impurities and enhance dye uptake. The cloth was washed thoroughly with distilled water. Mordanting was performed using alum (potassium aluminum sulfate) at a 5% (w/v) concentration, heated at 80°C for 1 hour, followed by washing and drying.

Dyeing Procedure:

The prepared cotton cloths were dyed using aqueous or organic solvent-based pomegranate peel extracts. The fabric-to-extract ratio was maintained at 1:20 (w/v). Dyeing was carried out at 60–80°C for 60–90 minutes under continuous stirring. After dyeing, cloth samples were washed with distilled water to remove unbound dye and air-dried in shade. The color fastness was tested using standard procedures against washing, rubbing, and light exposure.

RESULTS AND DISCUSSION

MORPHOLGICAL CHARACTERISTICS

RM01 formed aerobic, white colour colonies and was identified as Gram-positive. RM02 formed aerobic, transparent colonies and was identified as Gram-negative. RM03 formed aerobic, yellow colour colonies and was identified as Gram-positive. RM04 formed aerobic, pale yellow colour colonies and was identified as Gram-positive. These properties were studied according to methods of Shirling and Gotlieb and Bergey. Results are tabulated. (Table 1,2)

BIOCHEMICAL CHARACTERISTICS

Biochemical assays performed on the pure isolate provided insights into its metabolic capabilities (Table 3).

ANTIMICROBIAL ACTIVITY OF POMEGRANATE PEEL EXTRACT AGAINST SKIN ISOLATES IN DIFFERENT SOLVENT EXTRACTS:

The antimicrobial activity results for different solvent extracts (crude, chloroform, ethyl acetate, and isopropyl) at varying concentrations (20–80 µL) revealed a concentration-dependent increase in the zone of inhibition for all skin samples (white, translucent, yellow, and pale yellow)(Table 4). Among the extracts, the crude and chloroform fractions exhibited the highest antimicrobial activity, with maximum inhibition zones up to 18 mm at higher concentrations, indicating strong bioactive potential. Ethyl acetate and isopropyl extracts also showed moderate to good antimicrobial effects, suggesting the presence of secondary metabolites such as phenolics, flavonoids, and terpenoids that are more soluble in semi-polar solvents (Harborne, 1998). The variation in inhibition zones among different skin types may be attributed to differences in pigment-associated phytochemicals that influence antibacterial efficacy. The overall findings indicate that the bioactivity of extracts increases with concentration and depends on the solvent polarity, aligning with previous studies showing that polar and semi-polar solvents enhance the extraction of antimicrobial

compounds (Cowan, 1999; Parekh & Chanda, 2007). Thus, these results demonstrate that plant-derived extracts, particularly crude and chloroform fractions, possess promising antimicrobial potential for pharmaceutical or preservative applications. (TABLE 5) (Fig 38-43)

ANTI-OXIDANT ACTIVITY:

The antioxidant activity results for the isopropanol, chloroform, and extract samples demonstrate a clear concentration-dependent increase in antioxidant potential, as measured by the absorbance at 695 nm and expressed in % antioxidant activity relative to ascorbic acid (standard, 1.80 absorbance). At 250 µg/ml, all samples reached a plateau of 97.22% activity, indicating that the antioxidant potential nears saturation at higher concentrations. Among the solvents, chloroform initially showed lower activity at 50 µg/ml (57.77%) but quickly matched isopropanol and extract at 150 µg/ml and above. The extract sample exhibited relatively high activity even at 50 µg/ml (84.44%), suggesting it contains potent antioxidant compounds. These results align with previous studies indicating that phenolic and flavonoid compounds present in plant extracts contribute significantly to antioxidant activity by donating electrons to neutralize free radicals (Pham-Huy, He, & Pham-Huy, 2008). The FRAP (Ferric Reducing Antioxidant Power) assay used here is a reliable method to evaluate reducing power, and the strong performance of the extract suggests it is a promising natural antioxidant source (Benzie & Strain, 1996). Overall, the findings support the potential application of the extract as a natural antioxidant in pharmaceutical or food industries. (TABLE 5) (Fig 38-43)

ANTI INFLAMMATORY ACTIVITY

The anti-inflammatory activity of pomegranate peel extracts was evaluated using the protein denaturation inhibition assay with aspirin as the standard. All extracts showed a concentration-dependent increase in inhibition. Among them, the **chloroform extract** exhibited the highest activity (80% at 250 µg/ml), followed by the **isopropanol extract** (78.94%) and **ethyl acetate extract** (29.47%). The strong activity of chloroform and isopropanol extracts suggests the presence of potent bioactive compounds such as flavonoids, tannins, and phenolic acids that prevent protein denaturation and reduce inflammation (Mizushima & Kobayashi, 1968; Singh et al., 2019). These findings agree with previous reports showing that pomegranate peel contains ellagitannins and phenolics with significant anti-inflammatory properties (Fawole et al., 2012; Sadeghipour et al., 2020). Overall, the results indicate that pomegranate peel extracts, particularly the chloroform extract, possess notable anti-inflammatory potential comparable to aspirin. (TABLE 6) (Fig 44-49)

ANTI-DIABETIC ACTIVITY

The α-amylase inhibitory activity of the three extracts—**isopropanol**, **chloroform**, and **crude extract**—increased in a concentration-dependent manner, indicating potential antidiabetic properties. Among them, the **isopropanol extract** exhibited the highest % inhibition at 250 µg/ml (73.55%), followed by the chloroform extract (67.74%) and the crude extract (62.58%). This suggests that isopropanol is a more efficient solvent for extracting bioactive compounds with antidiabetic potential. These findings are consistent with previous studies demonstrating that plant-derived secondary metabolites such as flavonoids, alkaloids, and phenolics can inhibit carbohydrate-hydrolyzing enzymes like α-amylase (Kazeem et al., 2013; Sales et al., 2012). The dose-dependent increase further supports the hypothesis that these extracts interfere with starch breakdown, potentially leading to reduced postprandial hyperglycemia (Ali et al., 2006). Therefore, the isopropanol extract may serve as a promising candidate for the development of plant-based antidiabetic therapies. (TABLE 7) (Fig 50-55)

ANTI-CANCER ACTIVITY

MTT ASSAY:

A photomicrograph (20x) represents morphological changes in SKMEL3 cells, such as shrinkage, detachment, membrane blebbing, and distorted shape, induced by sample Powder treatment (400 & 600 µg/ml for 24 h) compared with the control. The control showed normal intact cell morphology, and their images were captured by a Biorad Fluorescent microscope. (TABLE 8,9) (Fig 56-60)

APOPTOSIS ASSAY:

SKMEL3 cancer cells were treated with Sample (400 and 600 µg/ml) for 24 hours, stained with dual dye AO/EB and then analyzed by fluorescence microscopy (Zoe Fluorescent Cell Imager, Biorad). Live cells show green fluorescence with a normal nuclear appearance. Early apoptotic cells with fragmented nuclear show yellow fluorescence with condensed chromatin. Late apoptotic cells show orange fluorescence with chromatin condensation or fragmentation (uniformly red/orange-stained cell nuclei).

Dye calculation

Dye strength – 10%
Alum - 15%
Salt - 5%
Liquor ratio (Fabric :water) = 1:20

Formula:

Dye (g) = Fabric weight (g)×dye %
Alum (g) = Fabric weight(g)×Alum%
Salt (g) =Fabric weight (g)×Salt %
Bath volume (L) =(Fabric weight/1000)×liquid

Ratio

Calculation:

For 350 g Fabric sample;

Dye (10%) = 350×0.10
= 35 g

Alum (15%) = 350×0.15
=52.5 g

Salt (5%) =350 × 0.05
= 17.5 g

Bath (1:20) =(350 /1000)×20
=7 L

TABLE 1:MORPHOLOGICAL CHARACTERISTICS OF SKIN ISOLATES

S. NO	SAMPLE	COLONY APPEARANCE	CELL SHAPE	GRAM STAINING
1	RM 01	White	Bacilli	Gram Positive
2	RM 02	Transparent	Bacilli	Gram Negative
3	RM 03	Yellow	Cocci	Gram Positive
4	RM 04	Pale yellow	Cocci	Gram Positive

TABLE 2: DETERMINATION OF TYPE OF RESPIRATION IN SKIN ISOLATES

S. NO	SAMPLE	RESPIRATION TYPE	ORGANISM
1	RM 01	Aerobic	*Staphylococcus epidermis*
2	RM 02	Aerobic	*Pseudomonas aeroginosa*
3	RM 03	Aerobic	*Staphylococcus aureus*
4	RM 04	Aerobic	*Micrococcus luteus*

TABLE 3: BIOCHEMICAL CHARACTERISTICS OF SKIN ISOLATES

BIOCHEMICAL TEST	RM 01	RM 02	RM 03	RM 04
Indole test	-	-	+	-
Methyl Red test	+	+	+	+
Voges Proskaeur test	+	-	-	-
Citrate test	+	+	+	+
Oxidase Test	+	+	+	+
Catalase Test	+	-	-	-
Urease Test	+	+	+	+

TABLE 4: QUANTIFICATION OF ANTI BACTERIAL ACTIVITY OF POMEGRANATE PEEL EXTRACT AGAINST SKIN ISOLATES IN DIFFERENT SOLVENT EXTRACTS:

TEST ORGANISMS	ZONE OF INHIBITION IN MM			
	CRUDE	ISOPROPYL	CHLOROFORM	ETHYL ACETATE
Staphylococcus epidermis	18	15	15	-
Pseudomonas aeroginosa	16	15	15	16
Staphylococcus aureus	18	14	16	18
Micrococcus luteus	18	15	15	15

Fig 1 - Sample Collection

SKIN CULTURES

Fig 2 - WHITE COLOUR COLONIES – RM 01

Fig 3 - TRANSPARENY COLONIES – RM 02.

Fig 4-YELLOW COLOUR COLONIES – RM 03

Fig 5-PALE YELLOW COLOUR COLONIES – RM 04

BIOCHEMICAL TEST

Fig 6 -Indole Test – RM 01

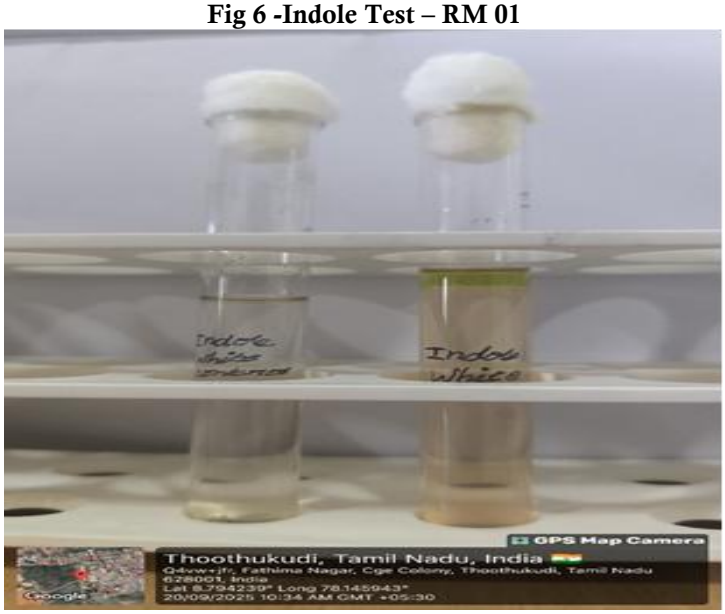


Fig 7 -Indole Test – RM 02

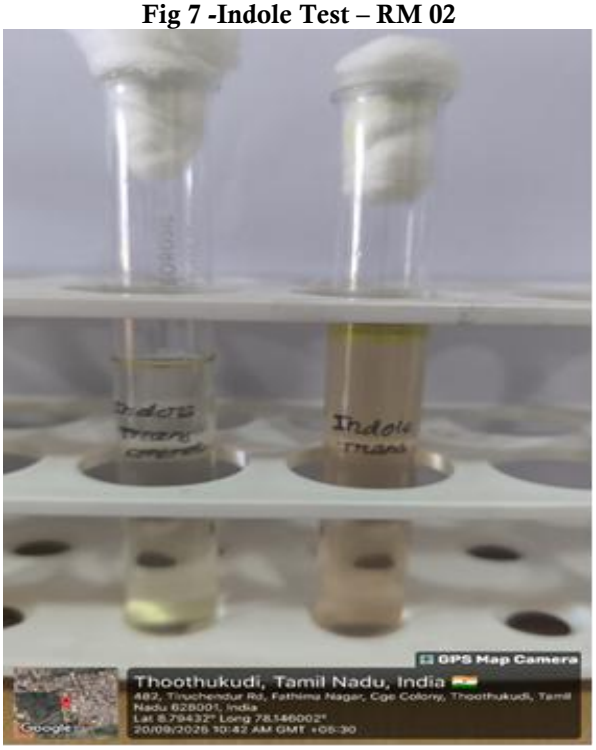


Fig 8- Indole Test – RM 03

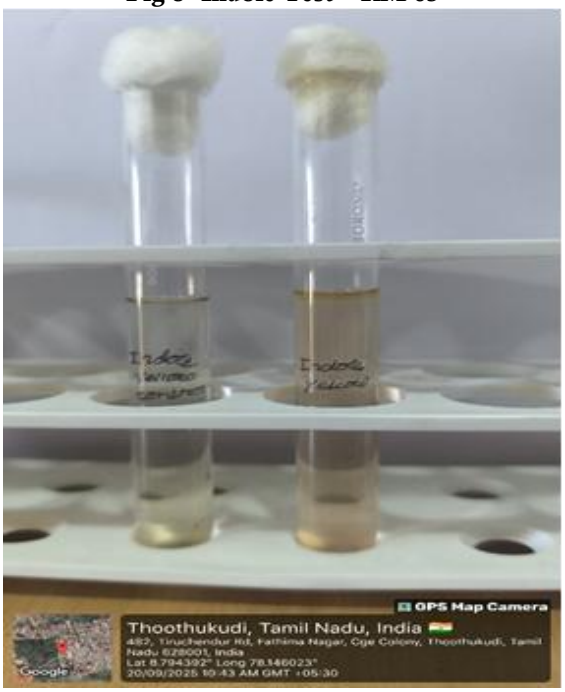


Fig 9-Indole Test – RM 04

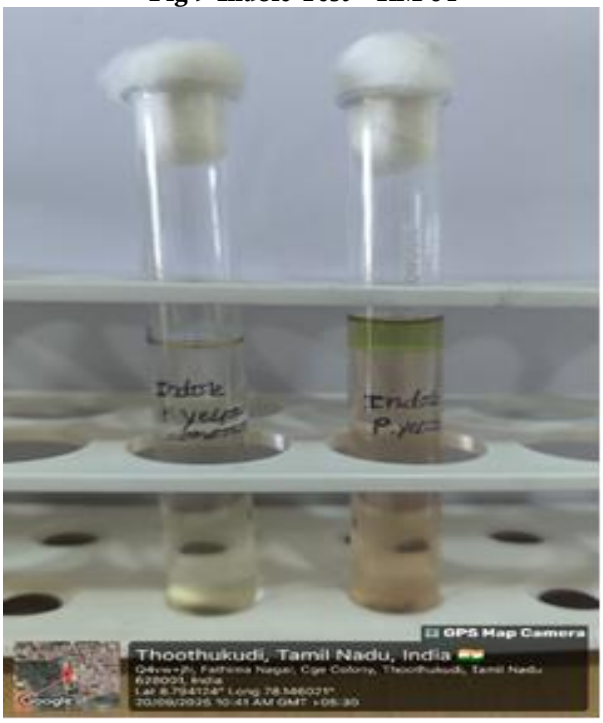


Fig 10-Methyl Red Test – RM 01

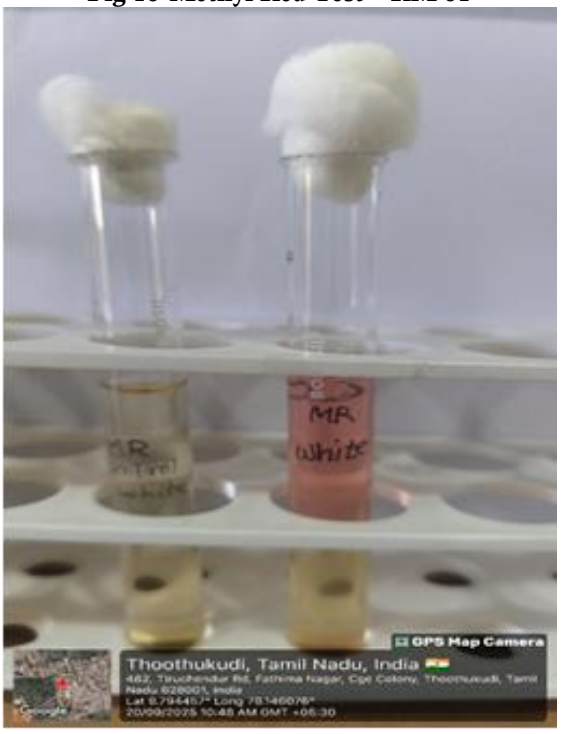


Fig 11 - Methyl Red Test – RM 02

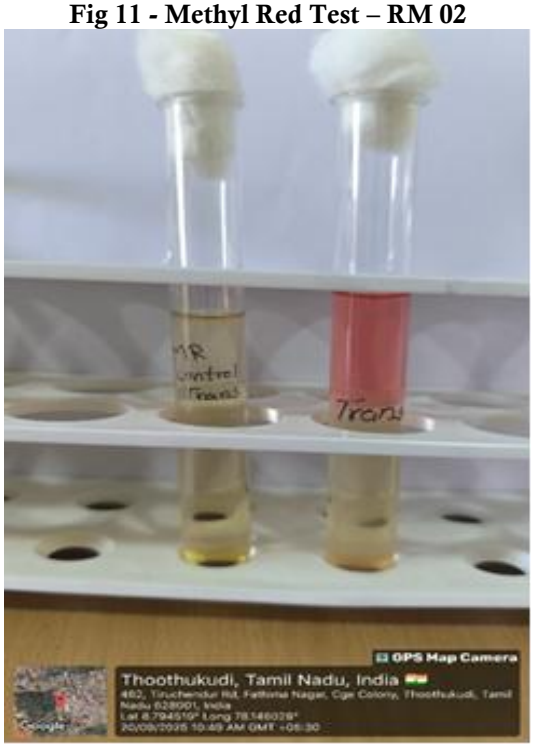


Fig 12 - Methyl Red Test – RM 03

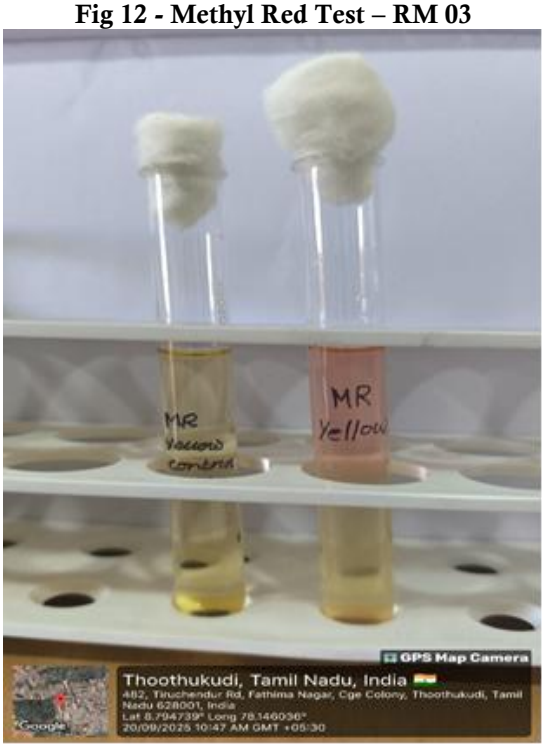


Fig 13- Methyl Red Test – RM 04

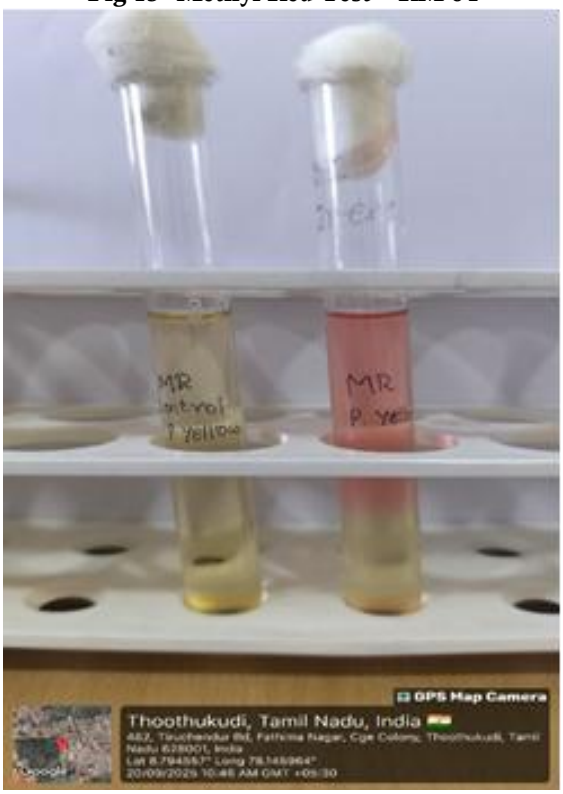


Fig 14 - Vogos Proskauer – RM 01

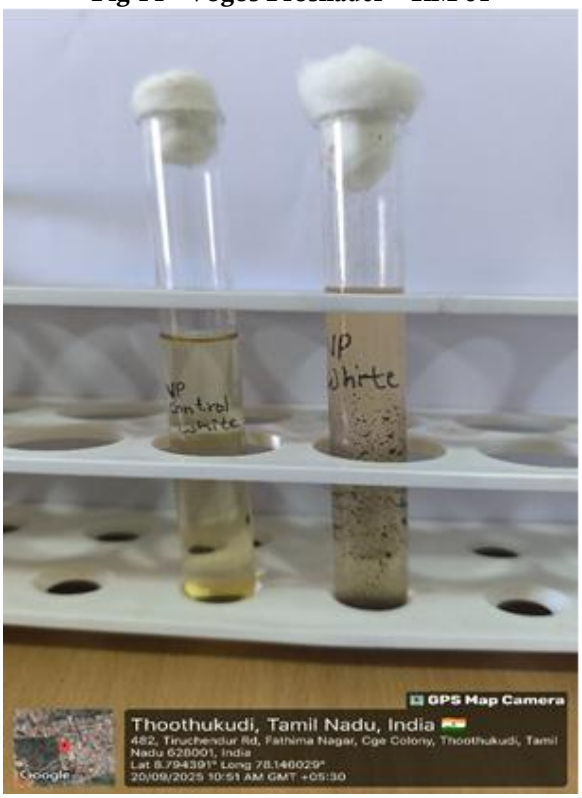


Fig 15 - Vogos Proskauer – RM 02

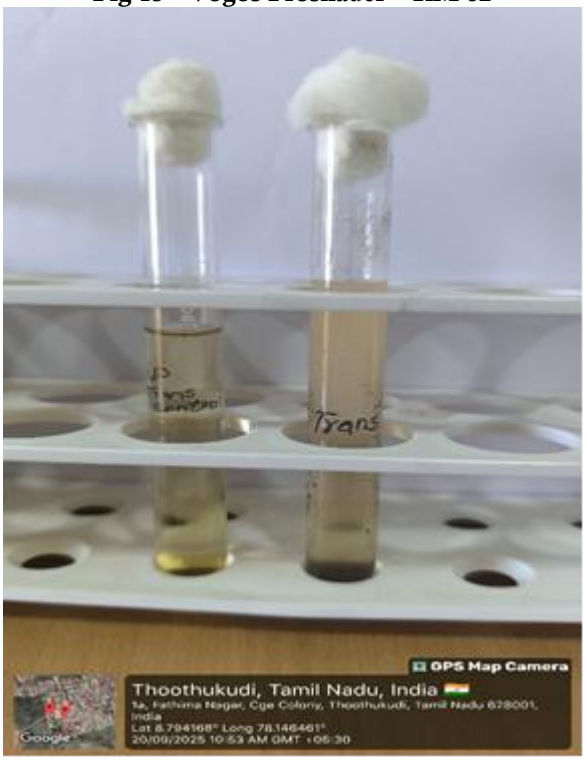


Fig 16 - Vogos Proskauer – RM 03

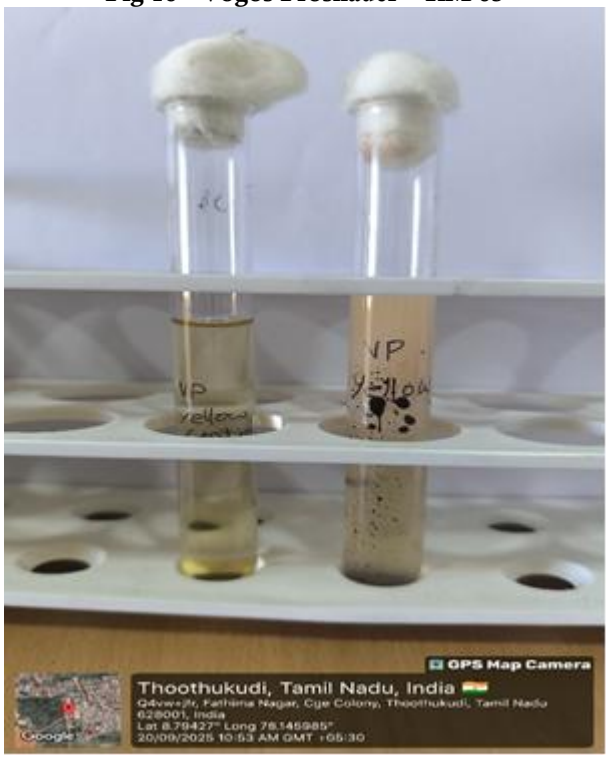


Fig 17 - Vogos Proskauer – RM 04

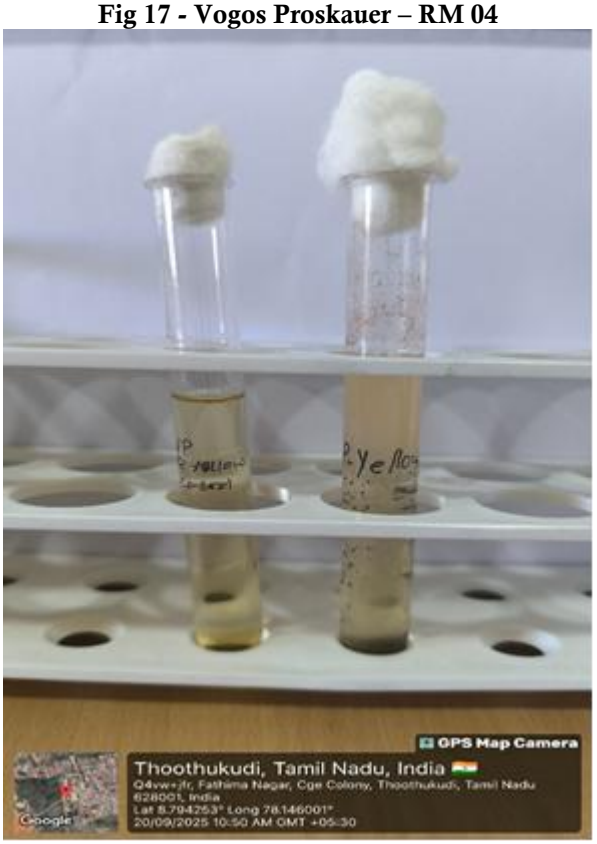


Fig 18 - Citrate Test – RM 01

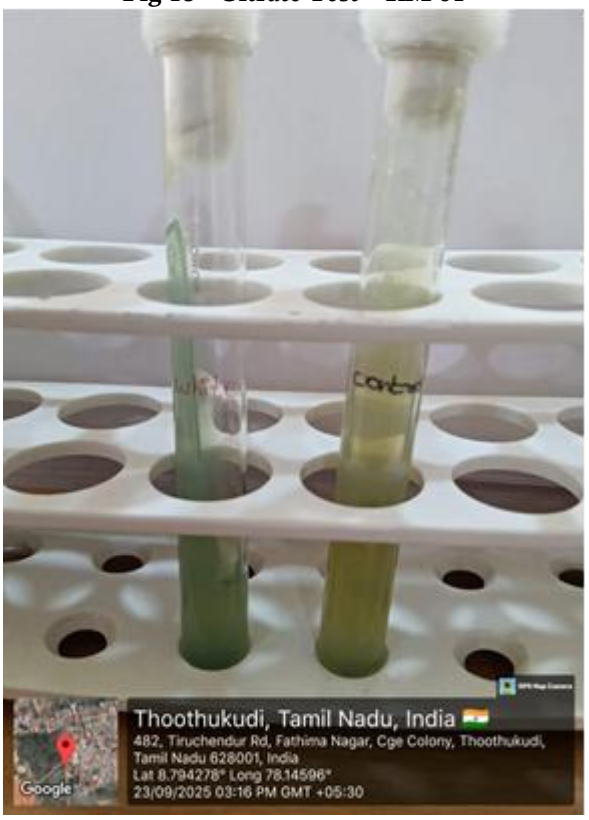


Fig 19 - Citrate Test – RM 02

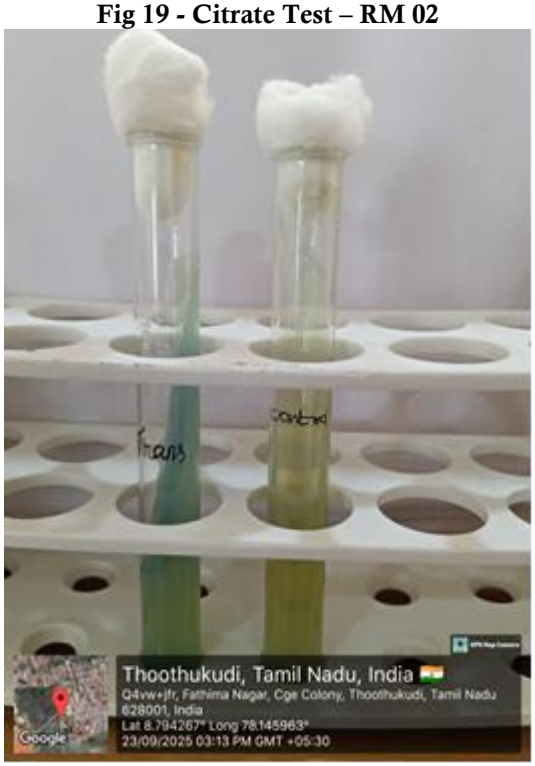


Fig 20-Citrate Test – RM 03

Fig 21 - Citrate Test – RM 04

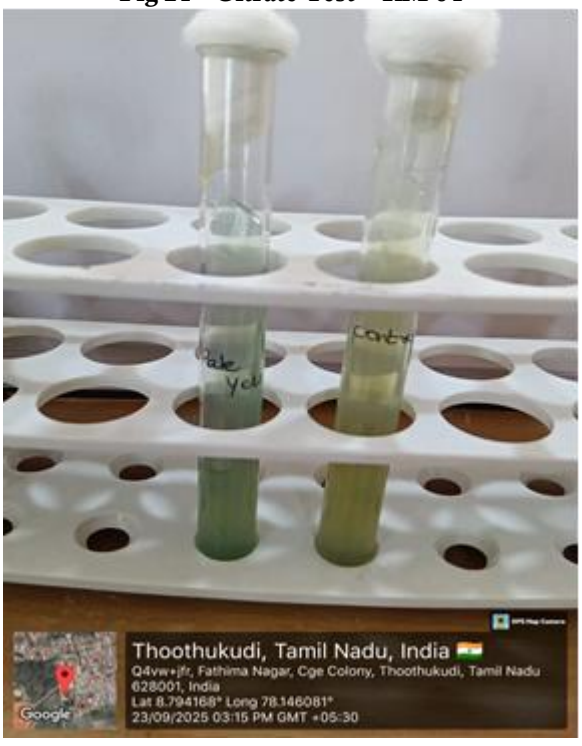


Fig 22 - Catalase Test RM 01

Fig 23 -Catalase Test RM 02

Fig 24 -Catalase Test RM 03

Fig 25 - Catalase Test RM 04

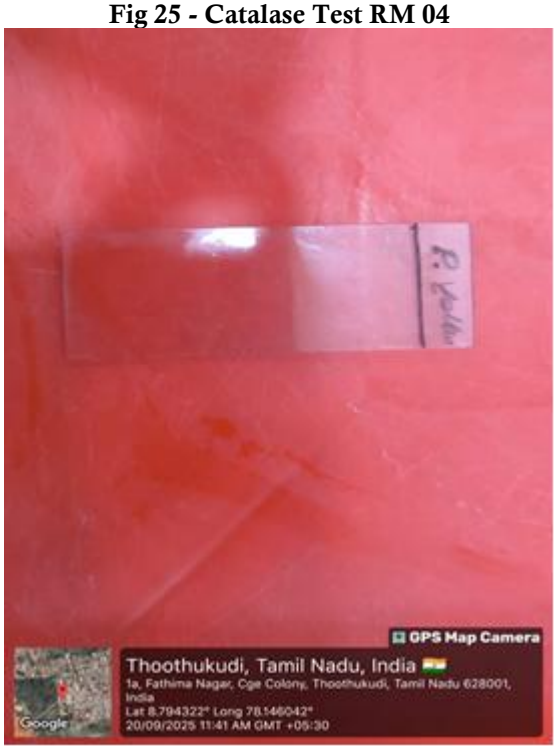


Fig 26 - Oxidase Test – RM 01

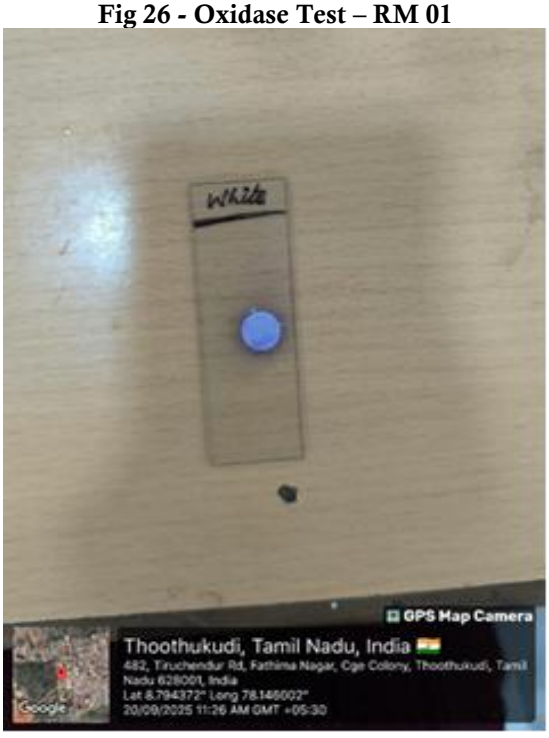


Fig 27 - Oxidase Test – RM-02

Fig 28-Oxidase Test – RM 03

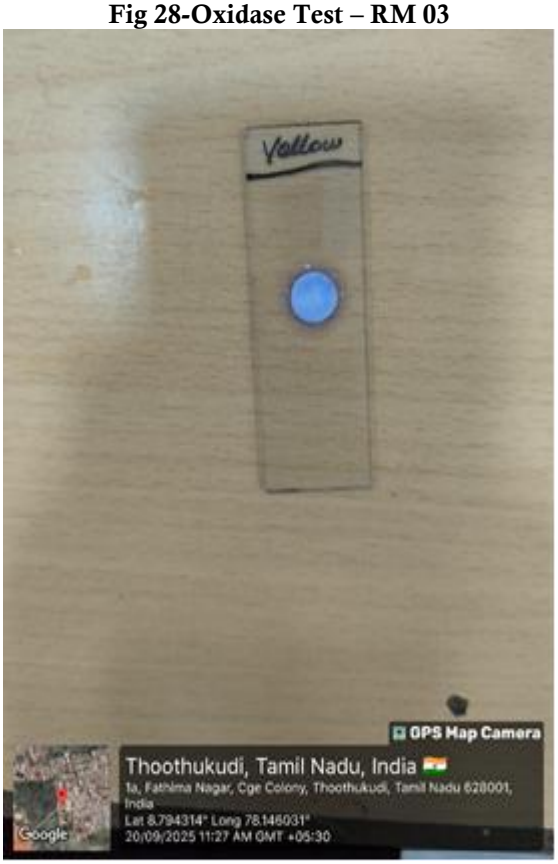


Fig 29 - Oxidase Test – RM 04

Fig 30- Urease test – RM 01

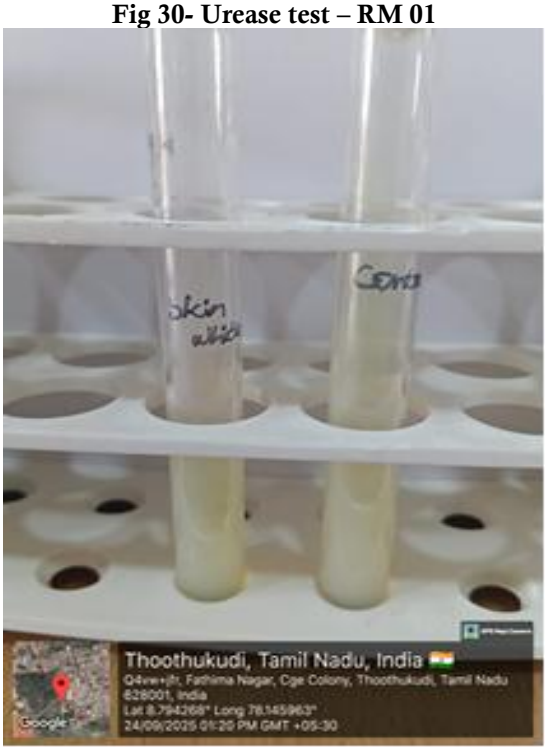


Fig 31 - Urease test – RM 02

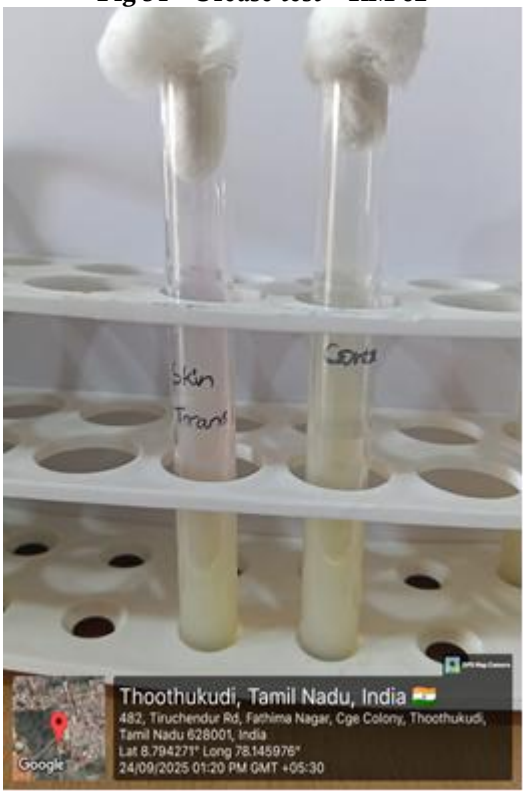


Fig 32 - Urease test – RM 03

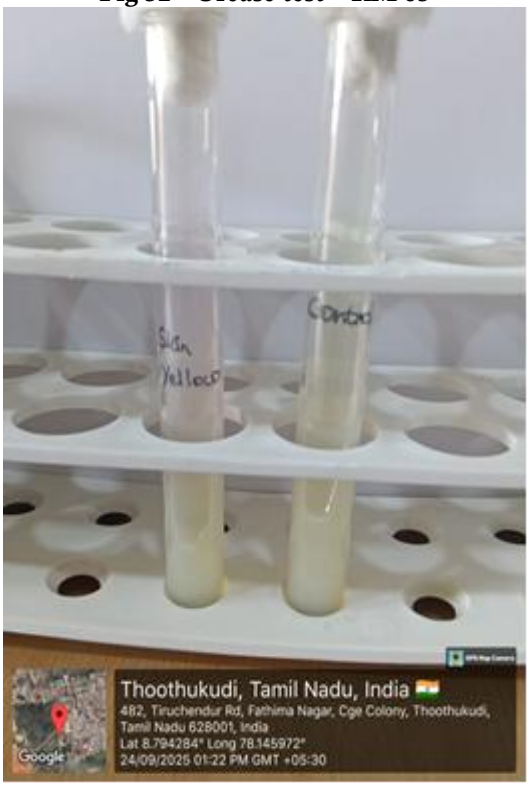


Fig 33 - Urease test – RM 04

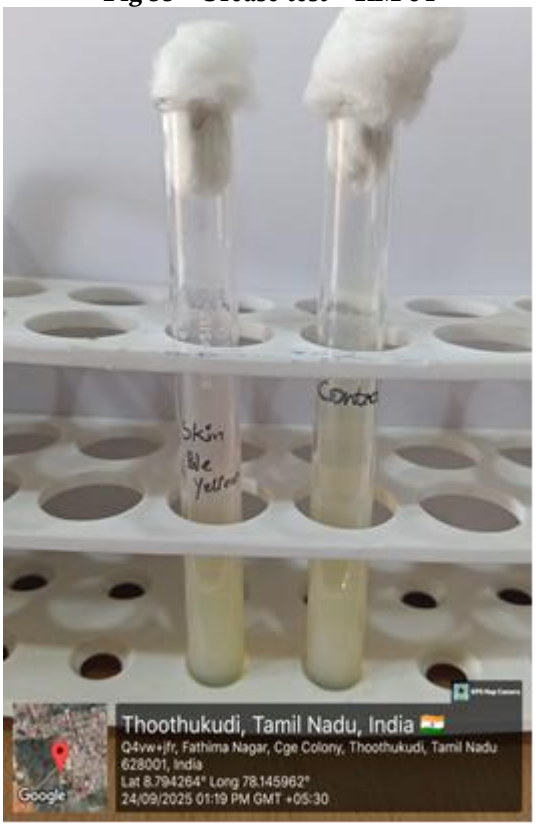


Fig 34 - Antimicrobial activity using Chloroform

Fig 35 - Antimicrobial activity using Ethyl acetate

Fig 36 - Antimicrobial activity using Isopropyl

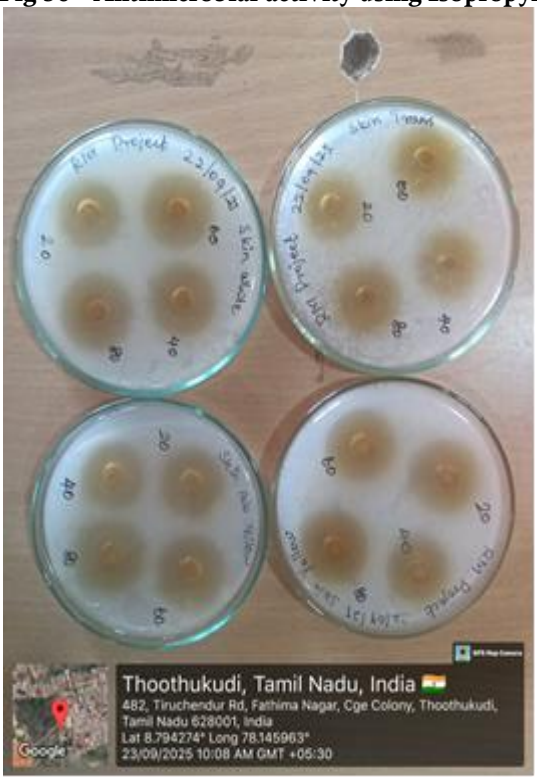


Fig 37 - Antimicrobial activity in Crude

ANTIOXIDANT ACTIVITY

Fig.38 Antioxidant activity of isopropanol extract

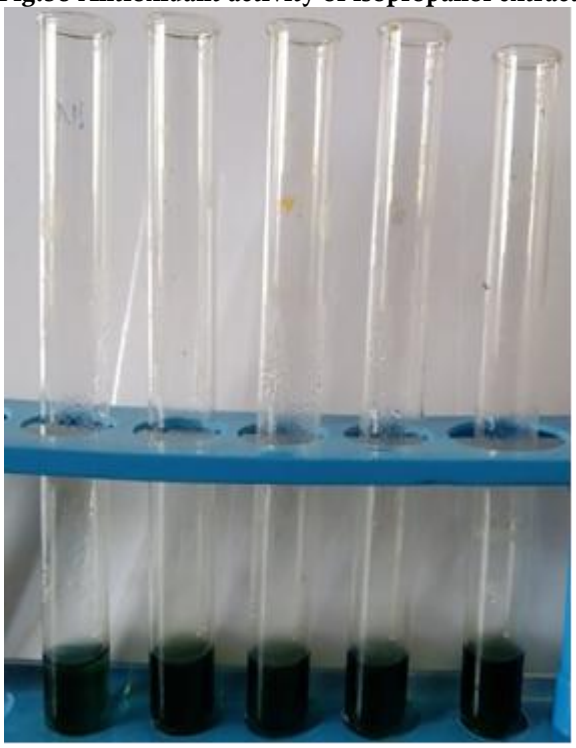


Fig. 39 Antioxidant activity of Chloroform extract

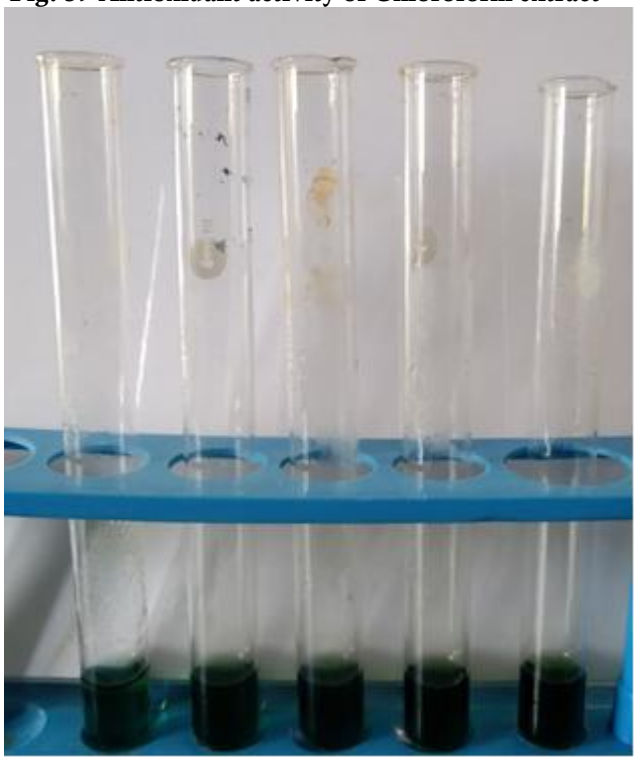


Fig. 40 Antioxidant activity of ethyl acetate extract

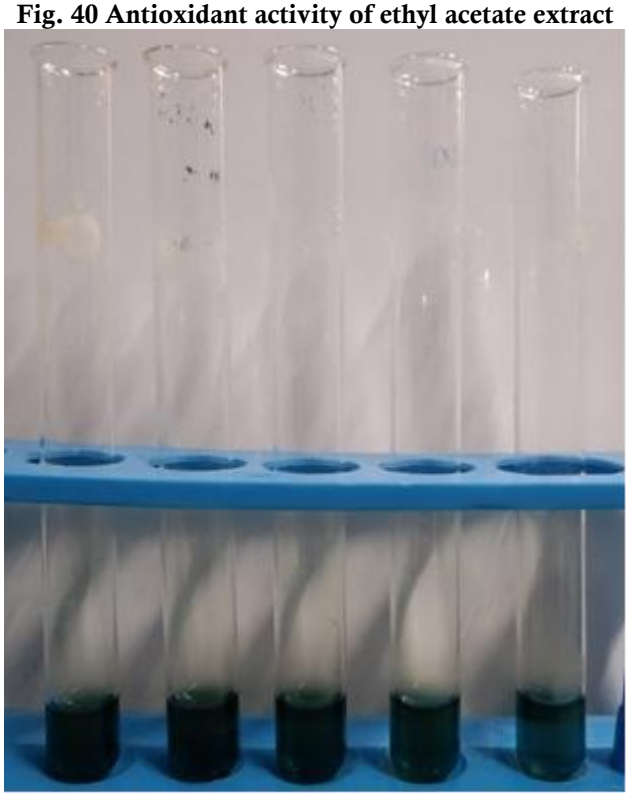


Table 5 : Antioxidant activity

Sample	Sample concentration µg/ml	Absorbance @ 695 nm	% Antioxidant activity= sample Absorbance /Standard Absorbance × 100
Ascorbic acid	1000	1.80	
Isopropanol	50	1.22	67.77
	100	1.40	77.77
	150	1.66	92.22
	200	1.75	97.22
	250	1.75	97.22
Chloroform	50	1.04	57.77
	100	1.52	84.44
	150	1.75	97.22
	200	1.75	97.22
	250	1.75	97.22
Ethyl acetate extract	50	1.52	84.44
	100	1.52	84.44
	150	1.75	97.22
	200	1.75	97.22
	250	1.75	97.22

Fig.41 Antioxidant activity of isopropanol extract

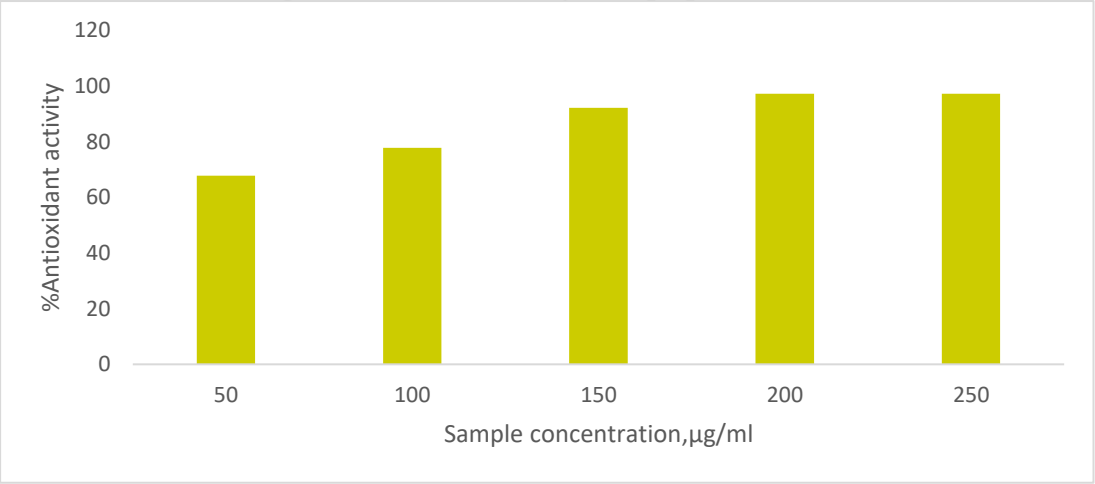


Fig.42 Antioxidant activity of chloroform extract

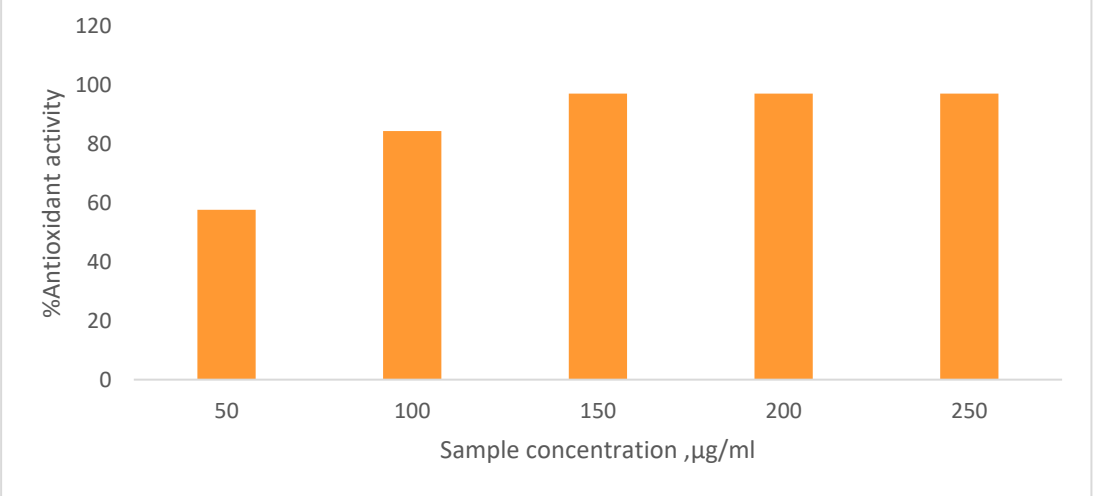
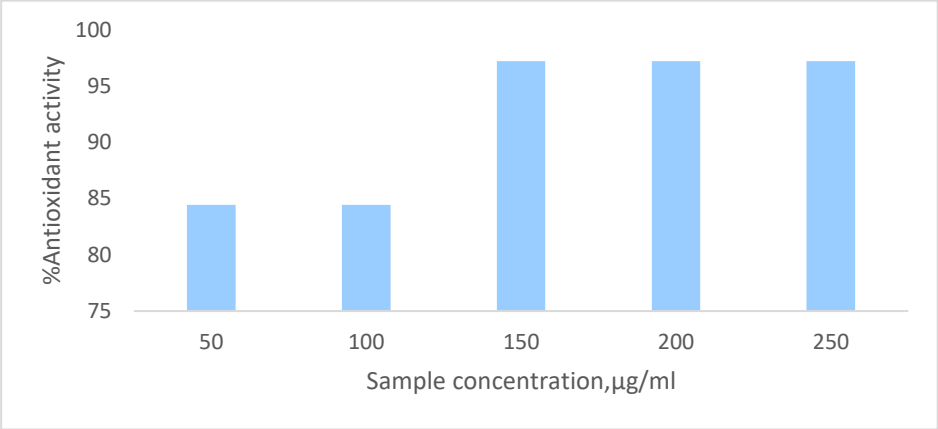


Fig.43 Antioxidant activity of ethyl acetate extract



Antiinflammatory activity

Fig. 44 Antiinflammatory activity of isopropanol extract

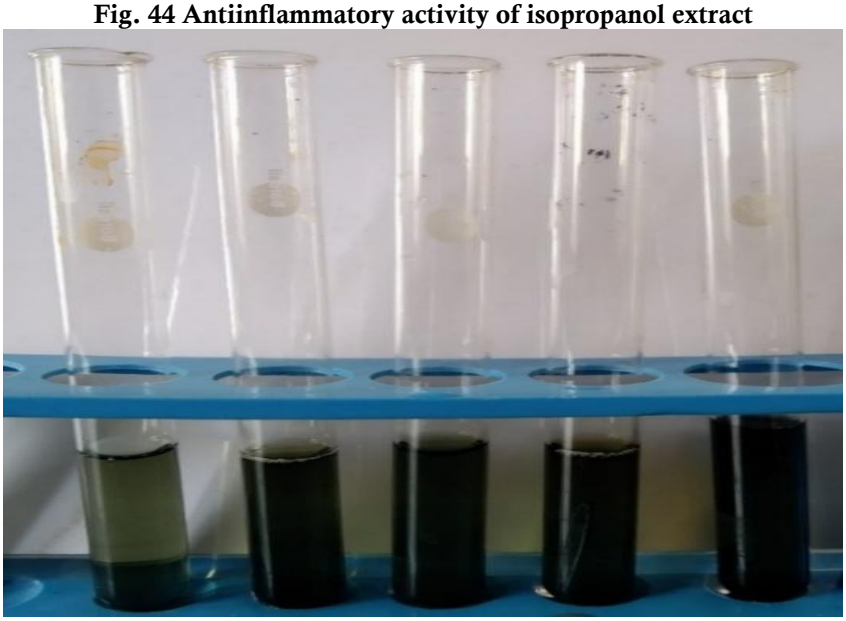


Fig.45 Antiinflammatory activity of chloroform extract

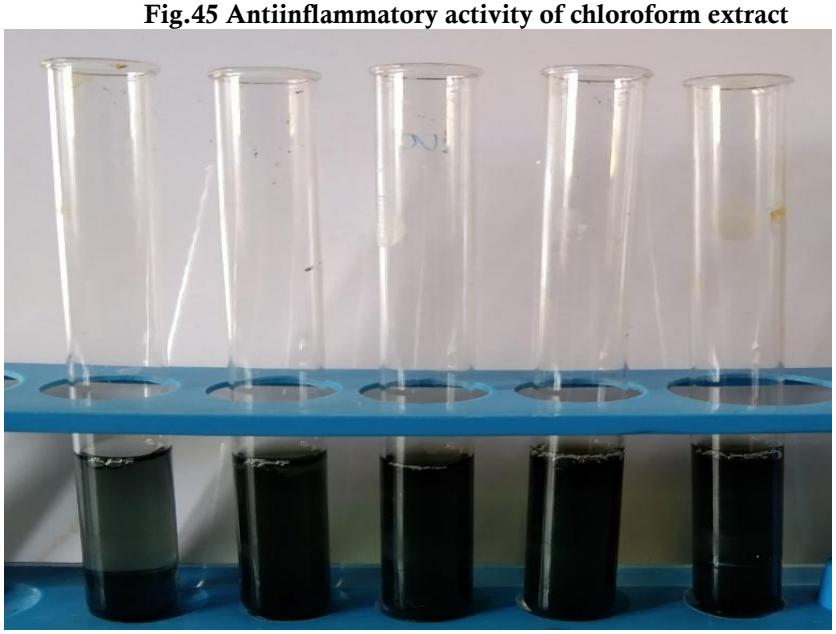


Fig. 46 Antiinflammatory activity of ethyl acetate extract

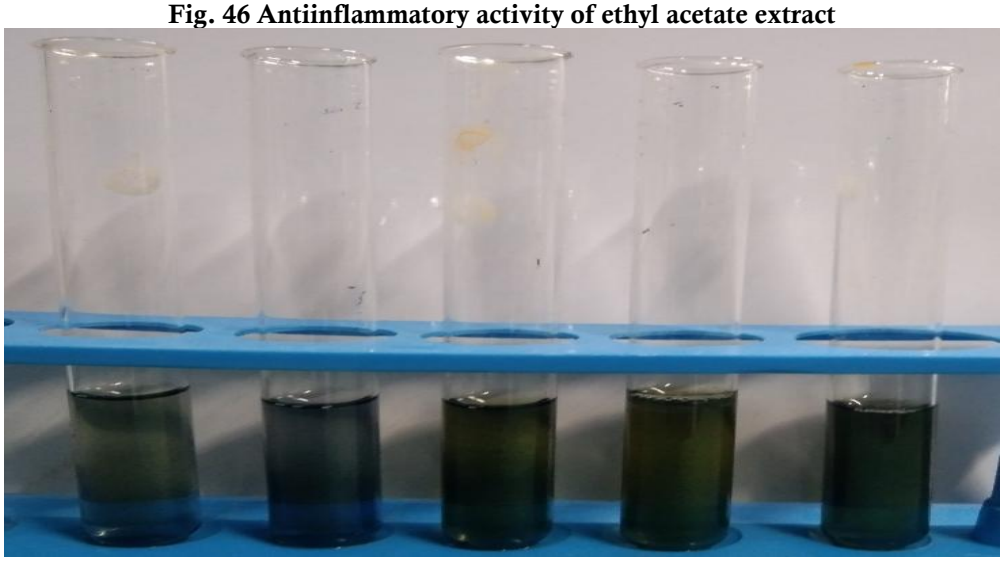


Table 6 : Antiinflammatory activity

Sample Concentration µg/ml	Absorbance at 660nm	%Inhibition
Aspirin	1.90	
Isopropanol extract		
50	0.40	21.05
100	0.82	43.16
150	0.92	48.42
200	0.92	48.42
250	1.50	78.94
Chloroform		
50	0.61	32.11
100	1.04	54.75
150	1.22	64.21
200	1.22	64.21
250	1.52	80
Ethyl acetate		
50	0.31	16.32
100	0.37	19.47
150	0.44	23.16
200	0.52	27.37
250	0.56	29.47

Fig. 47 Antiinflammatory activity of isopropanol extract

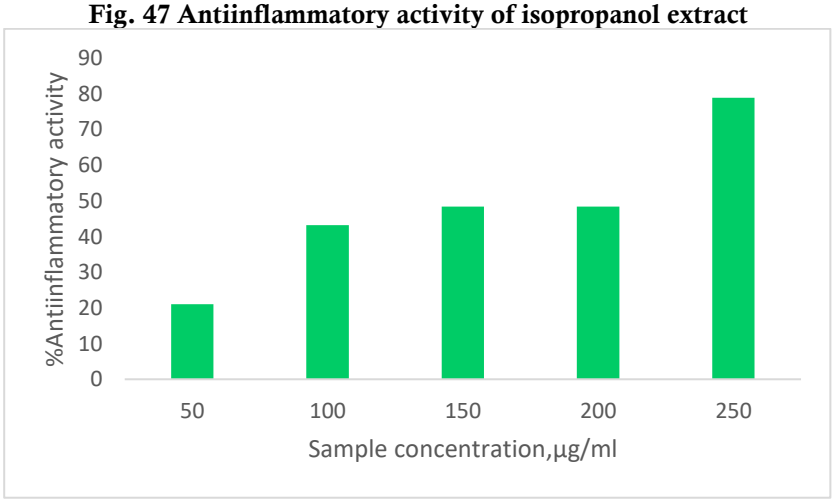


Fig. 48 Antiinflammatory activity of chloroform extract

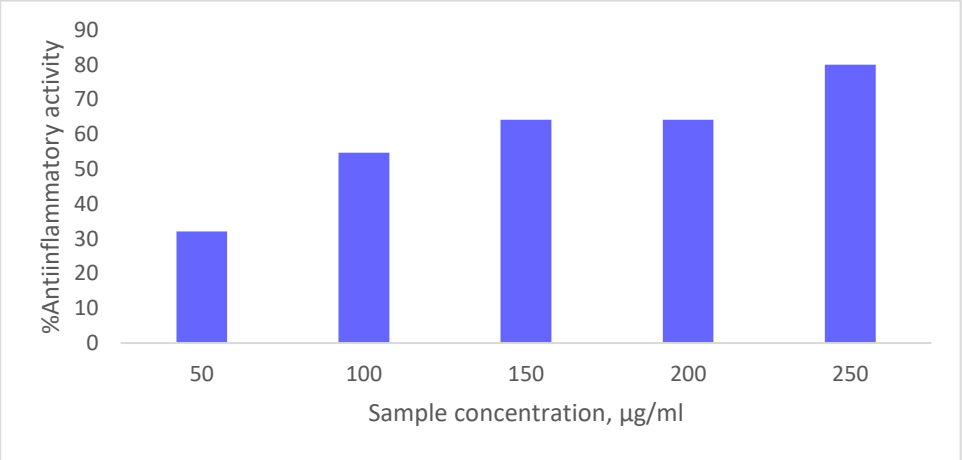
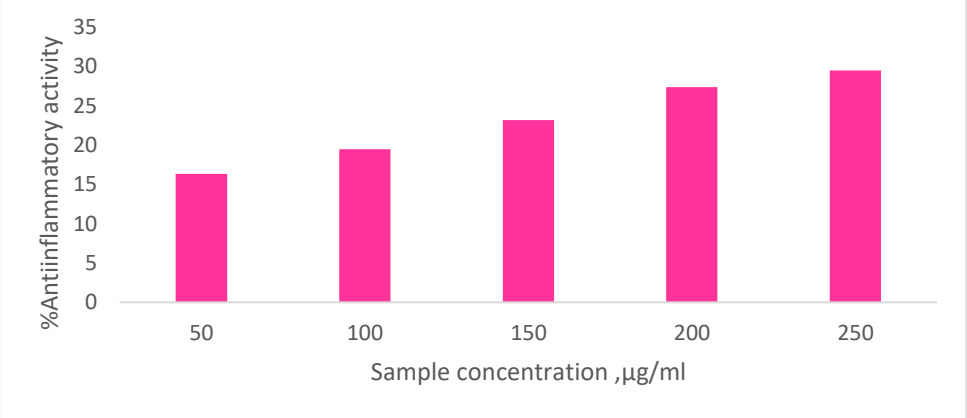


Fig. 49 Antiinflammatory activity of ethyl acetate extract



Antidiabetic activity

Fig. 50 Antidiabetic activity of isopropanol extract

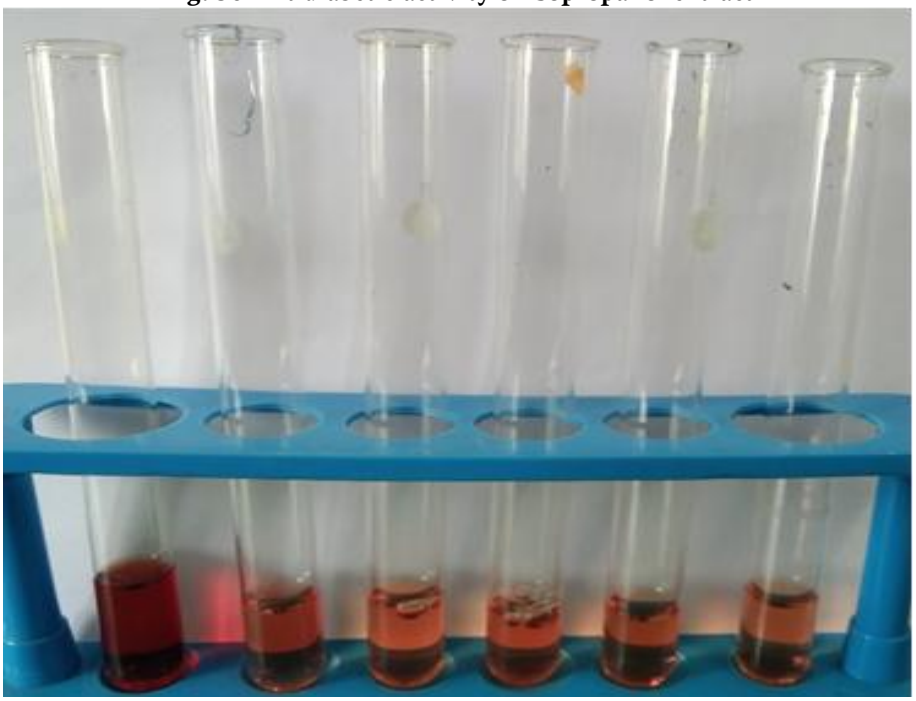


Fig. 51 Antidiabetic activity of chloroform extract

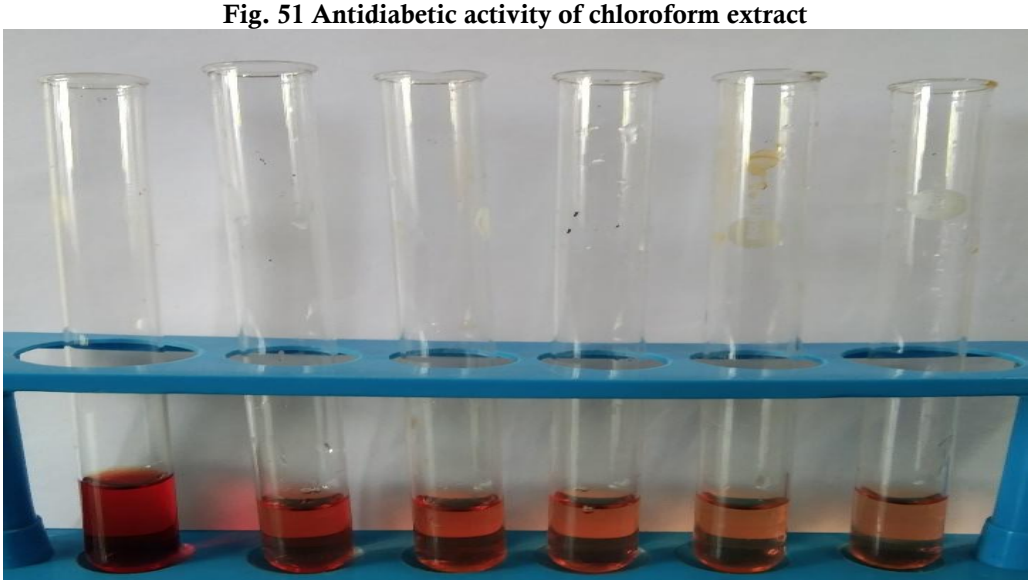


Fig. 52 Antidiabetic activity of ethyl acetate extract

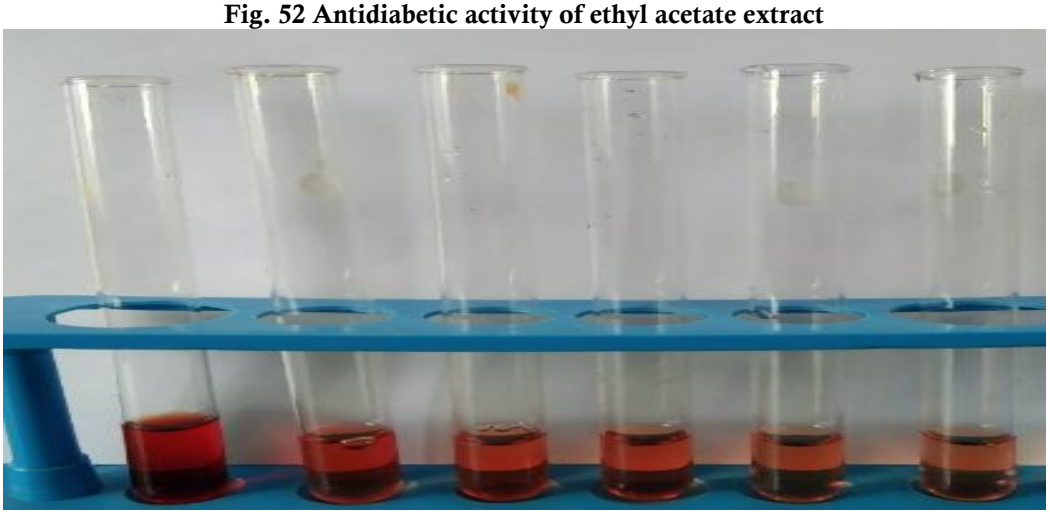


Table.7 : Antidiabetic activity

Sample µg/ml	Absorbance at 510nm	% Inhibition
Control	1.55	
Isopropanol		
50	0.95	38.71
100	0.70	54.84
150	0.66	57.42
200	0.52	63.23
250	0.41	73.55
Chloroform		
50	0.90	41.94
100	0.75	51.61
150	0.60	61.29
200	0.58	62.58
250	0.50	67.74
Ethyl acetate		
50	1.05	29.03
100	0.92	40.65
150	0.85	45.16
200	0.74	52.26
250	0.58	62.58

Fig.53 Antidiabetic activity of isopropanol extract

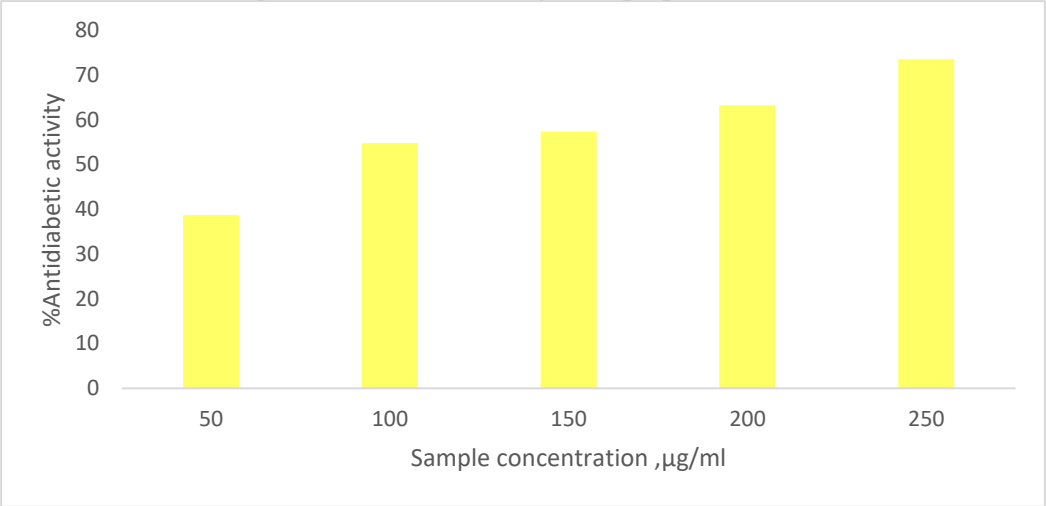


Fig. 54 Antidiabetic activity of chloroform extract

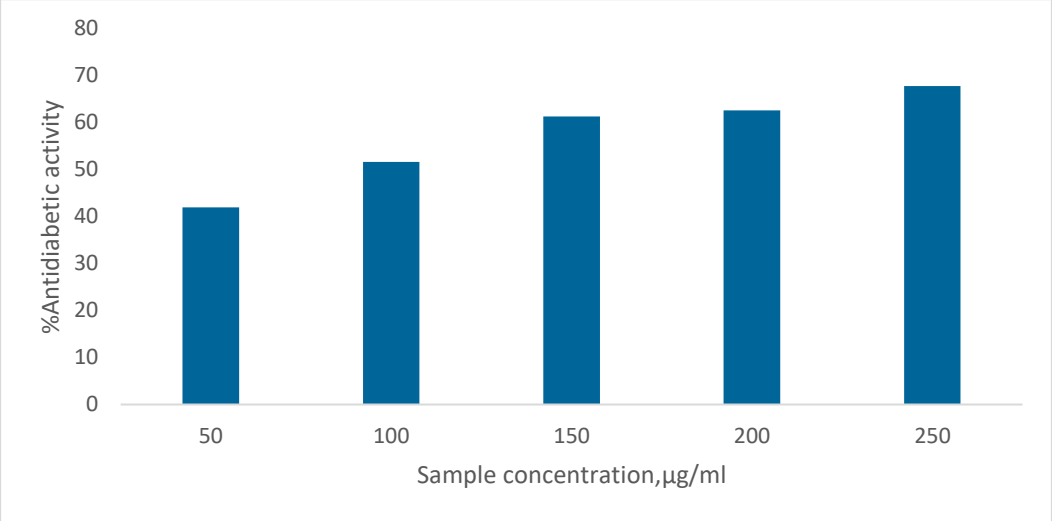
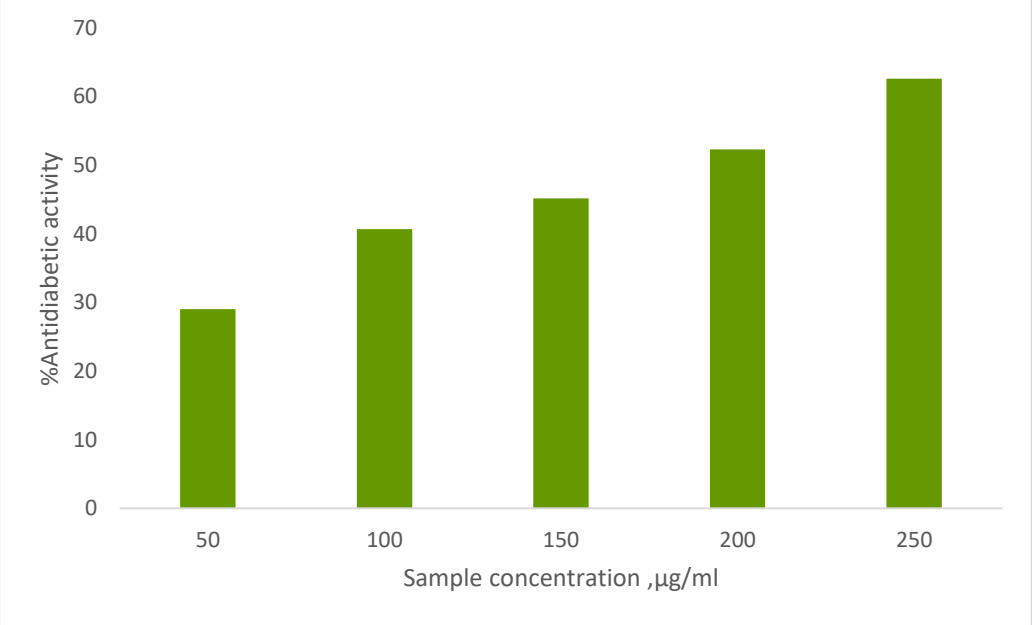


Fig.55 Antidiabetic activity of extract



ANTI CANCER ACTIVITY:-

Fig 56 Morphological changes in control and sample treated SKMEL3 cells for 24 h.

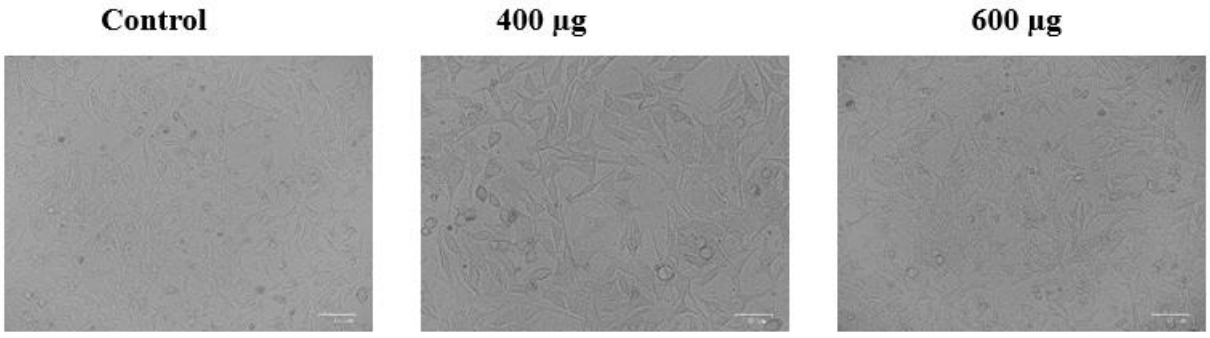


Table 8 : Cell Viability

	Control	100ug	20[0ug	400ug	600ug	800ug
	100	90.72	82.16	67.44	49.93	44.16
	100	92.01	79.18	69.93	53.42	39.19
	100	88.13	84.45	65.05	49.24	37.30
Average	**100**	**90.29**	**81.93**	**67.47**	**50.86**	**40.22**
SD	-	1.98	2.64	2.44	2.24	3.55

Table 9 : Cell Inhibition

Control	100ug	200ug	400ug	600ug	800ug
0	9.28	17.84	32.56	50.07	55.84
0	7.99	20.82	30.07	46.58	60.81
0	11.87	15.55	34.95	50.76	62.70

Fig 57 Cell Viability

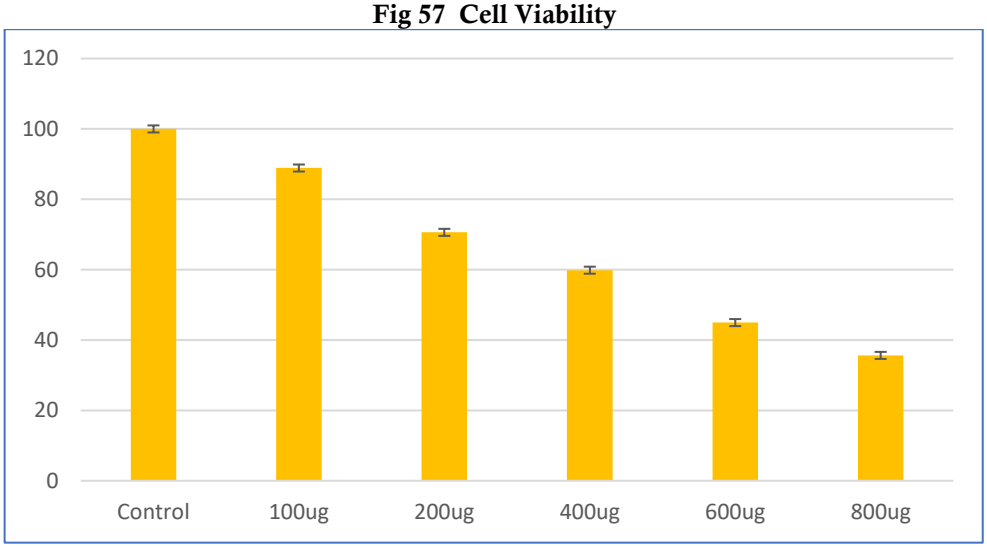


Fig 58 Cell Inhibition

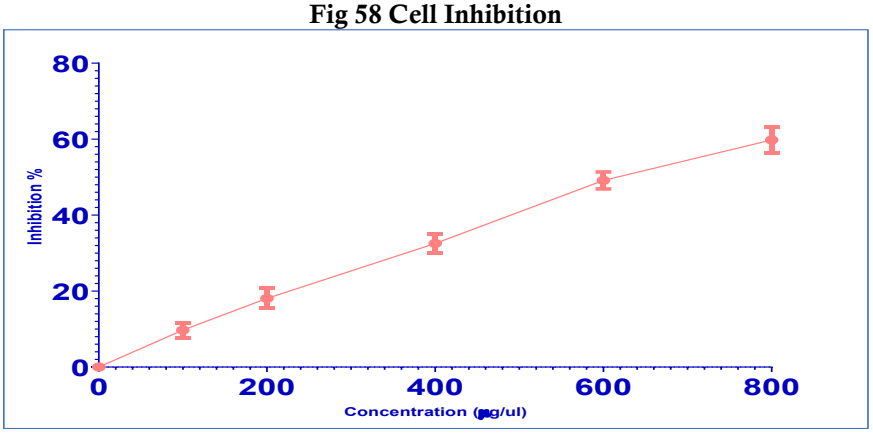


Fig 59 ANTICANCER ACTIVITY

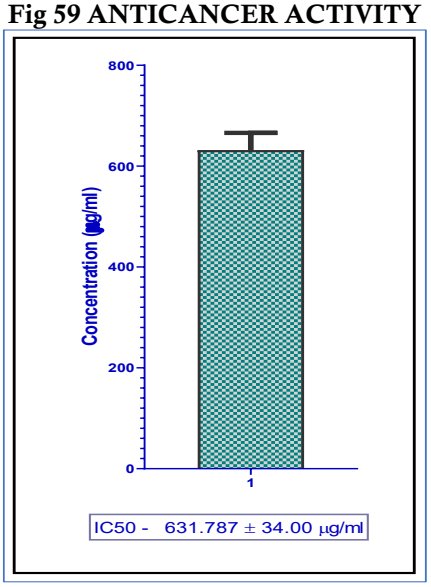
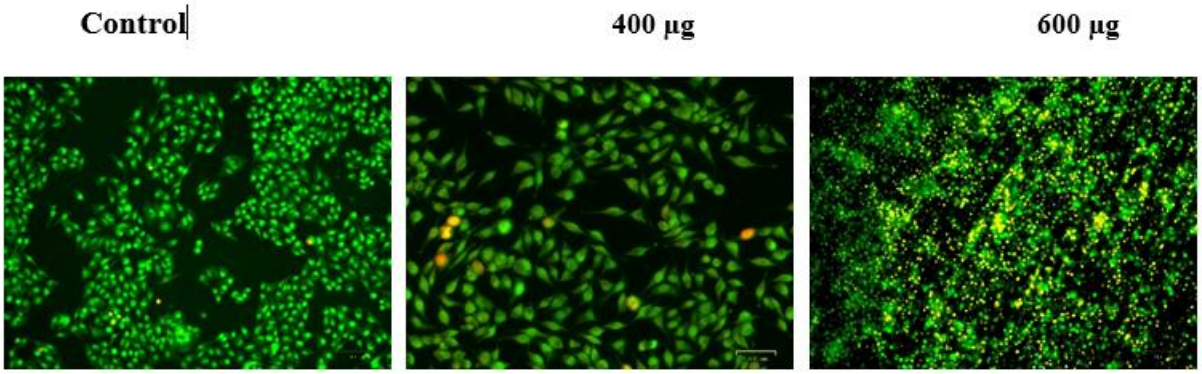


Fig 60 Apoptotic Staining – Control & sample treated SKMEL3 cells for 24 h.



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