J. Mountain Res. P-ISSN: 0974-3030, E-ISSN: 2582-5011 DOI: https://doi.org/10.51220/jmr.v20-i2.35
Vol. 20(2), (2025), 307-318

Anti-inflammatory & Anti-microbial activities of the bark extract of *Jatropha gossypiifolia* and isolated compound -Tripalmitoliene

M.C. Purohit[1] • Shikha Sayal[1] • Reena Purohit[2*]

[1]*Department of Chemistry, H.N.B. Garhwal University (A Central University) BGR Campus, Pauri, Uttarakhand, India*
[2]*Department of Chemistry. Bal Ganga Mahavidhyalay Sendul Kemar SDSUV. Tehri Uttarakhand India:*

[*]Corresponding Author's email Id: reenapurohit108@gmail.com

Received: 6.10.2025; Revised: 11.12.2025; Accepted: 12.12.2025

Abstract: The *Jatropha gossypifolia* commonly known as bellyache bush is a rich source of bioactive molecules with broad spectrum of biological activities. Its use requires pharmacological validation for therapeutic use. It is medicinal plant traditionally used for various ailments& it's a rich source of phytochemicals such as terpenoids, steroids, flavonoids, glycosides, phenol compounds, saponins fatty acids etc. This study demonstrated Tripalmitoliene one of the compounds isolated from Jatropha gossypifolia has demonstrated antimicrobial property against some bacteria, fungus & broad spectrum of anti-inflammatory activity. In present study of the EtOH extract of ***Jatropha gossypifolia*** bark four compounds were isolated. The ethanol extract (30gm) was fractioned initially on 100cm x5.0cm Sephadex LH-20 column, using CH_3OH and $CHCl_3$ as a mobile phase and 85 fractions (15 ml each) were obtained. The fractions were chromatographed by reverse phase column chromatography over silica gel eluted with chloroform and methanol (98:2→90:10) affording compound **1**, **2**, 3 and compound 4. The **compound 3,** named as Tripalmitoliene sample concentration 200 µg/100 µl. The ethanol extract and its isolated compound exhibited broad-spectrum antimicrobial activity against various microorganisms. Additionally, the ethanol, chloroform, and petroleum ether extracts, along with Tripalmitoliene, demonstrated broad-spectrum anti-inflammatory activity.

Keywords: Euphorbiaceae • Microorganisms • Phytochemicals • Biological activities.

Introduction

The plant has long cornerstone & source of bioactive molecule for modern drug discovery (Atanasov et al 2021) . Among these *jatropha gossypifolia* L., known as black physicnut, or bellyache bush or Ratanjoti belong to the Euphorbiaceae family that has enhance phytochemicals & broad spectrum of pharmacological activities (Kumar & Mandal 2017). *Jatropha gossypifolia* indigenous to the tropical and subtropical regions & costal area (Akinmoladun et al 2020). Several part of plant such as bark ,leaves, fruit, latex have been exploring pharmacological properties (Nair et al 2013).The bark of *jatropha gossypifolia* relatively underexplored while other parts have been broadly used in folk medicine for treating various ailments wandering from normal skin diseases, inflammation to gastrointestinal disorders (Devappa et al 2011; Umar et al 2015).

The recent investigations from bark of *jatropha gossypifolia* is a bank of bioactive molecules including flavoalkaloids, saponins, tannins ,terpenoids, phenolic compounds ,great number of which are unite with antimicrobial, antioxidant, anti-inflammatory, wound - healing activities (Oliveira et al 2020; Kumar & Mandal 2017). Ethnobotanical study of plant suggest that the infusions have been used in fever, rheumatism ,snakebites, diarrhoea, as in traditional healing system, specify the presence of robust bioactive molecule or constituents capable of interacting with biological markers (Rafatullah et al 1991; Nair et al 2013). These implementations offer a premium starting point for pharmacological

validation and standardization of drug development.

Recent phytochemical investigation has uncovered that the bark of *jatropha gossypifolia* contains secondary metabolites cyclic triterpenes, jatrophone, Flav glycosides and other phenolic compound, which are known to possess multiple pharmacological actions (Akinmoladun et al 2020; Umar et al 2015).

For instance, diterpenoid, found in jatropha species are widely recognized for antiviral, anti-inflammatory effect (Oskoueian et al 2012). Similarly, the tripalmitin or Tripalmitoliene exert antimicrobial effect .the molecule matrix of the bark suggests a synergistic mechanism of action that could explain its wide range of biological properties (Oliveira et al 2020). The studies exploring the anti-inflammatory and antimicrobial activity of *jatropha gossypifolia* bark extract and compound tripalmitin have demonstrated significant inhibitory effects against bacterial and fungal strains, this is particularly valuable in the current context of increasing antimicrobial resistance, which necessitates the discovery of ingenious agents from natural sources (Kumar & Mandal 2017; Al-Hazmi & Al-Essa 2020).

In-vivo Anti-inflammatory properties of isolated compound have also been documented , with bark extracts showing favourable activity in models of inflammation through the inhibition of pro-inflammatory mediators (Al-Hazmi & Al-Essa 2020). Which modulate inflammatory pathways &offer a natural alternative to synthetic anti-inflammatory drug that often have adverse side effects? Despite its potential therapeutic profile, the application of *jatropha gossypifolia* bark is not without concerns. Few pars of the plant, especially in the seeds &latex are known to be toxic because of the presence of phorbol esters (Devappa et al 2011). Hence, careful toxicological assessment & standardization are pivotal before any therapeutic application can be considered. It is crucial that studies focus on isolating &characterizing the active concept, understanding their mechanism of action ,and assessing their safety profile in both in vitro& in-vivo models. In conclusion *jatropha gossypifolia* bark contains a valuable yet underutilized natural resource with a wide array of biological activities supported by both traditional &emerging scientific evidence (Akinmoladun et al 2020; Atanasov et al 2021).

Its phytochemical diversity and pharmacological potential make a favourable candidate for the evolution of novel plant based Medicinals. However structured pharmacological, toxicological studies are needed to fully harness its medicinal potential .As attentiveness in ethnopharmacology & natural product-based drug discovery constant to grow, *jatropha* gossypiifolia bark stand out as an important botanical species deserving of further attention.

Review of Literature:-The research of natural products for their therapeutic potential has pinched significant interest, in particular from plants known in traditional medicine system (Atanasov et al 2021). *Jatropha gossypiifolia* commonly known as Ratanjoti belong to the Euphorbiaceae family & has been widely studied for its medicinal properties(Kumar & Mandal 2017; Akinmoladun et al 2020). Among the bioactive pharmaceuticals identified from the bark is tripalmitin (glyceryl tripalmitate),or Tripalmitoliene, a triglyceride composed of glycerol esterified with three molecules of palmitic acid. It's already reported in other species of jatropha such *jatropha curcas* (Devappa et al., 2011 Oskoueian et al 2012).Tripalmitin or Tripalmitoliene is a lipid based bioactive compound ,saturated triglyceride that occurs naturally in several plant species and animal fats. Though not exclusive *to jatropha gossifolia*, its separation from bark has added a new aspect to the understanding of lipid -based secondary metabolite in ethnomedicinal plants (Oliveira et al 2020). Traditionally, triglyceride like tripalmitin were mainly

considered as energy storage molecules; however, emerging evidence advise that they also take over significant pharmacological activities ,depending on their origin ,molecular structure& biological interactions (Al-Hazmi & Al-Essa 2020).

The Tripalmitoliene phytochemical isolated from *jatropha gossypiifolia* petroleum ether bark 's extract studies typically involve sequential solvent extraction followed by chromatographic separation and characterization using spectroscopic techniques like NMR,FTIR, & mass spectrometry(Umar et al 2015). The presence of Tripalmitoliene in the bark suggests the plant's lipid rich chemical makeup and supply a bioactive molecule rationale for some of its traditional application, especially in the treatment of inflammatory and microbe's infectious diseases (Nair et al 2013; Oliveira et al 2020). The biological activity investigations show though Tripalmitoliene itself is not as extensively studied as other phytoconstituents, available reports indicate a variety of pharmacological activities (Oskoueian et al 2012).

One of the most remarkable activities of tripalmitin is its cytotoxic effect on cancer cell lines. Studies have shown the tripalmitin can interfere with cell proliferation, potentially by altering membrane disrupting lipid metabolism in rapidly dividing cells. A study by (Wang et al 2013) demonstrated that Tripalmitoliene induced apoptosis in hepatocellular carcinoma cell through mitochondrial dysfunction. Although this work supports the anticancer potential of Tripalmitoliene isolated from plant like *J. gossypiifolia.*

On the basis of numerous medicinal properties of *jatropha gossypiifolia* these studies investigate antimicrobial and an anti-inflammatory activity, which is already, reported other species of Jatropha (Akinmoladun et al 2020; Kumar & Mandal 2017). The studies suggest that Tripalmitoliene and similar lipid exert antimicrobial effect by incorporating into microbial membrane, altering their permeability and ultimately causing cell lysis (Nair et al 2013) .while presented research demonstrated Tripalmitoliene from *jatropha gossypiifolia* extract rich in saturated fatty acid esters have been shown to inhibit Gram-positive and Gram-negative bacteria including *Staphylococcus aureus* and *Escherichia coli* (Umar et al., 2015; Oskoueian et al 2012).

The presented studies also show Tripalmitoliene also contribute to anti-inflammatory activity like standard drug ,Tripalmitoliene have demonstrated notable anti-inflammatory effect with carrageenan -induced pow edema models in rats(Al-Hazmi & Al-Essa 2020) although Tripalmitoliene have been associated with wound healing due to their emollient and barrier -forming properties, tripalmitin have been traditionally used to treat cuts, burns,& ulcers(Oliveira et al 2020). Tripalmitoliene is a naturally occurring &recognized safe compound in food &pharmaceutical formulations, some studies suggest that high doses of systematic exposure might have pro-inflammatory or metabolic effects, (Devappa et al 2011).Hence the pharmacological benefits must be weighed against potential side effects, especially when derived from toxic plant like *jatropha* species.

The limitation &research gap is despite its promising biological effects, tripalmitin remains under investigated as a standalone compound in the context of *jatropha gossypiifolia* (Akinmoladun et al 2020).Most studies focus on crude extracts, making it difficult to attribute the observed effects solely to Tripalmitoliene. Moreover, mechanistic insights into its molecular interactions are lacking .there is also limited information on its pharmacokinetics, metabolism and in vivo toxicity profiles (Oskoueian et al 2012).

The conclusion of the studies Tripalmitoliene isolated from *jatropha gossypiifolia* represents a biologically significant lipid molecule with demonstrated potential in antimicrobial, anti-inflammatory applications. While the

compound contributes to the overall therapeutic profile of the plant, further investigations are needed to fully elucidate its biological properties, mode of mechanism action and safety (Atanasov et al 2021). As interest grows in lipid-based therapeutics and plant-derived triglycerides, Tripalmitoliene may emerge as a valuable lead compound in natural product drug development.

Material &Method:- 1.Plant Materials:-The mature bark of *jatropha gossypiifolia* were collected from Forest Research Institute Dehradun Uttarakhand, India. The collection was taking based on ethnopharmacological literature, and identified from Department of Botany Pauri campus HNB Garhwal University. Voucher specimens of plants were stored in the Institute. The herbarium reserved for future reference. The collection of plant took place in the flowering season .The crushed bark were dried in the shade in an open for 5-10 days in the department of University.

2. Extraction and Fractionation of Plant Material:- Gathered plant material was carefully separated then, thoroughly cleaned with distilled water, and dried under shade in open place to prevent oxidation. Once dried, the material was converted into powder form. A total of 6 kg of the crushed bark was subjected to extraction with ethanol solvent in a Soxhlet apparatus therefore resulting aqueous ethanolic extract was concentrated under reduced pressure to remove the ethanol solvent, providing the yield of 250 g dry residue.

The finally extract was then resolute to column chromatography (CC) over silica gel. A gradient of solvent systems was used for elution, starting with chloroform: methanol (95:5), followed by other combinations such as the following ethanol :water, diethyl ether :chloroform, and hexane :ethyl acetate :methanol: water, additionally the ethanol extract was fractionated by sequential process using open column chromatography over silica gel, employing a gradient mixture of petroleum ether and chloroform (6:1). This process gave a yield of four fractions. Based on similarity observed on thin-layer chromatography (TLC), fraction three (F-3) was further purified using Sephadex LH-20 column chromatography. The final purification step involved crystallization of F-3 using a mixture of chloroform and ethanol (5:1), resulting in an isolated pure compound(s). then after the isolated compound characterized using spectroscopic techniques such as Mass spectra, UV&FTIR, H-NMR,NMR, spectrometry while the compound identified as Tripalmitoliene.

Result and discussion

Biological Activities of *J .gossypiifolia* Bark Extract & Tripalmitoliene

Antimicrobial Activity:- For antimicrobial test Soyabean Casein Digest agar/broth(SCDA/B) used as culture media for antibacterial test, and Sabouraud's dextrose agar & broth were used for the antifungal assays. Bacterial strains were inoculated onto SCDB prepared plates and fungal strains were inoculated into Sabouraud's dextrose broth. The bacterial cultures were incubated at **37°C for 18 hours**, whereas the fungal broth cultures were incubated for **48–72 hours**.

Microorganisms used: Test cultures of (E.coli) *Escherichia coli* (including a locally isolated K-12 strain), (S.aureus) *Staphylococcus aureus*, (C.albicans) *Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae*, (S.pyogens) *Streptococcus pyogenes*, and *Penicillium notatum* were procured from IMTECH, Chandigarh, India, and the Department of Microbiology Laboratory, Sai Institute of Paramedical and Allied Sciences, Dehradun, Uttarakhand, India.

Calculation of Zone of Inhibition by Agar Well Diffusion Method: The agar well diffusion method (Perez et al. 1993) was used with minute modifications. Soybean Casein Digest Medium (SCDM) were employed for culturing bacterial strains. The bacterial suspensions, first prepared in nutrient broth

and then inoculated into the cultured medium. Sabouraud's dextrose agar and broth was used for fungal cultures, which were separately suspended in Sabouraud's dextrose broth prior to inoculation.Wells of **8 mm diameter** punched into the solidified agar and now filled with plant extracts and solvent blanks. Ethanol introduced as the negative control, while chloramphenicol (1 mg/mL) was used as the positive control for antibacterial testing. The plates were incubated at **37°C for 18 hours** and antibacterial activity was assessed by measuring the diameter of the zones of inhibition in the plates. For the antifungal activity, Sabouraud's dextrose agar/broth plates were used as the same procedure. The zones of inhibition of the plates were measured after **48–72 hours** of incubation. Fluconazole (1 mg/mL) introduced as the standard antifungal agent. All assays were performed in **triplicate** to ensure accuracy and reproducibility of the inhibition zone measurements for each test organism showed **Table-1 &Graph** &See in the **Figs 1,2,3**

Test Organism	**Positive Control (Chloramphenicol) For Bacteria**		**Positive Control (Fucanazol) For Fungus**		***Jatropha gossypiifolia* Ethanol Extract**		**Tripalmitoliene (Isolated compound)**		**Negative Control(Ethanol)**	
	DZOI	**MIC**	**DZOI**	**MIC**	**DZOI**	**MIC**	**DZOI**	**MIC**	**DZOI**	**MIC**
E. Coli	26	0.625	--	--	30	0.039	35	0.039	NA	NA
S. Aureus	33	0.625	--	--	28	0.625	31	0.039	NA	NA
S. Pyogens	28	0.039	--	--	18	1.26	26	0.625	NA	NA
A. Niger	--	--	26	0.625	28	0.625	26	0.625	NA	NA
C. Albicans	--	--	28	0.625	32	0.625	25	0.625	NA	NA
P .Notatum	--	--	27	0.625	28	0.625	32	0.039	NA	NA
S.Cerevisae	--	--	30	0.038	34	0.039	25	0.625	NA	NA



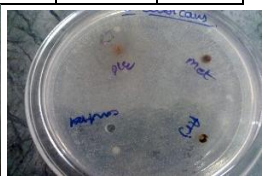
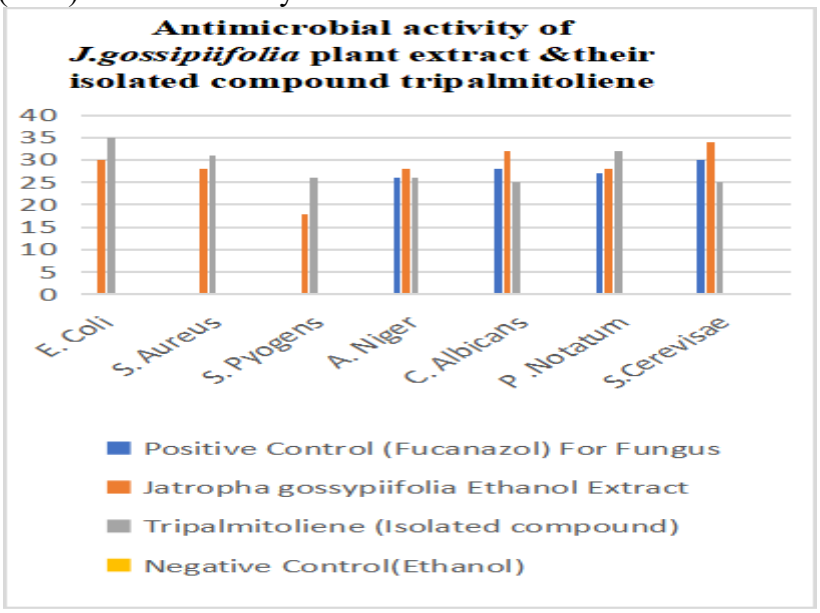


Fig 1 **Fig 2** **Fig 3**

Figs 1,2,3 : Tripalmitoliene Showed Zone of Inhibition by the Agar Well Diffusion

Table -1: Antimicrobial activity of the controls ,solvent ,plant extract &isolated compound by well diffusion method Diameter of zone of inhibition in mm (DZOI) &Minimum Inhibitory Concentration (MIC). NA-No activity



Graph 1: Antimicrobial activity of the controls ,solvent ,plant extract &isolated compound.

Results

Determination of Minimum Inhibitory Concentration (MIC):- MIC value of mighty plant extracts was determined by the method take on by (Vollekova *et al*., 2001 and Usman *et al.,* 2007), with few modifications. Plant extract was prepared in peak level concentration (200 µg/ml) with sterile distilled water and is se diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 48 h. The minimum dose at which bacterial growth is completely inhibited, with no turbidity observed, is referred to as the Minimum Lethal Concentration (MLC). In contrast, slight turbidity appears at lower concentrations where bacterial growth is not fully suppressed. the minimum amount of dose of plant extract which inhibits the growth of bacteria is known as Minimum Inhibitory Concentration (MIC).

2. Determination of Anti-Inflammatory Activity

Measurement of Paw Volume and Induction of Inflammation- Anti-inflammatory activity was performed by using Plethysmograph. Previous to drug administration, the paw volume of each animal was measured using the mercury displacement method. The animals were singly weighed, numbered, and marked on the right hind paw just distal to the tibio-tarsal junction. This mark assured consistent immersion depth during each measurement, maintaining uniformity in paw volume assessment. The initial paw volume was recorded for each rat using the mercury displacement technique. A 0.1% suspension of carrageenan was prepared in sodium carboxymethyl cellulose (C.M.C.). Subsequently, 0.1 mL of this suspension was injected into the sub plantar region of the left hind paw of each rat to induce inflammation.

The Diclofenac sodium (10 mg/kg) as the standard drug and the test compounds (100 mg/kg) were administered orally by gavage to the animals, 30 minutes prior to carrageenan administration. After 2 hrs of carrageenan administration, the paw volumes of control and drug treated rats was recorded by Plethysmograph. Edema was calculated as the difference between the two measurements.

Inhibition of swelling was determined by the comparing the change in hind paw volume in compounds and vehicle –treated rats. The anti-inflammatory activity was determined as the percentage of inhibition of inflammation after. It was induced by carrageenan. To calculate the percentage inhibition, the following formula was applied

Percentage Inhibition = {[(Vt-Vo) Control-(V_t-Vo) Test]/Vt-Vo Control} X 100

Where, V_t= Final paw volume; Vo= Initial paw volume

Groups: Animals: Albino rats of either sex weighing 150 -300 g. Animals were divided into 7groups with 6 animals in each group.

Group-I: 0. 1 % Sodium C.M.C (Negative Control). – Received normal saline (volume equivalent to standard drug) orally by gavages in a volume of 10 ml/kg, half hour after carrageenan (0.1 ml of 1% suspension in sodium C.M.C.) administration in the sub plantar region of left hind paw of each rat.

Group-II : Diclofenac suspension in 0.1 % Sodium CMC (10 mg/kg). Received diclofenac sodium suspension in 0.1% sodium C. M. C. (10 mg/kg) orally by gavages, half hour after carrageenan (0.1ml of 1%) administration in the sub plantar region of left hind paw of each rat.

Group-III :Sample 1 suspension (Ethanolic extract of *Jatropha gossypiifolia*) in 0.1% Sodium C.M.C. (100 mg/kg) Received sample 1 suspension in 0.1 % sodium C.M.C. (100mg/kg) orally by gavages, half hour after carrageenan (0.1ml of 1%) administration in the sub plantar region of left hind paw of each rat.

Group-IV Sample 2 suspension (Tripalmitoliene isolated from *Jatropha gossypiifolia*) in 0.1% Sodium C.M.C. (100 mg/kg). Received sample 2 suspension in 0.1 % sodium C.M.C. (100mg/kg) orally by gavages, half hour after carrageenan (0.1ml of 1%) administration in the sub plantar region of left hind paw of each rat.

Group-V: Sample 3 suspension (Chloroform extract of *Jatropha gossypiifolia*) in 0.1% Sodium C.M.C. (100 mg/kg). Received sample 3 suspension in 0.1 % sodium C.M.C. (100mg/kg) orally by gavages, half hour after carrageenan (0.1ml of 1%) administration in the sub plantar region of left hind paw of each rat.

Group-VI: Sample 4 suspension (Diethyl ether extract of *Jatropha gossypiifolia*) in 0.1% Sodium C.M.C. (100 mg/kg). Received sample 1 suspension in 0.1 % sodium C.M.C. (100mg/kg) orally by gavages, half hour after carrageenan (0.1ml of 1%) administration in the sub plantar region of left hind paw of each rat.

Group-VI: water (ml/kg). Received **water** (ml/kg) orally by gavages.

Result:- The anti-inflammatory activity of various extracts and the compound Tripalmitoliene isolated from *Jatropha gossypiifolia* bark was evaluate using the carrageenan-induced paw edema model in albino rats. The paw volume was measured before and 2 hours after carrageenan injection. The percentage inhibition of inflammation was calculated for each group of rats, and the data were examine using one-way ANOVA followed by Dunnett's multiple comparison test.

A statistically significant difference in paw edema volume was noticed among the seven groups ($p < 0.0001$), indicating effective anti-inflammatory reaction in the treatment groups. The ANOVA revealed a high F-value of 78.27 and an R^2 of 0.9306, demonstrating strong treatment effects covering the groups. Although Bartlett's test ($p = 0.0114$) indicated unequal variances, the ANOVA remains valid due to equal sample sizes.

Among the treatments, the standard drug diclofenac sodium (10 mg/kg) showed the highest depletion in paw volume (mean = 0.093 mL), followed by the ethanol extract (0.150 mL), the isolated compound Tripalmitoliene (0.192 mL), and the chloroform extract (0.135 mL). All treatment groups demonstrated statistically significant depletion in paw edema compared to the negative control (mean = 0.312 mL) ($p < 0.05$).

Dunnett's post hoc analysis committed remarkable differences between the control and each treatment group: **shown in table**

Control vs Standard: ***p < 0.001***

Control vs Ethanol extract: ***p < 0.001***

Control vs Tripalmitoliene: ***p < 0.001***

Control vs Chloroform extract: ***p < 0.001***

Control vs Petroleum ether extract: ***p < 0.001***

Control vs Water: ***p < 0.001***

Table Analysed	*Data 1*						
One-way analysis of variance							
P value	*< 0.0001*						
P value summary	****						
Are means signif. different? (P < 0.05)	*Yes*						
Number of groups	*7*						
F	*78.27*						
Bartlett's test for equal							

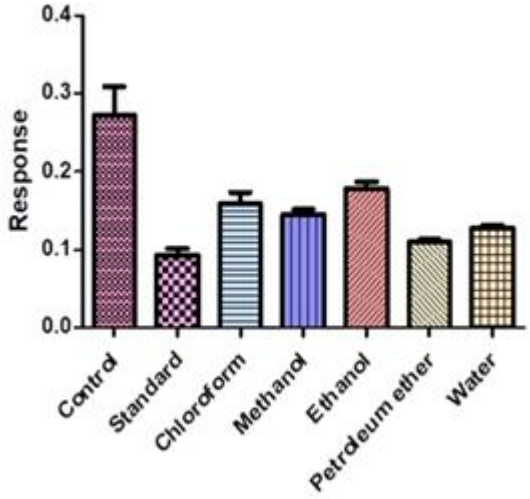
variances							
Bartlett's statistic (corrected)	*16.48*						
P value	*0.0114*						
P value summary	*						
Do the variances differ signif. (P < 0.05)	***Yes***						
ANOVA Table	*SS*	*df*	*MS*				
Treatment (between columns)	*0.1986*	*6*	*0.03310*	*Treatment (between columns)*	*0.1986*		
Residual (within columns)	*0.0148*	*35*	*0.0004229*	*Residual (within columns)*	*0.0148*		
Total	*0.2134*	*41*		*Total*	*0.2134*		
Dunnett's Multiple Comparison Test	***Mean Diff.***	***q***	***Significant? P < 0.05?***	***Summary***	***95% CI of diff***		
Control vs Standard	*0.2183*	*18.39*	*Yes*	***	*0.1863 to 0.2504*		
Control vs Ethanol	*0.1617*	*13.62*	*Yes*	***	*0.1296 to 0.1937*		
Control vs Tripalmitoliene	*0.1200*	*10.11*	*Yes*	***	*0.08794 to 0.1521*		
Control vs Chloroform	*0.1767*	*14.88*	*Yes*	***	*0.1446 to 0.2087*		
Control vs Petroleum ether	*0.2067*	*17.41*	*Yes*	***	*0.1746 to 0.2387*		
Control vs Water	*0.1817*	*15.30*	*Yes*	***	*0.1496 to 0.2137*		
Number of values	*6*	*6*	*6*	*6*	*6*	*6*	*6*
Minimum	*0.2600*	*0.0600*	*0.1300*	*0.1700*	*0.1000*	*0.1000*	*0.1100*
25% Percentile	*0.2750*	*0.0750*	*0.1375*	*0.1775*	*0.1225*	*0.1000*	*0.1250*
Median	*0.3150*	*0.1000*	*0.1500*	*0.1900*	*0.1300*	*0.1050*	*0.1300*
75% Percentile	*0.3450*	*0.1100*	*0.1625*	*0.2050*	*0.1600*	*0.1100*	*0.1400*
Maximum	*0.3600*	*0.1100*	*0.1700*	*0.2200*	*0.1600*	*0.1100*	*0.1400*
Mean	*0.3117*	*0.09333*	*0.1500*	*0.1917*	*0.1350*	*0.1050*	*0.1300*
Std. Deviation	*0.03710*	*0.01966*	*0.01549*	*0.01722*	*0.02258*	*0.005477*	*0.01095*
Std. Error	*0.01515*	*0.008028*	*0.006325*	*0.007032*	*0.009220*	*0.002236*	*0.004472*
Lower 95% CI	*0.2727*	*0.07270*	*0.1337*	*0.1736*	*0.1113*	*0.09925*	*0.1185*
Upper 95% CI	*0.3506*	*0.1140*	*0.1663*	*0.2097*	*0.1587*	*0.1107*	*0.1415*

The petroleum ether extracts also display a notable anti-inflammatory effect (mean = 0.105 mL), comparable to that of the standard drug. The water-treated group (mean = 0.130 mL), used as a vehicle control, showed a slight but still statistically significant difference from the control which is not treated.

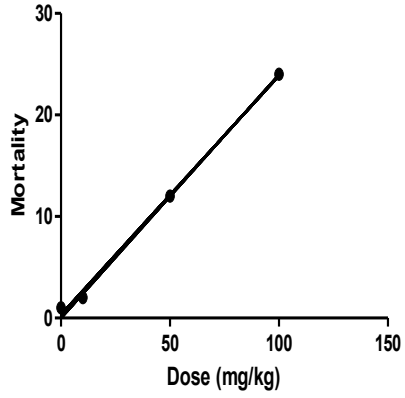
Statistics By One-Way ANOVA Method (Dunnett's Multiple Comparison Test):-

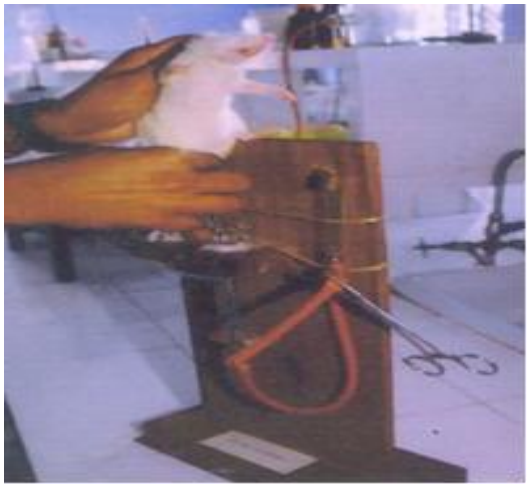
Control	Standard	Ethanolic extract of plant	Tripalmitoliene isolated from plant extract	Chloroform extract	Petroleum ether	Water
0.32	0.08	0.14	0.22	0.16	0.1	0.14
0.28	0.11	0.17	0.19	0.13	0.11	0.13
0.34	0.1	0.14	0.18	0.1	0.1	0.11
0.26	0.06	0.16	0.17	0.13	0.11	0.13
0.36	0.11	0.13	0.19	0.13	0.1	0.13
0.31	0.1	0.16	0.2	0.16	0.11	0.14

Statistics By One-Way ANOVA Method (Dunnett's Multiple Comparison Test (statics Table 2)



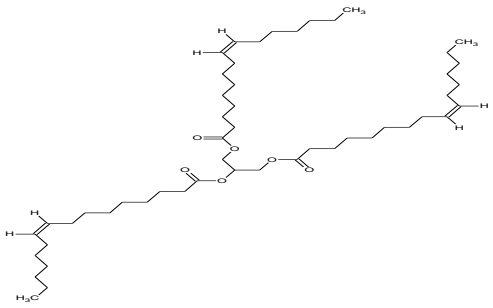
(Table 3 For Number of intervals) **ANOVA Graph 2 for different extract of plant**





Graph 3 Lethal Dose **Fig 4 Mercury displacement method for anti-inflammatory activity**

The ANOVA Graph 2 for different solvent extract, Graph 3 Lethal Dose for all plants extracts& isolated compound picture, Fig 4 Mercury displacement method for anti-inflammatory activity & below structure of Tripalmitoliene.



Discussion

Antimicrobial activities of plant extract & isolated compound:-

Broth dilution method was adopted for the determination of M.I.C values of the test sample. The specific bacterial inoculums (100 µl) were introduced separately in the test tubes having sterilized nutrient broth (N.B) ethanol solvent (negative control) was used for the dilution for the extract .the test tubes having separate bacterial culture were incubated overnight /24 h for 37^0C. The turbidity present within the test tube was recorded for determination of M.I.C) and M.B.C .the minimum amount of dose of the extract which kills the bacterial growth is known as minimum lethal concentration (M.L.C) and the minimum amount of the dose of the extract which inhibits the bacterial growth is known as minimum inhibitory concentration (M.I.C). The MIC results for the plant extracts are illustrated in **Table 1, 2 &pictures along with visual documentation**.

Anti-inflammatory activities of plant extracts & their isolated compound: - The present study signifies that *Jatropha gossypiifolia* bark extracts and the isolated compound Tripalmitoliene show significant anti-inflammatory activity in the inflammation of carrageenan-induced paw edema model (Winter et al., 1962; Vinegar et al., 1969). One-way ANOVA revealed the extremely significant differences among the treatment groups of rats, and post hoc collations supported the effectiveness of each tested sample compared to the negative control (Field, 2013) .

The ethanol extract showed the highly potent activity among the all-test compounds, second only to the standard drug diclofenac sodium. This suggests the presence of polar phytocompound with anti-inflammatory properties, likely acting over inhibition of mediators involved in severe inflammation, such as histamine, serotonin, and prostaglandins (Calixto et al., 2004; Vinegar et al., 1969).

Tripalmitoliene, an isolated compound from the *jatropha gossypiifolia* bark, also demonstrated marked anti-inflammatory effects. Although slightly less effective than the ethanol extract, its activity supports the hypothesis that this compound may be one of the active molecules responsible for the plant's therapeutic potential (Ramírez et al., 2013) .

The chloroform and petroleum ether extracts of bark *gossypiifolia* also bring down paw edema significantly, suggesting that both polar and non-polar compounds from the bark contribute to its pharmacological effect (Calixto et al., 2004). The variability of effectiveness between different extracts may reflect differences in phytochemical composition and solubility of active constituents (Akinmoladun et al., 2020) .

Conclusion: - The ethanol extract & isolated compound (Tripalmitoliene) of plant was tested for antibacterial and antifungal activity using agar well diffusion method at sample concentration 200 µg/100 µl. These all three extracts of plants showed strong and broad-spectrum antibacterial activity against *Staphylococcus aureus, S. pyrogenes and Escherichia coli* at 200 µg/100 µl. These three extracts of plants were found active against the

fungal pathogen *Candida albicans, Aspergillus niger, Saccharomyces cerevisae* and *Penicillium notatum* at 200 µg/100 µl. The results are shown in **Table 1** and **graph 1** the zones of inhibition in plates were shown in **pictures**.

The findings validate the traditional use of *Jatropha gossypiifolia* in inflammatory conditions and support further investigation into its bioactive components. These results provide a scientific basis for the anti-inflammatory potential of the plant and highlight Tripalmitoliene as a promising candidate for future drug & therapeutic development. All the sample extracts of *Jatropha gossipholia* and at a dose level of 100 mg/Kg, showed maximum inhibition of the oedema respectively which was significant ($p<0.05$) as compared to control. The lethal dose (LD50) of all the plant extracts was found to be 100 mg/Kg. **Tables 2 and 3,** along with **picture 2**, illustrate the results.

Research Gap & Opportunity:- Based on your research description to the help with the research gap for Jatropha gossypifolia (Bellyache Bush), break down what is already known and identify areas where more research could be beneficial. The gap is antimicrobial and anti-inflammatory activities are highlighted while the opportunity investigating the potential for anti-cancer, antioxidant, anti-diabetic, or anti-hypertensive activities of the plant's compounds the underlying pharmacological mechanisms of how these compounds work are still unclear. The opportunity investigating the molecular pathways through which compounds like Tripalmitoliene exert their effects. Are they interacting with specific proteins, enzymes, or receptors in human or microbial cells? Investigating the molecular pathways through which compounds like Tripalmitoliene exert their effects. The toxicity profile of the plant, particularly Tripalmitoliene, is not fully explored while compound showed good therapeutic strength. Investigating the toxicological effects through acute and chronic toxicity tests. This could include studies on cytotoxicity, genotoxicity, and potential organ-specific toxicity.

Acknowledgement:-The authors are thankful to the Sai Group of Institutions, Dehradun, and the SBS Institute, Dehradun, for providing the necessary facilities and assistance in conducting the antimicrobial and anti-inflammatory activities

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

References

Akinmoladun F O, Akinrinlola B L, Komolafe O A, Farombi E O & Olaleye M T (2020). Phytochemical composition and biological activities of *Jatropha* species (Euphorbiaceae): A review. *Journal of Ethnopharmacology, 256*, 112769.

Al-Hazmi H A.& Al-Essa M A (2020). *In vivo* anti-inflammatory and analgesic effects of flavonoids isolated from medicinal plants. *Saudi Pharmaceutical Journal, 28*(6), 798–803.

Atanasov A G, Zotchev S B, Dirsch V M & Supuran C T (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery, 20*(3), 200–216.

Calixto J B, Campos M M, Otuki M F & Santos A R S (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Medica, 70*(2), 93–103.

Devappa R K, Makkar H P S & Becker K (2011). *Jatropha* toxicity—A review of toxicity studies and mechanisms responsible for toxicity. *Environmental and Experimental Botany, 71*(3), 267–285.

Di Rosa, M., Giroud, J. P., & Willoughby, D. A. (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and

turpentine. *Journal of Pathology, 104*(1), 15–29.

Field A (2013). *Discovering statistics using IBM SPSS statistics* (4th ed.). Sage Publications.

Kumar N. & Mandal B (2017). Phytochemical and pharmacological review on *Jatropha gossypiifolia* L. (Euphorbiaceae). *Journal of Pharmacognosy and Phytochemistry, 6*(2), 220–223.

Nair A, Gupta R C & Keshri G K (2013). Phytochemical screening and antimicrobial potential of *Jatropha gossypifolia*. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(Suppl 4), 91–93.

Oliveira F A, Lima C F, Coelho R S & Silva R M (2020). Pharmacological potential and toxicological risks of *Jatropha* species: A systematic review. *Biomedicine & Pharmacotherapy, 132*, 110764.

Oskoueian E, Abdullah N & Oskoueian A (2012). Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. *International Journal of Molecular Sciences, 13*(5), 5935–5950.

Pérez C, Pauli M & Bazerque P (1993). An antibiotic assay by the agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis, 15*, 113–115.

Rafatullah S, Tariq M, Mossa J S, Al-Yahya M A, Al-Said M S & Ageel A M (1991). Antidiarrhoeal activity of *Jatropha curcas* in mice. *Journal of Ethnopharmacology, 35*(3), 243–247.

Ramírez A M, Rodríguez A & de la Rosa O (2013). Triterpenes as anti-inflammatory agents: New perspectives in the regulation of inflammation. *Current Medicinal Chemistry, 20*(8), 946–955.

Raut N A & Gaikwad P S (2006). Anti-inflammatory activity of various extracts of *Tagetes erecta* Linn. *International Journal of Green Pharmacy, 1*(1), 37.

Umar M T, Sule M I & Pateh U U (2015). Phytochemical and antimicrobial screening of petroleum ether extract of *Jatropha gossypifolia* Linn bark. *African Journal of Pharmacy and Pharmacology, 9*(3), 73–76.

Usman H, Abdulrahman, F I.& Usman A (2007). Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines, 4*(2), 124–131.

Vinegar R, Schreiber W & Hugo R (1969). Biphasic development of carrageenin edema in rats. *Journal of Pharmacology and Experimental Therapeutics, 166*(1), 96–103.

Volleková A, Kost'álová D & Sochorová R (2001). Isoquinoline alkaloids from *Mahonia aquifolium* stem bark are active against *Malassezia* species. *Folia Microbiologica, 46*(2), 107–111.

Winter C A, Risley E A & Nuss G W (1962). Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine, 111*(3), 544–547.