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PHARMACOGNOSTIC STUDY ,PHYTOCHEMICAL SCREENING AND FORMULATION DEVELOPMENT FROM *ARCHAS SAPOTA* BARK EXTRACTS FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT
By analyzing the pharmacognostic characteristics, phytochemical components, and wound healing potential of *Achras sapota* (Sapodilla) bark extracts, the current study aimed to develop a suitable herbal formulation for wound treatment. *Achras sapota* bark was collected, confirmed, and subjected to a comprehensive pharmacognostic analysis that included microscopic, macroscopic, and physicochemical analyses. Successive solvent extraction was carried out using more polar solvents, and the presence of bioactive phytoconstituents was assessed in the resultant extracts. Preliminary phytochemical studies identified tannins, flavonoids, alkaloids, glycosides, triterpenoids, steroids, and phenolic chemicals, all of which are known to promote tissue repair and antioxidant activity. The extracts were then analyzed using spectroscopic and chromatographic techniques to detect important phytoconstituents, such as phenolic compounds like gallic acid. Previous research has demonstrated the significant antioxidant properties of Achras sapota bark extract, supporting its promise as a wound healing treatment.

KEYWORDS: Bioactive, phytoconstituents, flavonoids, solvent extraction, and *Achras sapota*

INTRODUCTION:
Medicinal plants with therapeutic qualities have been used to cure human ailments since ancient times. As the knowledge, skills, and practice of holistic health care, traditional medicine is recognized and valued for its role in preserving health and treating ailments. Its base is indigenous concepts, beliefs, and experiences that have been passed down through the generations. Due to the high cost of allopathic medication and its potential side effects, people are often encouraged to take traditional medicines, especially those from rural and underprivileged areas [7]. Systematic research on medicinal plants could successfully aid in the identification of new bioactive compounds and their application as medicines to treat a variety of illnesses, as evidenced by the growing demand for plant extracts in the food, pharmaceutical, and cosmetics industries (Nostro et al., 2001). Due to pathogen resistance to current antibiotics and the adoption of traditional medicine as an alternative form of treatment, research on the biological activities of medicinal plants has lately opened up new avenues (Arias et al., 2004) [1]. In developing countries, almost 80% of people use herbal remedies for primary healthcare. Similar to the current process of discovering novel drugs, plants are used as sources of active compounds. Man has developed methods to separate the active ingredients from the entire medicinal plant after extracting its essence. This approach has led to the successful development of many chemicals that are still in widespread use today. Numerous substances, such as taxol, artemesinin, vinca alkaloids, quinine, quinidine, ajmaline, brucine, reserpine, and strychnine, have been created by this pathway. From these refined molecules or compounds, several new chemicals have been created and used to treat a variety of diseases [1]. The literature makes clear that traditional healers have utilized a number of plant species from the Chotanagpur plateau, a tribal area of India, to cure a variety of ailments, including pyrexia, inflammation, sores and ulcers, diarrhea, and other skin disorders (Kirtikar and Basu, 1935) [5]

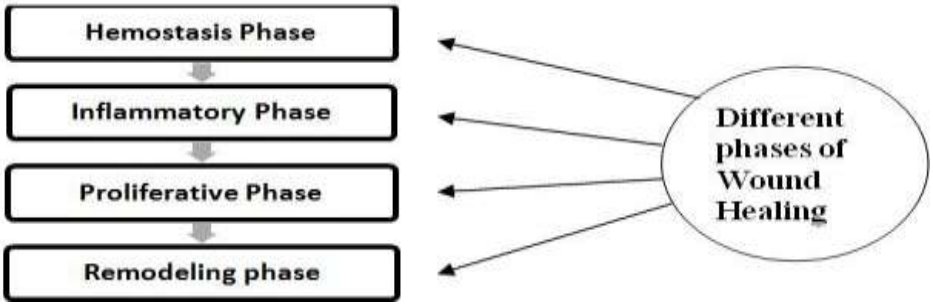


Fig no 1 :Phase of wound healing

Achras Sapota [2]

Achras Sapota, Sprengor (Chironjitree), is a medium-sized deciduous tree that can grow up to around fifty feet in height. It has a straight trunk. It can be identified by its red-blazed, dark gray, 1.25–1.75 cm thick crocodile bark that is broken into distinct squares.

Taxonomy

Table no :1 Taxonomy

Taxonomic Rank	Classification
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Viridiplantae
Phylum	Magnoliophyta
Subphylum	Euphyllophytina
Infraphylum	Radiatopses
Class	Magnoliopsida
Subclass	Rosidae
Superorder	Rutanae
Order	Burserales
Family	Anacardiaceae
Subfamily	Anacardioideae
Genus	Buchanania
Species	Buchanania lanzan Spreng.
Synonym	Achras sapota Spreng.

Leaves [2]

Simple, roughly oblong, obtuse, 15–25 cm long, densely coriaceous, with a rounded base and pointy tip. Ten to twenty pairs of parallel, straight veins are seen on leaves.

Stem [2]

The greenish-brown, relatively hard-wooded stems have noticeable rough bark that is tessellated in large squares.

Fruits [2]

Achras Sapota's seeds are called Chironji, while its fruits are called Char. One of the tastiest wild fruits with a hard shell is the Chironji fruit. Fruits stay on the tree for a considerable amount of time after ripening in April and May. Sweets are garnished with the kernel found inside the shells.

Figure 2: Photograph of *Achras Sapota* spreng

Figure 3 Photograph of *Achras Sapota* spreng

Applications in Ethnomedicine

The stem's bark

Bark powder is used to treat STDs, according to the ethnobotanical survey. Stem bark is pounded and applied for snakebite, sunstroke, and gum irritation [5].

Fruits

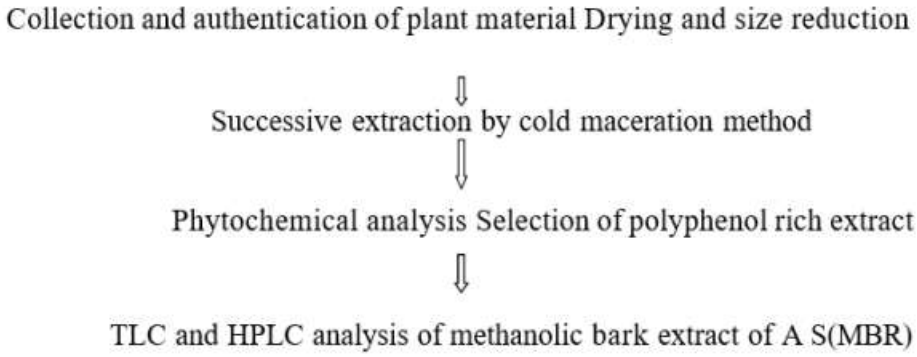
According to Shiddamallayya et al. (2010), Achras sapota fruits are used as an Ayurvedic raw remedy for a number of

ailments, such as pain, diarrhea, and tonic. Survase and Raut (2011) state that seed paste is used to treat skin diseases and fruit is used to cure asthma and cough [6].

METHODS AND MATERIALS:

Achras sapota bark extraction and standardization [8]

The cold maceration procedure was used to dry and remove the Achras Sapota plant's dried bark. After that, a preliminary phytochemical study was performed on the extract. The most active fraction was then chosen by evaluating the various extracts utilizing various in vitro and in vivo investigations.



Collection of plant material

Bark from Achras Sapota (Anacardiaceae) was gathered in September and October at the Meera nursery in Pune, Maharashtra.

Recognition and Verification

The Botanical Garden at SPPU University Pune provided the identification and authentication of the plant [No.-CNH/I-I (81)/2005-Tech. II. /1134]. The plant specimen was stored at SPPU University Pune's Department of Pharmaceutical Sciences and Technology.

Size reduction and drying [8]

The bark was gently dried at ambient temperature (32°±2°C) in the shade. After being completely dry, barks were ground and sieved to reduce their size. The bark's powdered MBRs were used in the extraction process that followed.

Getting the extract ready [9]

This extraction process was completed using Harborne's (1998) approach. In order to recover the organic components from the dried plant materials, the powdered plant materials were removed using a traditional chemical process. Petroleum ether, chloroform, ethyl acetate, and methanol were used as solvents in the cold maceration method of successive extraction. Petroleum ether (B.P. 60-80ºC) was used to defat the powdered barks (3.5 kg) by soaking them in the solvent and then extracting them.

Materials

We bought carbopol 940, methyl paraben, propyl paraben, propylene glycol, and triethanolamine from Merck Ltd. (India). The topical hydrogel known as "Megaheal" was acquired from Aristo Pharmaceutical Pvt. Ltd. in India. The remaining chemicals came from RANKEM Chemicals in India and were of analytical quality.

Preparation of Topical Gel

The bioactive fraction (F4) (0.5%w/w) of MBR was utilized for formulation of hydrogels using different concentrations of polymer (carbopol 940) and the formulation have been detailed in Table.

Table -2 Composition of the gel formulations prepared with MEBL

Formulat-ions	Bioactivefraction - (mg)	Carbpol 940 (mg)	Methylparaben (mg)	Propylparaben(mg)	Propyleneglycol (ml)	Triethanolamine (ml)	Purified water (ml)
S1	250	300	25	5	10	Up to 2.5	Q.S up to
		(0.3%)					50
S2	250	400	25	5	10	Up to 2.5	Q.S up to
		(0.4%)					50
S3	250	500	25	5	10	Up to 2.5	Q.S up to

		(0.5%)					50
S4	250	600	25	5	10	Up to 2.5	Q.S up to
		(0.6%)					50
S5	250	800	25	5	10	Up to 2.5	Q.S up to
		(0.8%)					50

Method for preparation of gel containing active fraction of extract

Carbopol 940, propylene glycol 400, methylparaben, propyl paraben, tri-ethanolamine, and distilled water were used in sufficient amounts to create 50g of gel. Two portions of water were needed for these formulas. In one section, precisely 250 mg of bioactive fraction F4 was dissolved, and then determined amounts of ethanol and propylene glycol-400 were added. In another section, carbopol-940 was dissolved, and then methylparaben and propylparaben were added. To achieve the required pH (6.8–7.0) and gel consistency, each of these solutions were combined in beakers and triethanolamine was added dropwise (Kaur et al., 2010, Pawar and Shamkuwar, 2013).

HPLC Analysis of MBR

The Pallaufa et al. (2008) method was used for the HPLC analysis of MBR. The most popular techniques for identifying the phenolic and flavonoid structures found in samples of methanolic bark extract rely on reversed phase high-performance liquid chromatography (RP-HPLC) with C18-columns as the stationary phase connected to an ultraviolet–visible (UV–Vis) detection system. Using a UV detector set to 280 nm, a 6:4 water/acetonitrile combination was used as the mobile phase at a flow rate of 1 milliliter per minute at 30 degrees Celsius.

Table no 3 :HPLC analysis of MBR

Parameter	Condition
Column	C18 column (150 × 4.6 mm, 5 µm particle size)
Mobile Phase	Solvent A: Water; Solvent B: Acetonitrile (A:B = 6:4)
Flow Rate	1 mL/min
Detection Wavelength	280 nm
Gradient Programme	Solvent A (0–5 min); Solvent A : Solvent B (5–55 min)
Sample Preparation	1 mg/mL solution

DPPH radical scavenging activity

Using a slightly modified version of Lee et al.'s (2003) 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test, the free radical scavenging ability of MBR was assessed in vitro. In short, 3 ml of 10µg/ml and 50µg/ml of MBR and ascorbic acid (standard) solution were combined with 1 ml of 0.3 mM DPPH solution (in 100% ethanol). A spectrophotometer (UV-1800, Shimadzu-UV spectrophotometer) was used to measure the absorbance at 517 nm after the mixture was agitated and left to remain at room temperature for 30 minutes. The assay had corresponding blanks. At 517 nm, the absorbance of DPPH was measured as a control. Higher radical scavenging activity was shown by the reaction mixture's lower absorbance. The following formula was used to calculate the scavenging effect (%):

$$\text{Scavenging Activity } (\%) = \frac{\text{Abs}_{Control} - \text{Abs}_{Test}}{\text{Abs}_{Control}} \times 100$$

Estimation of antibacterial activity of MBR

Strains of Bacteria

Both gram-positive (S. aureus MTCC 96 and B. subtilis MTCC 441) and gram-negative (E. coli MTCC 2939 and P. aeruginosa MTCC 2453) bacteria were employed as test microorganisms in this investigation. The organisms were cultivated for 18 to 24 hours at 37°C in brand-new nutrient agar (NA) plates. Single colonies from the NA plates were utilized to inoculate a suitable medium (5 ml) for antimicrobial testing.

Assay for Antimicrobials

This experiment was carried out using Andrews' (2001) methodology. The broth microdilution method was used to calculate MIC and MBC. In short, twofold serially diluted MBR was introduced to the suitable growth medium along with a standardized inoculum (10 µ l of a 1–5×105 CFU/ml suspension). The negative control was DMSO (0.1% v/v). The optical density was measured at 590 nm (UV-1800, Shimadzu-UV spectrophotometer) following a 16-hour incubation period. The MIC (minimum inhibitory concentration) was defined as the lowest concentration of the material that completely inhibited bacterial growth.The broth from the several clear microplate wells was spread out on nutritional agar

plates and incubated at 37°C for 24 hours in order to determine the minimum bactericidal concentration (MBC). The MBC was defined as the lowest sample concentration at which the visible bacterial colony was decreased to 99.9%.

Research on antibiofilms

MBR's impact on the development of biofilms

This experiment was carried out using the Pompilio et al. (2011) approach. Broth (MHB) with and without MBR (treated and non-treated controls). The positive controls were gentamicin and ciprofloxacin. Every test agent was examined at sub-lethal concentrations (1/2×, 1/4×, and 1/8×MIC). The non-adherent bacteria were eliminated from the plates by washing them with PBS (pH 7.2) after 16 hours of incubation. After fixing the biofilm (slime and adherent cells) for 30 minutes at 60°C, the cells were stained with 150 µ l of 1% crystal violet solution for 5 minutes at room temperature.

In-vivo research

Models of animals

The study used male albino Swiss mice of either sex that weighed between 23 and 26 grams. The Birla Institute of Technology, Mesra's Laboratory Animal House provided the animals (Reg. no.: 621/02/ac/CPCSEA). The institutional animal ethics committee's consent was rigorously adhered to in all animal studies (Reg. no.: 621/02/ac/CPCSEA). The animals were housed in polyacrylic cages with 12-hour light-dark cycles and conventional housing conditions of temperature (24–27°C) and humidity (60–65%). Before the experiment, they were acclimated to the lab setting. Dry pellets were used as food, and water was freely available.

Studies on acute toxicity

According to Adeneye et al. (2006), the acute toxicity investigations were conducted in accordance with OECD guidelines-425. MBR at 1500 mg/kg was administered orally by gavage to mice of either sex (three females and three males, weight: 24–35 gm, age: 6–8 weeks). The animals were observed for signs of toxicity. After 24 hours, the number of survival was recorded, and the animals were subsequently monitored for an additional 13 days.

Acute toxicity to the skin

According to OECD recommendations (1987), the study was conducted to investigate the skin irritation caused by MBR (ointment). The mice's shaved dorsal area was treated with an ointment containing 5% (w/w) MBR in order to investigate the acute skin toxicity of the substance.

Assessment of MBR analgesic research

Test for writhing caused by acetic acid

The procedure outlined by Koster et al. (1959) was followed in conducting this experiment. Five groups of mice were given either the usual medication Aspirin (100 mg/kg) or the control vehicle MBR (200 and 400 mg/kg) intraperitoneally (i.p.). Thirty minutes later, each animal received an injection of 1% (v/v) acetic acid (10 ml/kg, i.p.). Each animal's number of writhing reactions was counted throughout a 15-minute period. Positive analgesic responses were defined as a significant decrease in writhing reaction relative to control.

Assessment of MBR's anti-inflammatory research

Rat paw edema produced by carrageenan

Rats with carrageenan-induced paw oedema were used to test MBR's anti-inflammatory properties (Winter et al., 1962). Three groups of five experimental rats weighing between 100 and 120 grams each were created. Thirty minutes before the injection of carrageenan, the control vehicle (normal saline), MBR (200 mg/kg and 400 mg/kg), and standard medication (diclofenac sodium 10 mg/kg) were given intraperitoneally (i.p.) to several groups. Each rat received an intradermal injection (0.1 ml/rat) of carrageenan (Type I; 1% w/v in normal saline) at the plantar side of its right hind paw.

Animal grouping

Following the production of wounds, the experimental animals were split up into four groups, each with five male mice.

Table 4 - Grouping of animals

Group	Treatment
Group A	Control (Ointment base)
Group B	5% w/w plant extract ointment
Group C	10% w/w plant extract ointment
Group D	Standard drug – Framycetin (topical application)

Histological evaluation

The procedure of Lee (1968) and Sadaf et al. (2006) was followed in the histopathological investigation. On the eleventh day, skin samples from each mouse's various wounds were taken. The samples were processed, blocked with paraffin, and fixed in 10% neutral buffered formalin. Hematoxylin and eosin was used to stain five micrometer slices. An area below the epidermis in each skin part was chosen at random. Under a 400x magnification microscope, the tissue was qualitatively evaluated, and many aspects of wound healing were examined.

In vitro investigations

Assays for Antioxidants

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test was used to measure MBR's antioxidant activity. The outcomes are shown below.

DPPH radical scavenging test

MBR generated a dose-dependent scavenging activity in the DPPH radical scavenging assay model, which was seen at 30 and 60 minutes, respectively. At the various time periods, it was discovered that ascorbic acid (standard) had a greater scavenging activity than MBR.

Table 5 DPPH radical scavenging assay of MBR

Drug	Concentration (µg/mL)	% Scavenging (Mean ± SEM)	
		at 30 min	60 min
MBR	10	34.17 ± 2.6	36.17 ± 2.1
MBR	50	43.35 ± 2.8	48.36 ± 2.7
Ascorbic Acid	10	97.30 ± 2.03	97.03 ± 3.2
Ascorbic Acid	50	97.67 ± 3.1	97.31 ± 2.4

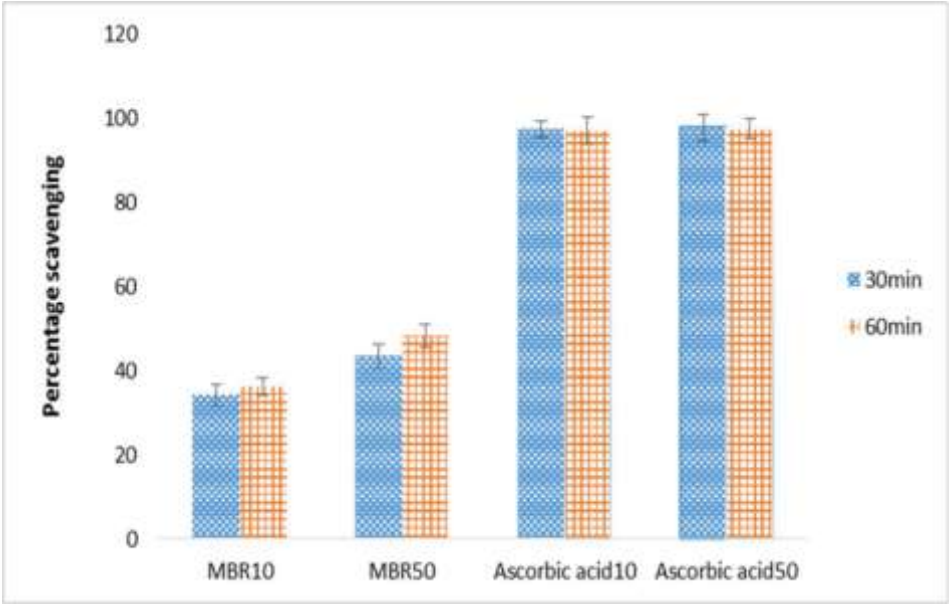


Fig. 4DPPH radical scavenging assay of MBR .Values are expressed as mean ± S.E.M; (n=3).

Evaluation of MBR's antibacterial activity

The broth microdilution method and the antibiofilm assay (biofilm generation and destruction of preformed biofilm) were used to assess the antibacterial efficacy of MBR. The checker board microdilution experiment was used to investigate the synergistic activity of MBR. The outcomes are shown below.

MBR antimicrobial screening

The microdilution method was used to investigate the antibacterial properties of MBR. Several strains of gram positive (S. aureus; MIC 0.625 mg/ml; B. subtilis; MIC 0.625 mg/ml) and gram negative (E. coli; MIC 1.25 mg/ml; P. aeruginosa; MIC 0.625 mg/ml) bacteria were discovered to be susceptible to MBR. Against the various bacterial strains, MBR showed bactericidal action.

Table 6 MIC and MBC determination of the MBR and its comparison with standard antibiotics against the bacterial strains

Organism	MBR		Ciprofloxacin		Gentamicin	
	MIC (mg/ml)	MBC (mg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
S. aureus	0.625	0.625	0.125	0.25	0.250	0.500
B. subtilis	0.625	1.250	0.250	0.50	0.125	0.250
E. coli	1.250	2.500	0.250	0.50	0.125	0.125
P. aeruginosa	0.625	1.250	0.125	0.25	0.062	0.250

Antibiofilm studies

Effect of MBR on biofilm formation

MBR's ability to prevent gram-negative bacteria (E. Coli, P. aeruginosa) from forming biofilms was assessed. A concentration-dependent decrease in biofilm development was shown by the experiment (Fig. 3). E. coli biofilm formation was inhibited by MBR at concentrations of 1/2, 1/4, and 1/8 MIC (62.18%±5.23%, 33.16%±3.84%, and 14.56%±1.82%, respectively). In contrast, MBR at concentrations of 1/2 and 1/4 MIC showed comparable activity against P.aeruginosa, with an extent of 44.18% ±2.07% and 17.4% ±1.05%, respectively. When compared to MBR, ciprofloxacin showed superior inhibitory action (1/2MIC-86.15% ± 2.88%; 1/4MIC -58.67% ± 3.41%; and 1/8MIC -28.60 ± 1.38%), while the standard medication gentamicin was shown to be ineffective in inhibiting biofilm formation.

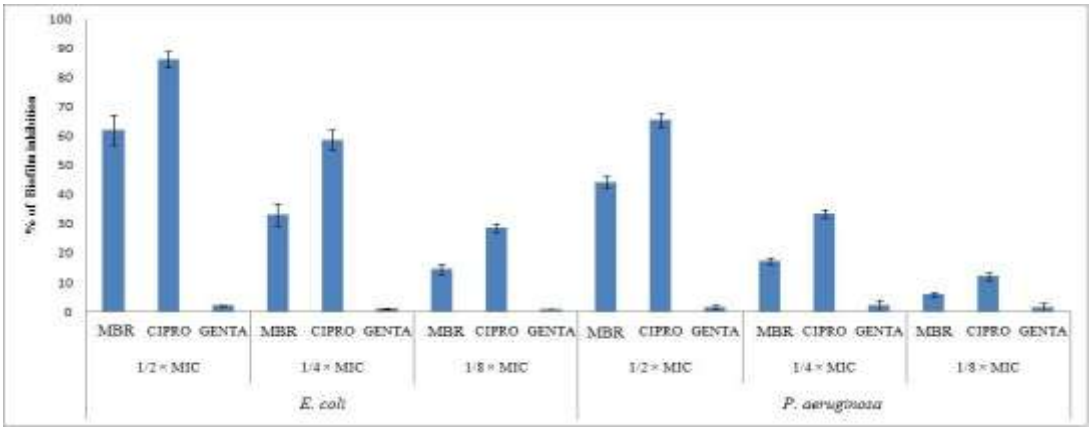


Fig. 5 Effect of MBR on biofilm formation (*E.coli* and *P.aeuriginosa*).

Effect of MBR on preformed biofilm

Additionally, it was discovered that MBR was highly successful in breaking up the preformed biofilms that certain Gram-negative bacteria (such as P. aeruginosa and E. coli) created. When MBR (1×, 5×, and 10× MIC) was applied to mature biofilms (E. coli), the viable cell count decreased by 48.14%, 65.55%, and 79.81%, respectively. By contrast, the viable cell count was reduced by 32.73% and 93.22%, respectively, by gentamicin (10× MIC) and ciprofloxacin (10× MIC).

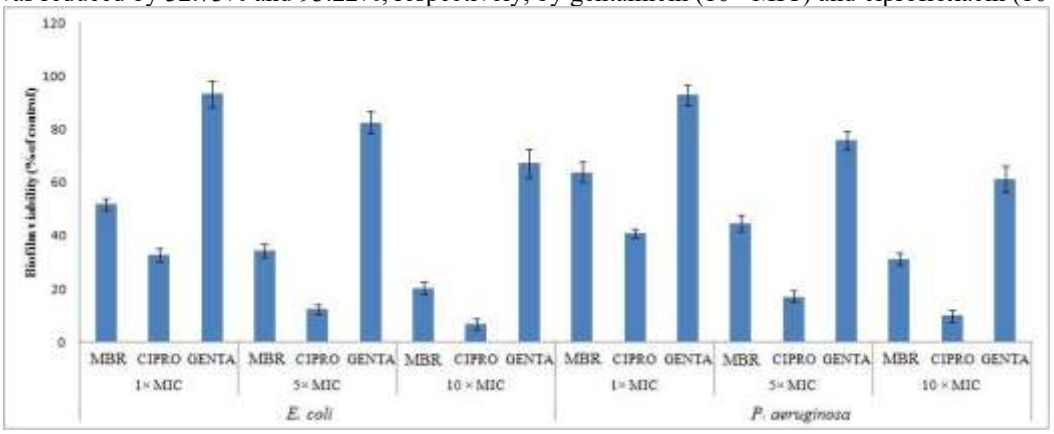


Fig . 6 Effect of MBR on preformed biofilm formation (*E.coli* and *P.aeuriginosa*) .

Effect of MBR on Synergistic study

When MBR was combined with other antibiotics (Amikacin, Gentamycin, Ciprofloxacin, and Polymyxin B), the checker board microtiter plate assay demonstrated a synergistic effect against S. aureus (FIC<0.5).MBR showed additive activity ($5 < \sum FIC \leq 1$) when combined with ampicillin and tetracycline in the instance of E. coli (Table 4.4); however, MBR was found to have a synergistic impact when combined with other antibiotics.

Table no 7 :*In vitro* interaction between MBR and different antibiotics (determined by FIC1)

Name of the antibiotics	∑FIC (Interpretation)	
	S.aureus	***E.coli***
Amikacin	0.5 (SYN)	0.43 (SYN)
Gentamycin	0.337 (SYN)	0.387 (SYN)
Ciprofloxacin	0.186 (SYN)	0.46 (SYN)
Ampicillin	0.236 (SYN)	0.80 (ADD)
Tetracycline	0.30 (SYN)	0.89 (ADD)
Polymyxin B	0.38 (SYN)	0.141 (SYN)

SYN = synergy: Synergy was defined as an FIC1< 0.5; ADD= Additive: $5 < \sum FIC \leq 1$

***In-vivo* studies**

Studies on acute toxicity

The animals were determined to be normal even after a 24-hour observation period, and the MBR was found to be safe up to 1500 mg/kg (p.o.). The animals' attentiveness, sedation, urination, convulsions, and locomotion were all found to be unaffected, and there were neither any deaths nor any indications of toxicity.

Acute toxicity to the skin

MBR ointment did not cause any kind of irritation in the skin irritation trial, and the area of skin exposed to MBR did not exhibit any discernible inflammation.

MBR's analgesic effects on mice's writhing reflex generated by acetic acid.

When compared to the control, the analgesic investigation showed that MBR significantly inhibited writhing caused by acetic acid at both doses. Nevertheless, MBR (400 mg/kg) showed more analgesic activity (64.81%) than MBR (200 mg/kg) (42.59%).

Table 8 Analgesic activity of MBR by acetic acid-induced writhing response in mice

Treatment group	Dose(mg/kg)	Number of writhings (mean ± S.E.M)	Inhibition (%)
Control	NS	54±0.708	
MBR	200	31±0.71*	42.59
MBR	400	19±0.55**	64.81
Aspirin	100	9±1.13**	83.33

Values are expressed as mean ± S.E.M; (n=5). *p<0.05, **p<0.01 (Vs. control), NS-Normal Saline.

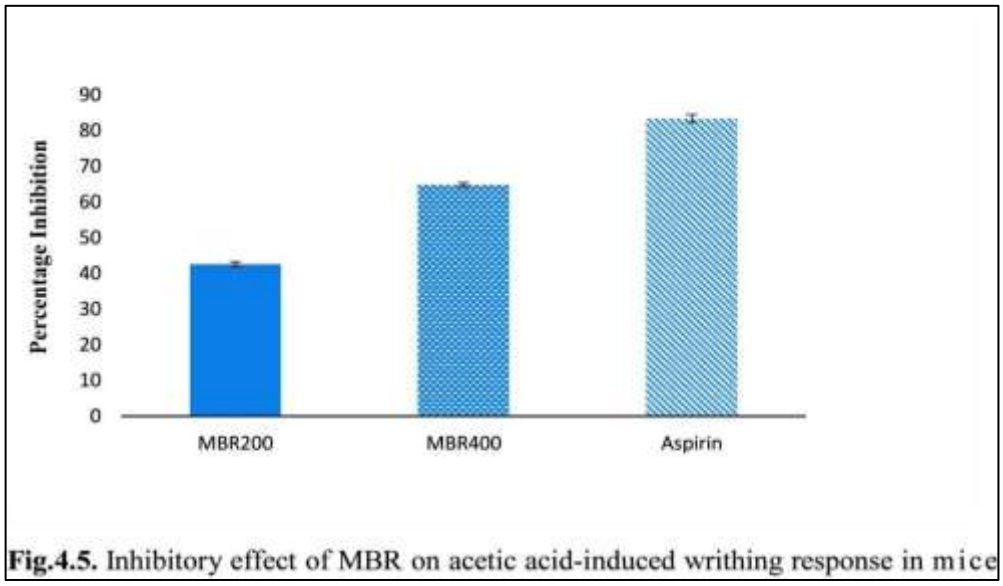


Fig no 7 :Inhibitory effects of MBR on acetic acid induced writhhing response in mice

Anti-inflammatory activity of MBR

When MBR's anti-inflammatory qualities were assessed, it was shown that oral MBR administration resulted in a dose-dependent reduction of rat paw oedema caused by carrageenan. At a dosage of 400 mg/kg, MBR exhibited an early start (30 minutes) and shown notable anti-inflammatory action for up to three hours. In contrast, MBR (200 mg/kg) showed noticeable inhibitory effect only in the second and third hours, respectively (Table 4.6, Fig. 4.6).

Table 9 Anti-inflammatory activity of MBR against carrageenan induced rat paw oedema.

S. No	Group	Dose (mg/kg)	Reduction of paw volume (ml) mean ± SEM (Percentage Inhibition)			
			30 min (Mean ± SEM)	1 hr (Mean ± SEM)	2 hr (Mean ± SEM)	3 hr (Mean ± SEM)
1	Control (N.S.)	–	0.36 ± 0.071	0.71 ± 0.08	0.74 ± 0.04	0.62 ± 0.03
2	MBR	200	0.31 ± 0.05 (13.8)	0.59 ± 0.03 (16.9)	0.52 ± 0.05* (29.72)	0.41 ± 0.041* (33.8)
3	MBR	400	0.26 ± 0.091* (27.7)	0.47 ± 0.06* (33.8)	0.41 ± 0.02* (44.5)	0.32 ± 0.025* (48.38)
4	Diclofenac Sodium	10	0.21 ± 0.03* (41.6)	0.25 ± 0.04** (64.78)	0.19 ± 0.04** (74.3)	0.11 ± 0.028** (82.25)

Values are expressed as mean ± S.E.M; (n=5). *p<0.05, **p<0.01 (Vs. control). NS- Normal Saline

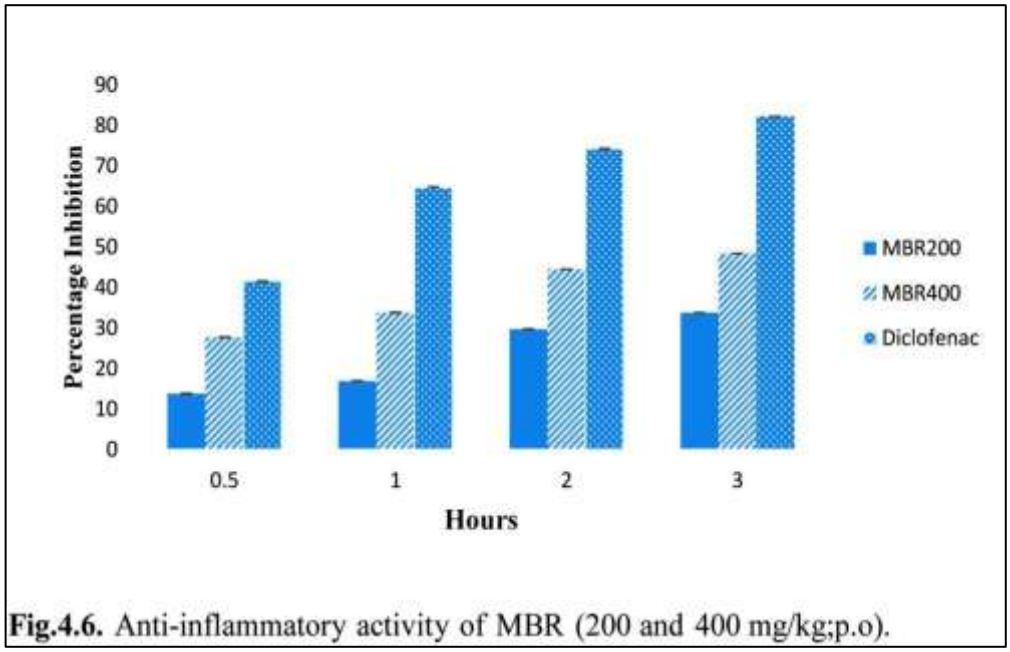


Fig.4.6. Anti-inflammatory activity of MBR (200 and 400 mg/kg;p.o).

Fig no 8 :Antiinflammatory activity of MBR (200 and 400 mg/kg:PO)

Impact of MBR topical treatment on mouse excision wounds

When compared to the control, topical treatment of MBR (10% w/w) resulted in a substantial increase in wound contraction on days nine, twelve, and fifteen.Nevertheless, there was no discernible wound contraction activity in MBR (5%w/w). When compared to control, framycetin (1%) significantly increased wound contraction from day six to day fifteen.

DISCUSSION

Several in vitro and in vivo test methods were employed to examine Buchanania Lanzan (BL), a traditional medicinal plant used in Indian folk medicine, in order to comprehend the pharmacological characteristics of the plant extract (MBR). Different types of inflammatory cells, including neutrophils, monocytes, and lymphocytes, produce a variety of substances as soon as the skin is wounded. The inflammatory process has been connected to wound healing difficulties in the majority of cases. Reactive oxygen species, including superoxide radical, hydroxyl radical, and non-radical hydrogen peroxide, are constantly produced as a result of inflammation (Kung et al., 2008). Reduced collagen synthesis and delayed excisional and incisional wound healing may result from elevated oxidative stress during inflammation (Kanta, 2011). It is also known that anti-inflammatory drugs speed up the healing of wounds. Numerous investigations on the in vitro and in vivo anti-inflammatory properties of Sambucusebulus leaves have been documented in the literature (Yesilada, 1997). Additionally, Ahmadiani et al. (1998) hypothesized that the plant's flavonoid concentration may be connected to Sambucusebulus's anti-inflammatory and wound-healing properties. Rat paw edema caused by carrageenan was inhibited by MBR in a dose-dependent manner. The current study offers more proof of MBR's anti-inflammatory properties. According to the paper, flavonoids are well-known potent antioxidant chemicals that inhibit lipid peroxidation, preventing cell damage and increasing collagen fibril viability (Getie et al., 2002; Shetty et al., 2008). In addition to the aforementioned natural compounds, Sussman (2007) reported that NSAIDs (non-steroidal anti-inflammatory drugs), ibuprofen, colchicine, corticosteroids, and antiplatelets (aspirin) are some of the widely accessible synthetic medications utilized in wound healing.

The use of antioxidants may partially aid in the healing of ischemic skin wounds because oxygen free radicals created during injury play a significant role in ischemia injury and exacerbate ischemic wounds (Senel et al., 1997). Antioxidants protect the tissue against the harmful effects of ROS (reactive oxygen species) and proteases, which are known to be linked to neutrophil increase in the wound region (Houghton et al., 2005). It has been demonstrated that flavonoids, a class of naturally occurring benzo pyrone derivatives, have a number of biological characteristics, such as hepatoprotective, antithrombotic, anti-inflammatory, and antiviral activities, many of which may be connected, at least in part, to their capacity to scavenge free radicals and act as antioxidants (Chen et al., 1990). Because of their ability to scavenge free radicals, flavonoid-rich natural products have reportedly been shown to lessen oxidative stress, which in turn reduces lipid peroxidation and cell death. Additionally, flavonoids' chelating qualities aid in the elimination of divently metal ions, which are believed to contribute to the production of hydroxyl radicals.

Consequently, the in vitro DPPH model was used to investigate MBR's antioxidant properties. The DPPH scavenging assay demonstrated a dose-dependent scavenging activity of MBR in our in vitro antioxidant experiments (34.17% and 43.35% at dosages of 10 and 50 µ g/ml, respectively).

The interruption of a tissue's normal anatomical structure and function is referred to as a wound. Inflammation, proliferation, and remodeling are the three phases of the dynamic, interactive activities that characterize the orderly and timely healing process of wounds (Singer and Clark, 1999). In vitro and in vivo test models can be used to evaluate the therapeutic benefits of natural compounds.

Based on in vivo wound models, the current study proved that MBR has the ability to repair wounds. When applied topically, MBR (10% w/w) significantly increased wound contraction on days 9, 12, and 15 compared to the control. Additionally, there was proof that MBR (10% w/w) significantly improved its ability to contract wounds through tensile strength. These results were corroborated by histopathology observations, which showed improvements in tissue regeneration, tensile strength, and epithelialization for MBR. Literature makes it clear that a number of natural products made of active ingredients such alkaloids, flavonoids, and triterpenes can aid in the healing of wounds (Sumitra et al., 2005). Proanthocyanidins and reseveratrol from Vitisvinifera (Khanna et al., 2002), quercetin, isorhamnetin, and kaempferol from Hippophaer hamnoides (Fu et al., 2005), β-sitosterol (Krishnan, 2006) from Aloe vera gel, and Asiaticoside from Centella asiatica (Shukla et al., 1999).

Procyanidin-B2 from CoMBRetumu cronatum (Kisseih et al., 2015) and acylated iridoid glycosides from Scrophulari anodosa (Stevenson et al., 2002) are two other significant plant-derived compounds that have been shown to exhibit wound healing properties, as demonstrated by numerous in vitro and in vivo investigations.

Microorganisms are known to obstruct the natural healing process of wounds, according to several published investigations. According to published reviews, an investigation of 584 wounds (from various places) revealed that the abundance of Staphylococcal and Clostiridium species has been confirmed (Brook and Randolph, 1981). Wound infections are frequently reported to be poly-microbial in nature. According to the findings that are now accessible, it is widely known that bacteria have a detrimental effect on the healing process of wounds. As a result, the antibacterial qualities of the methanolic extract were also assessed. In our study, MBR was discovered to be effective against both Gram-positive (S. aureus and B. subtilis) and Gram-negative (E. coli and P. aeruginosa) bacteria. We also found that MBR was more effective against S. aureus (0.625 mg/ml).

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