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A STUDY ON TRADITIONAL DICOTS AS COMBAT TO WOUND MICROBIAL CLUSTERS

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ABSTRACT: Antibiotic resistance emergence, as well as the Darwinism of up to the minute strains of ailment generating agents, is of grave concern to the global health community. Sustainable therapeutics of an ailment entails the amalgamation of contemporary pharmaceuticals or some potential prospective of novel drugs. Customarily employed medicative plants of Gopalganj community could be an enticing of drugs to fight this problem. This study is targeted at a reconnaissance of the antimicrobial properties of the plants that are customarily being used as traditional health implementation. The antimicrobial potential of five different plant extracts was screened against three pathogenic microorganisms, which were isolated from typical non-healing wounds of patients of Gopalganj district hospital. The microorganisms chosen to evaluate for antimicrobial activity were *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and Staphylococcus aureus. The favorable activity was shown by the extracts of *Tridex procumbens* L. was found to highest with zone of Inhibitions reaching to 18 mm, 19 mm and 20 they were nearly around the zone of inhibitions of positive control.

INTRODUCTION: Wounds can be broadly categorized as having either an acute or a chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasions, and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries [1]. Next-generation sequencing methods have allowed us to link alterations in the human body microbiome to the pathogenesis of autoimmune, metabolic, and atopic diseases [2, 3]. Despite the fact that similar proportions of the host to microbial cells exist on the cutaneous microbiome [4].

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The impact of the cutaneous microbiome on acute and chronic wound healing is less defined. Both *in-vivo* and *in-vitro* studies of the cutaneous microbiome have supported a general consensus that the microbial composition of skin wounds impacts wound healing. However, the conclusions drawn from these studies have been supporting that absence of microbiota initially could. Canesso *et al*. demonstrated that in the absence of commensal skin microbiota, Swiss mice demonstrated accelerated wound closure and epithelization with a significantly altered wound leukocyte profile [5].

In-vitro several studies have been made which to study antimicrobial efficacy of traditional medicinal plants and their different Phyto-constituents, which are responsible for combating microorganisms. Microflora has effectively targeted wounds [6], regardless of the idea of the cutaneous damage, intense injuries are relied upon to mend inside an anticipated time span, despite the

fact that the treatment required to encourage recuperating will shift as per the sort, site and profundity of an injury. The essential conclusion of a perfect, careful injury would be relied upon to require insignificant mediation to empower mending to advance normally and rapidly. In any case, in an increasingly serious horrible damage, for example, a consumed wound or shot injury, the nearness of devitalized tissue and pollution with feasible (*e.g*., bacterial) and nonviable outside material is probably going to require careful debridement and antimicrobial treatment to empower mending to advance through a characteristic arrangement of procedures, including aggravation and granulation, to definite epithelialization and rebuilding [1].

Thus, in light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [7]. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, the emergence and multiplication of multidrug-resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria [8].

MATERIALS AND METHODOLOGY:

Procurement of Samples: The samples were procured from the Hathwa region of Gopalganj and all the plants were identified by the Botanist team of Jai Prakash University (Bihar).Plants selected for the pharmacological studies we recollected from their natural habitat. The chosen plants are Acalypha indicaL., Emblica officinalis Gaertn.

Tridex procumbens L. Medicinally useful parts of the selected plants specimens such as tender leaves of *Acalypha indica* L., *Emblica officinalis* Gaertn and *Tridex procumbens* L. were washed with tap water, and then with distilled water. Cleaned plant specimens were then shade dried. Dried materials were powdered. Powdered materials were then sieved. 50 grams of sieved powder was packed inside filter paper in the Soxhlet tube and introduced in the extraction unit of Soxhlet extractor, and extraction was done with methanol and distilled water (30:70). The extracts were filtered by using Whattman filter paper No. 42, size 125 mm, and concentrated with a rotary evaporator. The concentrated product was then dried on a water bath as described by [9].

Test Microorganism: The organisms mentioned below of micro sizes were brought into the frame for the study for the screening of antibacterial activity. All the samples were tested against these [4] organisms throughout this study

Gram +ve Organisms:

- *Staphylococcus aureus* (MTCC3160)
- *Proteus mirabilis* (MTCC425)

Gram -ve organisms:

- *Escherichia coli* (MTCC40)
- *Pseudomonas aeruginosa* (MTCC 741)

The microorganisms were chosen on the basis of Literature studies, and they were procured from MTCC Chandigarh. For the sustenance of culture, they were maintained on Nutrient Agar media (M087) (HiMedia).

Justification for choosing of microorganism lies in that the above-mentioned microorganisms have proven to be an intricate part of the wound. Their presence worsens the wound milieu; therefore, they have been targeted. Their absence in wounds could really allow subcutaneous tissues to perform their action and heal the wound easily.

Preparation of Inoculums: A loop full of isolated colonies was inoculated into 4 ml of peptone water and incubated at 37 ºC for 4 h. This actively growing bacterial suspension was adjusted with peptone 78 water so as to obtain turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) sulphuric acid (H_2SO_4). This turbidity is equivalent to approximately 1 to 2 × 108 colony forming units per ml (CFU/ml). The inoculums thus prepared were used further for antibacterial sensitivity testing [10].

Standard and Sample Preparations to Quantify Different Antimicrobial Bioactive Components Present in Extracts:

Standard and Sample Preparation: Stigmasterol Quantitation in *Acalypha Indical* L: The stigmasterol standard (100 mg) was explicitly measured and drawn out with methanol (30 ml) at 45° for 12-16 h, the solvent being refreshed at the end of 4 h. Pooled extricates condensed on a rotary vacuum evaporator and further dried using an oven at 80°. The yield of extract was 5.25% w/w. Stock solutions of the reference compounds (1000 µg/ml) and test extract (5000 µg/ml) were prepared separately by dissolving them in methanol, and further dilutions were prepared from these stock solutions. The standard stock and working solutions were all prepared in calibrated flasks. The sample was prepared accordingly; already prepared extract of *Acalypha indica* L. was dried and made powder through Rota- evaporator and then was run in HPLC (Shimadzu VP 1606). The flow rate was 2.2 ml/min. The wavelength to identify Stigma sterol is 207 nm.

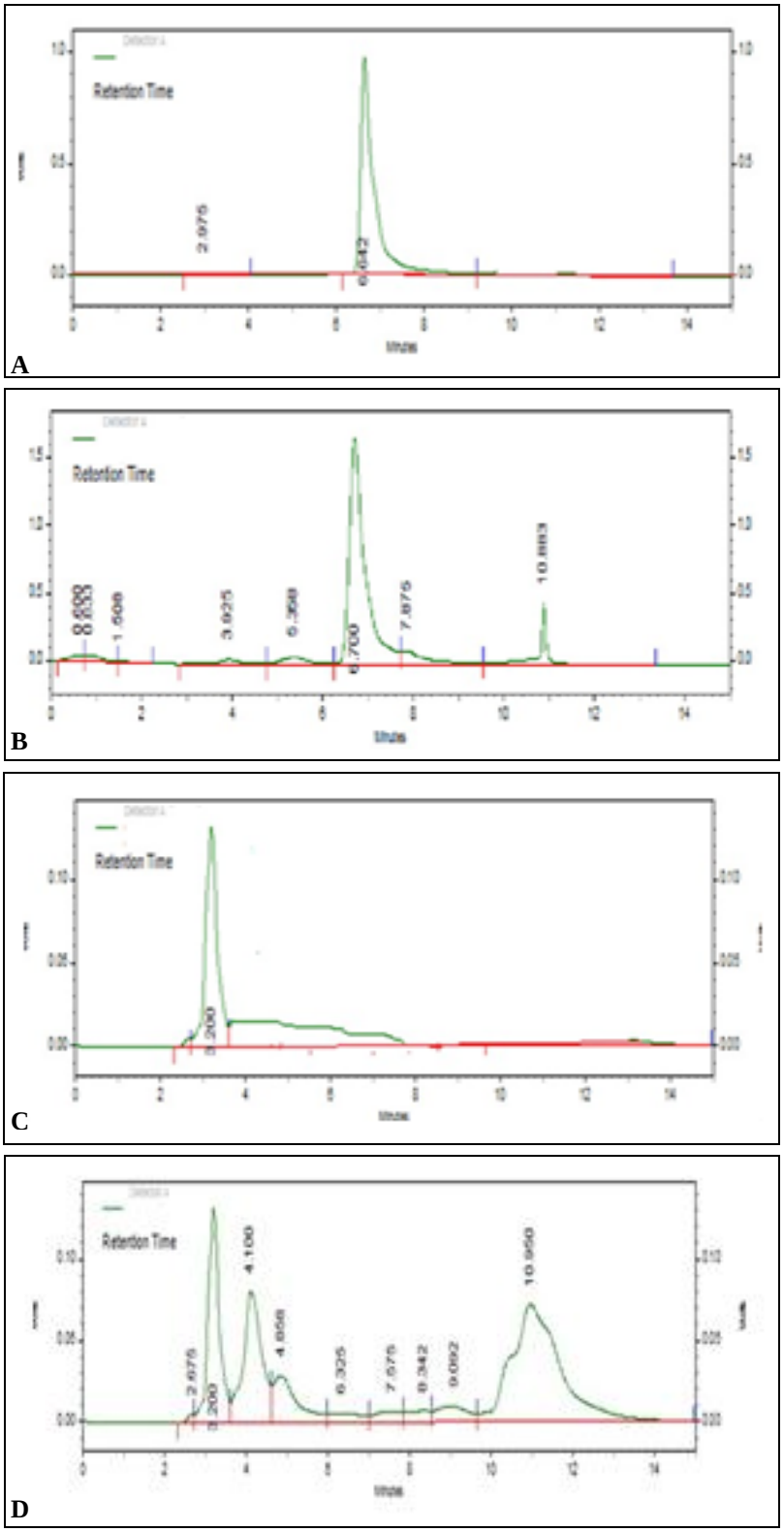
Standard Preparation of Daucosterol *Forem-blica Officinalisgaert:* Daucosterol (550 µg/mL) were prepared in 2% Dimethyl Sulfoxide in methanol, respectively, and stored below 4 ºC. The working standard solutions were prepared by appropriate dilution of stock solutions with methanol. These diluted working solutions were used for the establishment of calibration curves [11]. The extract already prepared by Soxhlet extrication was brought into use in this. The solvent was steamed out with the help of common Rota-evaporation viscous content and was brought to powdery form freeze-drying.

The dried sample was mixed in 4 mL 2% Dimethyl sulfoxide in methanol to bring uniformity. All sample solutions were filtered through a 0.45 µm membrane filter before HPLC analysis. Since, all the standards brought in implications were his mobile phase was composed of 0.1% TFA aqueous solution initial and ACN -final at a rated flow of 1.5 microlitres per every 60 seconds. The HPLC run was performed for 20 minutes. The volume of sample injected in HPLC comprised of 20 micro litres this wavelength was determined after standardization of the procedure at Double beam Spectrophotometer (Systronics Double beam spectrophotometer 2202). Beta sitosterol standard preparation and run in HPLC:

Run Conditions for *Tridex procumbens* and its Standard Stigmasterol: The beta sitosterol was prepared same as Stigmasterol. The HPLC analysis of *Tridex procumbens* L. included freeze drying of the extract obtained from the Soxhlet extraction and then further it was brought under scan at the wavelength of 206 nm.

Antimicrobial Screening: The antibacterial activities of the extracts were determined using the Agar well diffusion method. The bacteria used in the study were two-gram positive strains *i.e*., *Staphylococcus aureus* MTCC 3160 and Proteus mirabilis MTCC 425, and two gram-negative strains *i.e*., Escherichia coli MTCC 40 and *Pseudomonas aeruginosa* MTCC 741. The inoculums size of the test strain was standardized according to the Clinical and Laboratory Standards Institute guidelines [12]. One ml volume of the standard suspension of bacterial test strain was spread evenly on Mueller Hinton Agar (HiMedia M173) plate using a sterile cotton swab. The plates were allowed to dry at room temperature. Four wells with the size of 6 mm in each plate were aseptically punched in the agar with a sterile cork borer (6 mm diameter). Ethanolic extracts of four plant extracts were applied to the wells as 10, 20, 30, and 40 µl of 100 mg/ml of stock solution. Gentamicin (HiMedia, CMS461) (1 mg/ml) 40 µl was used as a positive experimental control, and 10% DMSO was used as a negative control. Plates were incubated at 37 °C for 48 h. Triplicate plates were maintained for each organism. At the end of the incubation period, the diameter of zone of inhibition was measured in millimeters. Each experiment was carried out in 3 replicates and the mean diameter of the inhibition zone of bacterial growth was recorded in mm. Zone sizes were recorded on the recording sheet. The three separate measurements from each plate of three test plates. Calculated the average and the standard deviation of the diameter of the zone of inhibition (ZOI) for each sample [13]. ZOI re-presented in terms of susceptible (S), intermediate (I), or resistant (R) based on the interpretation chart. Antimicrobial activity of this combination is carried out, and the results are noted in tabular form as well as graphical form.

RESULTS AND DISCUSSION: The results discussed in this writing regards the antimicrobial activity of plant extracts; the microbes mentioned in this study have been extensively observed while calculating the total microbial load on the wounds of the plants mentioned in the research works [15]. In extensive swill of Literatures, it was observed that phytosterols possess the property for inhibition of microbes. All three plants showed the superb presence of phytosterols such as Stigmasterol, Beta-sitosterol, and Daucosterol.



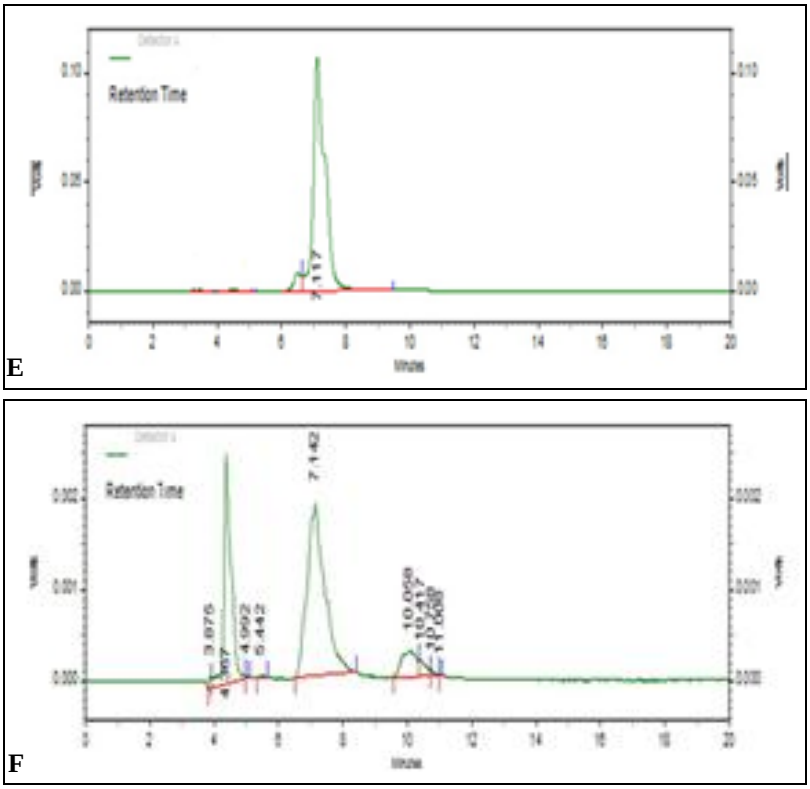


FIG. 1: (A) CHROMATOGRAM OF SIGMASTROL; (B) CHROMATOGRAM OF METHANOL EXTRACT OF *ACLYPHA INDICA* (C) CHROMATOGRAM OF PHYTOSTEROLS- DAUCOSTEROL (D) THE NEXT CHROMATOGRAM IS OF PLANT EXTRACT *EMBLICA OFFIINALIS* GAERTN, (E) CHROMATOGRAM OF BETA SITOSTEROL (F) CHROMATOGRAM REPRESENT METHENOLIC EXTRACT OF *TRIDEX PROCUMBENS* L.

The first chromatogram of the analysis is for Sigmasterol standard it is a phytosterols abundant in quantity in the plant sample of *Acalypha indica* retention time of Sigmasterol was observed to be 6.642 min. The next chromatogram represented is about the methanolic extract of *Acalypha indica* L. the peak at 6.642 min represented the dominant presence of Sigmasterol; when quantitated it was found to be 4.32% w/v. The next slot of Chromatogram is for Daucosterol when compared with the extract of *Emblica offiinalis* Gaertn in the standard single purified peak was observed at 3.20 minutes. Varied peaks were obtained while running the methanolic extract of *Emblica offiinalis Gaertn.* Confirming the presence of daucosterol in the sample it was obtained in 9.8 % w/v.

The third slot represented the chromatogram for Beta- sitosterol in the extracts of *Tridex procumbens* L. the presence of it was observed as 7. 9% / v. Out of the three extracts, the dominant one possessing phytosterols in larger amounts was *Tridex procumbens* L. Similar, amounts of Daucosterol were found in a work of Ivanescu [16.] *Emblica offiinalis* Gaertn had the highest content of Daucosterol in the other species he chosen. *A. annua* L. contained amounts of stigmasterol and β-sitosterol (119.5 mg/100 g DW), and free stigmasterol was found in larger quantities than free β-sitosterol in this plant, reported [17]. *Emblica officinalis* has been extensively studied as for its estimation in Vitamin C content [1]. Suitable content of phytosterols was observed in the extracts of *Emblica officinalis, Acalypha indica* L., and *Tridex procumbens* L.

Antibacterial Assay: The leaves extract of *Acalypha indica* L., *Emblica officinalis* Gaertn. *Tridex procumbens* L.tested against their capability to act antimicrobial against the microbes of wounds. The method followed in this procedure was the antimicrobial activity of *Acalypha indica* L., *Emblica officinalis* Gaertn. and *Tridex procumbens* L. against the isolate *P. aeruginosa*has been mentioned in the table below. The zones of inhibitions for all three concentrations were measured in triplicates. The diameter of the well was included while calculating the zone of inhibition 5 mm was the diameter of the well in the research work followed.

TABLE 1: THE BELOW MENTIONED TABLE REPRESENTS THE ANTIBACTERIAL ACTIVITY OF ALL THE THREE PLANT EXTRACTS AGAINST *PSEUDOMONAS AERUGINOSA*

Wells	Samples	Zone of Inhibition (mm)			Final ZOI ± S. D.
		C1 (a)	C1 (a)	C1 (a)	Inhibition Around the well
1	*Acalypha indica* L.	11	11	12	11.3333± 0.57735
2	*Emblica officinalis* Gaertn.	12	12	13	12.333±0.57735
3	*Tridex procumbens* L.	13	14	14	14.3±0.5775
(+)C	Levaquin (Hi media)	20	20	20	20±0.0
Wells	**Concentration of Plant Extracts (µg/ml)**	**Zone of Inhibition (mm)**			**Inhibition Around the well**
		C2 (a)	C2 (b)	C2 (c)	
1	*Acalypha indica* L.	12	13	15	12.667± 0.5775
2	*Emblica officinalis* Gaertn.	18	18	19	18.3345± 0.5775
3	*Tridex procumbens* L.	19	19	19	19±0
(+)C	Levaquin (Hi media)	25	25	25	25.0±0.0
Wells	**Concentration of Plant Extract (µg/ml)**	**Zone of Inhibition (mm)**			**Inhibition Around the well**
		C3 (a)	C3 (b)	C3 (c)	
1	*Acalypha indica* L.	17	18	18	17.7±0.6
2	*Emblica officinalis* Gaertn.	18	18	18	18.0±0.0
3	*Tridex procumbens* L.	20	20	19	19.7±0.6
(+)C	Levaquin (Hi media)	25	26	25	25.3±0.6

In the above table C1, C2, C3 corresponds to three different concentrations 500 ppm, 1000 ppm and 1500 ppm, respectively

In the above chart antimicrobial invasion was checked for the three plant leaves extract *viz*. *Acalypha indica* L., *Emblica officinalis* Gaertn., *Tridex procumbens* L. *Tridex procumbens* L. showed maximum inhibitory action against *Pseudomonas aeruginosa* the maximum of the value was obtained at the concentration of 1000 and 1500 ppm, which appeared to be relatively very high in comparison to the other plant extracts of same concentrations [18]. While the extracts of *Acalypha indica* L., *Emblica offiinalis* Gaertn. Showed a proper result against the strain of *Pseudomonas aeruginosa. Emblica offiinalis* Gaertn .at 1500 ppm it was obtained to be 18 mm comparatively very less than the positive control (Levaquin - Hi media). Covering one gram-negative isolate the other targeted microorganism in our research was *E. coli*al; though they haven't been toxic to life form [19] but they have been easily procured from the food sample and have been observed on the wounds of the patients generally suffering from the incision. The positive control antibiotic used for *E. coli* is Cephalosporin, and the plant extracts showed a comparatively very high zone of Inhibition. Proteus species are frequently recovered from infected wounds. They contaminate wounds and thus cause infections, also; in the study brought up by [19] reported that the Proteus species in 158 isolates, 23% were Proteus mirabilis this was handled in University of Berlin.

TABLE 2: THE ANTIMICROBIAL ACTIVITY OF *ACALYPHA INDICA*L., *EMBLICA OFFICINALIS*GAERTN.AND *TRIDEXPROCUMBENS*L. AGAINST THE ISOLATE *E. COLI* HAS BEEN MENTIONED IN THE TABLE BELOW

Wells	Samples	Zone of Inhibition (mm)			Final ZOI±S.D.
		C1 (a)	C1(b)	C1(c)	Inhibition Around the well
1	*Acalypha indica* L.	9	12	13	12.66± 2.134
2	*Emblica officinalis* Gaertn.	12	13	13	12.973± 1.206
3	*Tridex procumbens* L.	13	14	15	14.3±0.6
(+)C	Levofloxcacin	20	20	20	20±0.0
Wells	**Concentration of Plant Extracts (µg/ml)**	**Zone of Inhibition (mm)**			
		C2 (a)	C2(b)	C2 (c)	Inhibition Around the well
1	*Acalypha indica* L.	09	13	18	16.3±2.9
2	*Emblica officinalis* Gaertn.	18	18	19	18.3±0.6
3	*Tridex procumbens* L.	19	19	18	18.7±0.6
(+)C	Levofloxcacin	25	25	25	25.0±0.0
Wells	**Concentration of Plant Extract (µg/ml)**	**Zone of Inhibition (mm)**			**Inhibition Around the well**
		C3 (a)	C3 (b)	C3 (c)	(mm)*
1	*Acalypha indica* L.	17	18	18	17.7±0.6
2	*Emblica officinalis* Gaertn.	18	18	18	18.0±0.0
3	*Tridex procumbens* L.	20	20	19	19.7±0.6
(+)C	Levofloxcacin	25	26	25	25.3±0.6

TABLE 3: THE ANTIMICROBIAL ACTIVITY OF *ACALYPHA INDICA* L., *EMBLICA OFFIINALIS* GAERTN. AND *TRIDEX PROCUMBENS* L. AGAINST THE ISOLATE PROTEUS MIRABILIS HAS BEEN MENTIONED IN THE TABLE BELOW

Wells	Samples	Zone of Inhibition (mm)			
		C1 (a)	C1 (b)	C1 (c)	Inhibition Around the well
1	*Acalypha indica* L.	9	11	13	11±1.632993
2	*Emblica officinalis* Gaertn.	10	11	13	11.333± 1.247
3	*Tridex procumbens* L.	14	14	15	14.333± 0,471405
(+)C	Cephalosporins	20	20	20	20±0
Wells	**Concentration of Plant Extracts (µg/ml)**	**Zone of Inhibition (mm)**			
		C2 (a)	**C2 (b)**	**C2 (c)**	**Inhibition Around the well**
1	*Acalypha indica* L.	18	18	18	18± 0
2	*Emblica officinalis* Gaertn.	18	18	18	18±0
3	*Tridex procumbens* L.	19	19	19	19±0
(+)C	Cephalosporins	20	20	20	20±0
Wells	**Concentration of Plant Extract (µg/ml)**	**Zone of Inhibition (mm)**			
		C3 (a)	**C3 (b)**	**C3 (c)**	**Inhibition Around the well**
1	*Acalypha indica* L.	17	18	18	17.6667 ± 0.471405
2	*Emblica officinalis* Gaertn.	18	18	18	18±0
3	*Tridex procumbens* L.	20	20	19	19.667± 0.471405
(+)C	Cephalosporins	20	20	20	20± 0

For the isolate of *Proteus mirabilis* the 1000 ppm and 1500 ppm of all the extracts displayed common attack; therefore, both the concentration possess common action towards it. *Tridex procumbens* L. in all three showed the maximum zone of inhibition which meant a similar response. Similar outcomes were estimated in the work of Scheers [20]. The next targeted isolate was *Staphylococcus aureus*; Elemental Literature review and metasyntheses of a handful number of wound analyses proclaimed that there is around 78.2% outspread of biofilms in crucial wound [21]. A rapidly growing body of evidence establishes biofilm infection as a major cause of delayed wound healing. *Staphylococcus* is one of the predominant (65%) causes of persistent infections in chronic wounds. Bacteria of the *Staphylococcus* genus are highly efficient in establishing biofilms resulting in persistent infection [22]. Gram-positive *Staphylococcus* is a nonmotile and nonperforming bacterium that readily adheres to highly proteinaceous surfaces of chronic wounds and forms matrix-encased communities resulting in persistent chronic wound infection that is recalcitrant to antibacterial therapies.

TABLE 4: THE ANTIMICROBIAL ACTIVITY OF *ACALYPHA INDICAL*., *EMBLICA OFFIINALIS* GAERTN.AND *TRIDEX PROCUMBENSL* AGAINST THE ISOLATE *STAPHYLOCOCCUS AUREUS* HAS BEEN MENTIONED IN THE TABLE BELOW

Wells	Samples	Zone of Inhibition (mm)			Final ZOI±S.D.
		C1(a)	C1 (b)	C1(c)	Inhibition Around the well
1	*Acalypha indica* L.	9	11	13	11± 1.632993
2	*Emblica officinalis* Gaertn.	10	11	13	11.333±1.247219
3	*Tridex procumbens*L.	14	14	15	14.333± 0.471405
(+)C	Levofloxcacin	20	20	20	20 ± 0
Wells	**Concentration of Plant Extracts (µg/ml)**	**Zone of Inhibition (mm)**			
		C2 (a)	**C2 (b)**	**C2 (c)**	**Inhibition in mm**
1	*Acalypha indica* L.	13	13	14	17.333± 0.5775
2	*Emblica officinalis* Gaertn.	12	13	14	15.222± 0.5775
3	*Tridex procumbens* L.	15	16	15	15.3423± 0.5755
(+)C	Levofloxcacin	25	25	25	25± 0
Wells	**Concentration of Plant Extract (µg/ml)**	**Zone of Inhibition (mm)**			
		C3 (a)	**C3 (b)**	**C4 (c)**	**Inhibition in mm**
1	*Acalypha indica* L.	18	18	19	18.453 ± 0.521
2	*Emblica officinalis* Gaertn.	18	19	18	18.123± 0.531
3	*Tridex procumbens* L.	20	20	21	20±0.5775
(+)C	Levofloxcacin	25	25	25	25 ±0

Microbial entrenchment of *Tridex procumbens L.*, *Emblica offiinalis* Gaertn and *Acalypha indica* L. was estimated as Zone of inhibition on bacterial spread plate of Mueller Hinton Agar (Hi media). *Staphylococcus aureus* and especially strains with resistance to antimicrobial agents, is an unabated challenge in community-acquired and nosocomial infections ranging from wound infections or osteomyelitis to life-threatening endocarditis, or septic shock [23].

Therefore it became very crucial to target *Staphylococcus aureus* for the analysis of anti-microbial convention. Extracts of *Emblica offiinalis* Gaertn showed a supreme response to encountering the isolates of *Staphylococcus aureus* [24.]

CONCLUSION: All the plant species assessed right now at present utilized generally for the treatment of skin and wound diseases. The positive discoveries from this examination give a logical premise to the conventional utilization of *Acalypha indica*, *Tridex procumbens* and *Emblica offiinalis Gaertn* treatment of skin and wound diseases.

The concentrates of *Tridex procumbens L.* have a promising antibacterial encounter independently at different concentrations against the disengages of wounds *viz Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus,* and *E. coli.*

At last, the aftereffects of this examination plainly clarify the antibacterial capability of these plants and give proof to help their utilization in society medication.

The plant extracts, through targeted, were only leaf extracts; therefore, in the future, bark extracts of trees, Flower extracts could be exploited for further evaluation of their ethnobotanical studies.

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