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Synergistic Effects of *Tridax procumbens* and *Tagetes erecta* in a Biodegradable Wound Healing Bandage

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Abstract - In the pursuit of sustainable and bioactive alternatives to conventional wound care, this study presents the development and evaluation of a novel herbal bandage infused with ethanol extracts of *Tridax procumbens* and *Tagetes erecta*. Addressing the limitations of synthetic dressings—such as poor biocompatibility, lack of therapeutic action, and environmental impact—this project integrates traditional medicinal plants into a modern wound-healing matrix. A 2:1 extract ratio was optimized based on antimicrobial efficacy against *Escherichia coli*, *Staphylococcus aureus*, and *Candida* spp. Phytochemical analysis confirmed the presence of flavonoids, tannins, glycosides, phenols, and terpenoids, contributing to the extract's multifaceted biological activity. FTIR spectroscopy further validated the biochemical functionality through the identification of phenolic, alcoholic, carbonyl, and ester groups. The formulated bandage demonstrated significant antioxidant activity (85.44% DPPH radical scavenging at 100 µg/mL) and anti-inflammatory potential (80.8% inhibition of protein denaturation at 50 µg/mL). In vitro antimicrobial tests revealed superior zones of inhibition in extract-coated fabrics, particularly in the 2:1 combination. Physiological assessments indicated no skin irritation or allergic reactions, supporting its dermatological safety. The bandage exhibited uniform spreadability, ideal pH (6), and favorable physical characteristics. This work highlights the synergistic potential of plant-based biomedical textiles as eco-friendly, biocompatible, and therapeutically active wound dressings, combining traditional knowledge with modern biomedical innovations. Further studies may explore scale-up and clinical applicability.
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Key Words: *Tridax procumbens*, *Tagetes erecta,* Wound healing, antimicrobial, synergistic.

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INTRODUCTION

A single Incision may look inconsequential, but underneath it is an elaborate biological struggle for healing, protection, and restoration." A wound, even though it is considered jumpy commonplace interference by just a 'break' in the protective skin-skin barrier, is the beginning of a long, complex biological problem to restore unvaried structural and functional integrity. A wound can be seen as a loss of continuity of the skin due to cuts or surgical procedures. Healing of the wound can be seen as a process that involves several phases, including hemostasis, inflammation, proliferation, and remodeling. Chronic hurting and even the growth of scars. This orderly process is easily affronted and challenged by microbial infection, oxidative stress, retardation of tissue regeneration, mishandling of wound cause, chronic hurting and even the growth of scars (Verma *et al.,* 2018).

Every year, accidents and injuries come first in rank as a cause of disability in most countries, with an estimated 5 million cases occurring on a global scale. In many situations, the outcome of wound healing depends on available resources and the timeliness of its therapy. A wide range of products, such as sutures, abrasions, dressings, and modern wound care products, have been developed and are commercially available to help protect the wound from infection whilst promoting healing. Of these, dressings are the most readily available and simple to use. A bandage is used to dress a particular area of the body, owing to its skin, to facilitate healing by preventing the area from exuding liquid or infecting other individuals.

It is in recognition of the rising problems of the difficulty of antimicrobial resistance, chronic wounds, and allergic reactions to man-made materials, finding more efficacious, safe, and biocompatible materials for wound healing has become a very urgent matter. That's because wound dressing does not only concern itself with covering a wound, but also reaches towards triggering and increasing its repair processes. Traditional bandages that are produced from synthetic materials such as cotton, polyester, or polyurethane have been the most significant part of wound care for more than a decade. Wound bandages work well as physical barriers and are easy to use, but they are not bioactive enough to promote healing (Shital Torkadi *et al.,* 2022). Additionally, the synthetic bandages can easily cause allergic reactions because of certain qualities they have. There is a high demand for more advanced wound treatment products that offer both coverage and treatment. However, wound dressing bandages-which are quite a basic yet dominating device-are now witnessed under a new scientific purview and rethinking. From very simple cotton gauze dressings to more advanced hydrocolloids, alginate pads, and nanofiber dressings, this vast expanse has massively evolved into contemporary wound dressings (Mandal *et al.,* 2021). But the real trouble of the problem is the satisfaction of showing very large volumes of bandages, but a good percentage of them are still most dependent on synthetic polymers, petrochemical-based adhesives, and chemical antimicrobials. And while those used have proved remarkably effective, they don't come without their fair share of adverse side effects.

Ever-increasing prevalence of skin irritation, hypersensitivity, wound healing inhibition, and disruption of the skin microbiome (Verma *et al.,* 2018).

In recent times, the need for organic and natural wound care materials has been intensifying. The development of herbal bandages augmented with bioactive factors, as root commodities of the plant, finds a place of innovation, promise, and ecological friendliness. The environmental cost of non-biodegradable synthetic dressing is an urgent one, coming as it does in the embrace of the worldwide interests in sustainability. This is a time crying out for a paradigm change-one that would redefine wound care in terms of biocompatibility, ecological harmony, and therapeutic effectiveness. This has further brought interest back in herbal and plant drugs for wound healing, not just as adjuvant therapies.

The combination of plant-based medicine with the physical efficacy of traditional bandages can be viewed as a comprehensive treatment approach to the wound problem. These herbal bandages are not just casual dressings; they are the mechanics of active support for healing. They augment wound healing by phenomenal phytochemicals endowed with antimicrobial, anti-inflammatory, antioxidative, and regenerative properties, apart from direct interactions with the wound site to heighten healing. They are capable of rendering dual functions: mechanical protection while slowly releasing therapeutic agents just at the wound site to diminish systemic complications and boost the rate of repair. Of numerous phytotherapeutic remedies, *Tridax procumbens* and *Tagetes erecta* have bright prospects. They have a long ethnobotanical history of wound healing; however, their joint use in herbal bandage, evidently an innovative area that has not been widely explored.

The coat buttons, commonly referred to as *Tridax procumbens*, are a creeping herb known for its traditional medicine in tropical greenery. *Tridax procumbens* is rich in flavonoids, tannins, and several other bioactive compounds offer traditional people the ability to use them for the treatment of cuts and wounds, as well as infections. The plant exhibits widespread traditional medicinal applications, particularly as an antimicrobial, antiseptic, and anti-inflammatory remedy, and is further endowed with a plethora of possibilities that encourage the scientific community to pursue it as a source of various therapeutic agents. Flavonoids act as antioxidants by neutralizing free radicals and stimulating cells required for wound healing, fibroblasts, which in turn produce collagen that acts as a scaffold in reconstituting the tissue. Alkaloids contribute to the antimicrobial effect by inhibiting the growth of pathogenic bacteria at the wound site. Moreover, tannins have an astringent action that causes contraction of the tissues with the effect of reducing exudation (Akhila *et al.,* 2021). The plant is also known for immediate facilitation of clot formation, which can, together with the aforementioned properties, provide wide-ranging support for relatively rapid wound healing. It has also been established that *Tridax procumbens* can help to limit the reproduction of microbes, which helps in lowering the chances of infections of a wound. Thus, this very practical use of the plant brings it to the foreground as one of the most important wound healing agents.

Tagetes erecta, much admired for its ornamental value with vibrant flower colour, yet it has also held quite an important place in traditional healing. Marigold has very beautiful yellow flowers and is known for its antiseptic, antimicrobial, and anti-inflammatory qualities as it consists of carotenoids, flavonoids, and even volatile oils, including limonene, ocimene, and lutein (Chaudhary *et al.,* 2021). These bioactive substances do protect the wound from damage but assist in the formation of granulation tissues and speed up the entire healing process. Traditionally applied as an antiseptic, anti-inflammatory, and a wound healer in herbal systems. The phytoconstituents in these compounds confer both antimicrobial and angiogenetic properties, such that the creation of new blood vessels brings nutrition and oxygen to developing tissues. The antioxidant capacity offered through this plant shields the wound area from oxidative deterioration, whilst the anti-inflammatory action simply modulates the activity of cytokines so that swelling and pain in the tissues may be lessened. An astringent further promotes quicker wound contraction, thus improving comfort in patients through its analgesic activity.

The Integration of these two herbal extracts in a bandage has a double benefit. It is hoped that together, the properties of *Tagetes erecta* and *Tridax procumbens* will give an excellent treatment for wounds. A combination of these two herbs would further provide synergism in therapy by sharing overlapping pharmacological actions, which amplify the healing response as a whole. The synergy equally matters for the care of the wounds in simultaneous antimicrobial shielding, controlling inflammation, reducing oxidative stress, and regeneration of tissues (Akhila *et al.,* 2021). Thus, the dual-extract herbal bandage becomes a very complicated solution for infection control whilst actively supporting healing at each phase. The bandage serves the need for protection and absorption, while the incorporated bioactive materials serve the function of treating the wound. Such a formulation overcomes the disadvantages of conventional synthetic bandages and takes advantage of the ability of medicinal plants to heal wounds.

The application of bandages can go beyond the minor cuts and wounds, as they can potentially treat chronic wounds, diabetic ulcers, surgical incisions, and burns. Concerning the plant-based bandage, benefits are likely to accrue not only to individual health but also to its impact on society as well as on the environment. Herbal dressings for wounds are commonly biodegradable, which helps minimize medical waste and lesser pollution to the environment (Mohanraj *et al.,* 2022). Furthermore, their bioactivity properties play an important role in fighting against pathogens resistant to drugs, which is a very vital problem in the modern world. Also, their biocompatibility means that there is a low risk of allergic reactions, so they can be used for all patient populations. They are also available at comparatively lower prices, especially in resource-limited areas of the globe where there is no access to synthetic dressings. Such essential generic products for wound care could propel small-scale industry, rural employment, and a possible revival of traditional medicine with focused monetary assistance to the fortunate.

This research aims to find a product that serves as both a bandage and a drug, effectively curing wounds and reducing the risk of complications, thereby contributing to a sustainable healthcare system.

The use of such kind of plant bandages is not limited to the conventional use of assisting wounds. Their decomposition properties also make them eco-friendly and address the increasing issues of medical waste. Furthermore, due to bio-compatibility and low cost, they are suitable for poor countries, giving them a substitute for high-cost synthetic.

Materials and methods:

Collection of Plant Materials:

Fresh leaves of *Tridax procumbens* and flowers of *Tagetes erecta* were selected for the present study and collected from agricultural areas around Coimbatore regions of 11.0168°N and 76.9558°E, 11.4549°N and 77.4365°E ° E, 11.1085°N ° N and 77.3411°E, respectively.

Plant extraction:

The plant materials were washed thoroughly in distilled water and dried in the shade for 7-10 days. The dried plants are ground into fine powder. The ground powder is stored in a clean, closed container. The plant extraction was carried out by the maceration process. 10 g of each powder was soaked in 100 ml of 80% ethanol and incubated at room temperature for 48-72 h with occasional stirring. The extract is filtered through Whatman No. 1 filter paper and the filtrate is stored at 4°C in bottles to prevent spoilage by exposure to light.

Phytochemical Analysis of Plant Extracts (Ajayi *et al.,* 2011)

Phytochemical analysis is carried out to detect the presence of biologically active compounds in the extract. The following tests are performed:

Test for Alkaloids:

1 ml of each extract is mixed with 5 drops of Mayer's reagent. A creamy white or yellow coloured precipitate indicates the presence of alkaloids.

Test for Glycosides:

Mix 1 ml of each extract with 1 ml of acetic acid, a few drops of $FeCl_3$ solution. Then add 1 ml of concentrated H_2SO_4, and confirm the presence of glycosides by the formation of a brown ring.

Test for Flavonoids:

Mix 1 ml of extract with 1 ml of 2N sodium hydroxide. The presence of flavonoids is indicated by yellow colour.

Test for Phenolic compounds:

To 1 ml of each extract, 2 ml of distilled water and a few drops of ferric chloride are added. A dark green or blue-black colour confirms the presence of phenols.

Test for Tannins:

Mix 0.5 ml of each extract 5% $FeCl_3$ solution. The presence of tannins can be indicated by the formation of a dark blue or greenish black.

Test for Phytosteroids:

Mix 0.5 ml sample with 1 ml of acetic anhydride and 2 ml of concentrated sulphuric acid. The presence of phytosteroids can be indicated by the formation of violet to blue or greenish colour.

Test for Terpenoids:

Mix 2 ml of extract with 2 ml of chloroform and 2 ml of concentrated H_2SO_4. A greyish solution confirms the presence of terpenoids.

Test for Quinones:

To 0.5 ml of each sample, add concentrated sulphuric acid. Formation of red colour indicates quinones.

Test for Coumarins:

To a 0.5 ml sample, add 0.5 ml of 10% NaOH solution. Formation of yellow colour indicates the presence of coumarins.

FTIR Analysis (Ragavendran *et al.,* 2011)

Fourier Transform Infrared Spectroscopy (FTIR) is an accurate, non-destructive, and sensitive analytical tool widely employed to determine the different functional groups present in plant extract phytochemicals. The working of FTIR is straightforward on the basis of infrared radiation being absorbed by molecules that leads to vibrational transitions like bond stretching, bending, twisting, or rocking. There is a unique infrared radiation absorption at a specific wavenumber for each functional group to generate an absorption spectrum or "chemical fingerprint" for the sample. The normal FTIR method involves sample preparation by grinding the sample and potassium bromide (KBr) in a 1:100 ratio to prepare a flawless powder. The powder is pressed into a thin disc form using a high-pressure hydraulic press (usually 6 bars). The pellet is placed in the sample holder of the FTIR spectrometer and scanned from 4000–400 cm^1. The spectra thus obtained are interpreted by matching peak positions with known ranges of absorption of functional groups. This process is routinely adopted in some phytochemical research and also attests to the efficiency of FTIR in identifying bioactive compounds behind antioxidant, antimicrobial, anti-inflammatory, and healing activity in several plant species.

Antimicrobial Analysis: (Vijayalakshmi *et al.,* 2024)

Antimicrobial activity is evaluated by the agar well diffusion method. The antimicrobial study was made against some of the pathogens, such as *Escherichia coli, Staphylococcus aureus, Pseudomonas,* and *Candida*. Mueller-Hinton agar was used to study the antimicrobial assay. To the sterile petri dishes, the agar is added. Once the agar is solidified, 20 to 25 µL of a homogeneous bacterial suspension, which is 18 hours old, is introduced using sterile cotton swabs. Using a cork borer, wells were made in the solid media of Mueller-Hinton agar. Subsequently, to each well, 10 to 50 µL of plant extracts is added and incubated at 37°C for 24 hours. The zone of inhibition around the disk was measured in millimeters to evaluate the antibacterial effect against the test organisms.

Test for Antioxidant Ability

DPPH radical scavenging assay (Asadujjaman *et al.,* 2013)

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most widely used methods for the assessment of antioxidant activity of plant extracts or compounds. DPPH is a stable free radical with a deep violet colour in a methanol solution, which fades with the action of an antioxidant. The antioxidant test is performed based on the protocol followed in Asadujjaman *et al.,* 2013, with slight modifications. In this case, a volume of 1 mL of DPPH solution (methyl alcohol 0.1 mM) is combined with 1 mL of the extract at different concentrations (for example, 10, 20, 40, 60, 80, and 100 µg/mL). The reaction mixture was then gently shaken, after which it was incubated in the dark for 30 minutes at room temperature. Then the absorbance was measured at 517 nm in a UV-Visible spectrophotometer. Control was performed by treating with 1 mL DPPH water solution and without methanol sample. A standard antioxidant such as ascorbic acid was used as the positive control. The percentage of DPPH radical scavenging activity was calculated with the following formula:

$$\% \text{ Scavenging Activity} = [(\text{Absorbance of Control} - \text{Absorbance of Sample}) / \text{Absorbance of Control}] \times 100$$

Analysis of Anti-Inflammatory Activity

Inhibition of protein denaturation assay (Kaddour *et al.,* 2020)

Anti-inflammatory activity is assessed by protein denaturation inhibition assay. It was done to identify the potential of the plant extract to alleviate swelling and redness. The sample was studied for its anti-inflammatory properties through the Bovine Serum Albumin (BSA) protein denaturation method using phosphate-buffered saline (PBS, pH 6.4). The reaction mixture had 2 mL of PBS, 1 mL of the plant extract sample at different concentrations (10, 20, 30, 40, and 50 µg/mL), and 1 mL of 1% BSA solution. Each mixture was incubated for 20 minutes at room temperature (37°C). Then the mixtures were heated to 70°C for 5 minutes to cause denaturation of the protein. The absorbance was measured at 660 nm using a UV-Visible spectrophotometer after cooling. For negative control, the plant extract was substituted with 1 mL of distilled water. Aspirin, otherwise known as acetylsalicylic acid, is used as a positive control. The inhibition percentage of protein denaturation was calculated with the following equation.

$$\% \text{ Inhibition} = [(\text{Absorbance of Control} - \text{Absorbance of Sample}) / \text{Absorbance of Control}] \times 100$$

Selection of fabric

The fabric used for herbal dressings should be breathable, absorbent, and biodegradable. Cotton woven fabrics/cotton gauze is chosen because of their high absorbency and flexibility. It is breathable and provides durability for a variety of applications. The traditional woven fabric is prepared by the weaving method (Deepa *et al.,* 2024). It is also a disposable product and is used in various industries. The fabrics are washed with distilled water, sterilized by autoclaving at 121°C for 15 minutes, and dried under aseptic conditions before use for impregnation with plant extracts. (Vijayalakshmi *et al.,* 2024)

Bandage preparation (Shital Torkadi *et al.,* 2022).

The plant extract is concentrated in the rotor. Now, the non-woven cotton fabric is soaked in the concentrated extract, and the syringes are used to load the extract uniformly in the fabric material. Then it is allowed to dry for 1 hour under aseptic conditions. An adhesive bandage consists of woven fabric and adhesive, which is used for holding the non-woven fabric that has been coated with the plant extract. The adhesive sticks to the body, and the non-woven fabric covers the wound. The fabric is cut into a suitable dimension. The plant-

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coated fabric is placed in the centre. At last, a butter paper is used to protect the prepared bandage from contamination. Butter paper is easily removable, and thereby it is used as a releasable label.

Evaluation parameters

Evaluating the conc. Liquid extract:

Colour: The colour of the extracted plant was observed.

pH: Using a pH paper, the pH of the conc. Liquid extract and also the pH of the sample obtained by dissolving 1 ml of concentrated solution. Plant extract in 10 ml of distilled water was noted. The wound healing efficiency is improved in the acidic medium (Shital Torkadi *et al.,* 2022).

Physical parameters of non-woven fabric loaded with the plant extract:

Colour: The colour of the non-woven fabric material loaded with the plant extract was observed.

pH: By placing the fabric infused with the plant extract in 10 ml of distilled water, the pH is determined.

Spreadability: Based on how the plant extract is spread uniformly in the fabric, the spreadability was observed.

RESULTS AND DISCUSSIONS

Collection of plant material

Fresh leaves of *Tridax procumbens* and flowers of *Tagetes erecta* were collected in Coimbatore, Tamil Nadu. The leaves of *Tridax procumbens* and flower petals of *Tagetes erecta* were thoroughly washed and dried in the shade for 7 days.

Figure 1: Dried leaves of *Tridax procumbens* and flowers of *Tagetes erecta*

Plant extraction

The dried leaves of *Tridax procumbens* and flowers of *Tagetes erecta* were powdered, and the plant extraction was done by the maceration process with 80 % ethanol (10 g powder in 100 ml of ethanol). It was stored for further studies. 80% ethanol was used as 100% ethanol is less effective in extracting low molecular weight phenolic compounds with high antioxidant capacity than compared to 80% or 95% ethanol (Suryawanshi *et al.,* 2021). Ethanol is been proven to produce a highly rich phytochemical extract of *Tridax procumbens.* Therefore, it is used for plant extraction.

Figure 2: Extracted plant samples

Figure 2: Extraction of Plant Materials

Phytochemical analysis of plant extracts

The secondary metabolites identified in the ethanolic extract of *Tridax procumbens* and *Tagetes erecta* are shown in Table 1. The results are visualized in Figures 3 and 4. The test for various secondary metabolites is carried out with common phytochemical methods. The presence or absence of phytochemical compounds is determined by a change in colour or by the formation of a precipitate. Phytochemical investigation of ethanolic extracts of *Tridax procumbens* and *Tagetes erecta* revealed the presence of several bioactive constituents, which can be attributed to their pharmacological activity. The ethanolic leaf extract of *Tridax procumbens* showed the presence of glycosides, flavonoids, phenolic compounds, tannins, phytosteroids, and terpenoids. Whereas alkaloids, quinones, and coumarins were absent. The ethanolic flower extract of *Tagetes erecta* showed the presence of alkaloids, glycosides, phenolic compounds, tannins, phytosteroids, and terpenoids. Whereas flavonoids, quinones, and coumarins were absent.

In the present study, flavonoids were present in *Tridax procumbens* but not in *Tagetes erecta*. It may have been because of the solvent or geographic variation between the plant varieties. Flavonoids have powerful antioxidant capacity and are mainly involved in cellular protection as well as the repair of tissue. Alkaloids are known to show pharmacological activities like antimicrobial, anticancer, and analgesic activity. Alkaloids were found in *Tagetes erecta* but not in *Tridax procumbens*. The lack of *Tridax procumbens* in this specific study could be attributed to variation in extraction processes or geographical differences in the phytochemistry of plants, and the presence of *Tagetes erecta* could be the cause for its antimicrobial activity. The presence in *Tridax procumbens* of glycosides, flavonoids, phenolic compounds, tannins, phytosterols, and terpenoids is reason enough for its age-old application in wound healing, since these are accountable for antimicrobial, antioxidant, and anti-inflammatory activities. Quinones and coumarins were not present in the extracts of both plants under this research. Coumarins, however, possess anti-coagulant and anti-cancer activity, while quinones are useful in the treatment of heart disease and osteoporosis. Their lack could reduce some of the therapeutic capability, but does not necessarily deny the extracts of any effectiveness, since the other phytochemicals contained together are active bioactive substances. The simultaneous presence of phytochemicals in both extracts enhances their synergistic effect. *Tridax procumbens* leaf extract contains flavonoids with antioxidant activity, and *Tagetes erecta* contains alkaloids that enhance antimicrobial activity. The simultaneous occurrence of glycosides, phenolics, tannins, phytosterols, and terpenoids in the two extracts is thought to enhance the action of herbal drugs for wound healing purposes by virtue of anti-inflammatory, antimicrobial, and healing activities of the tissues.

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Table 1: Results of phytochemical test

S. No.	Test	Ethanolic extract of *Tridax procumbens*	Ethanolic extract of *Tagetes erecta*
1.	Alkaloids	-	+
2.	Glycosides	+	+
3.	Flavonoids	+	-
4.	Phenolic compounds	+	+
5	Tannins	+	+
6.	Phytosteroids	+	+
7.	Terpenoids	+	+
8.	Quinones	-	-
9.	Coumarins	-	-

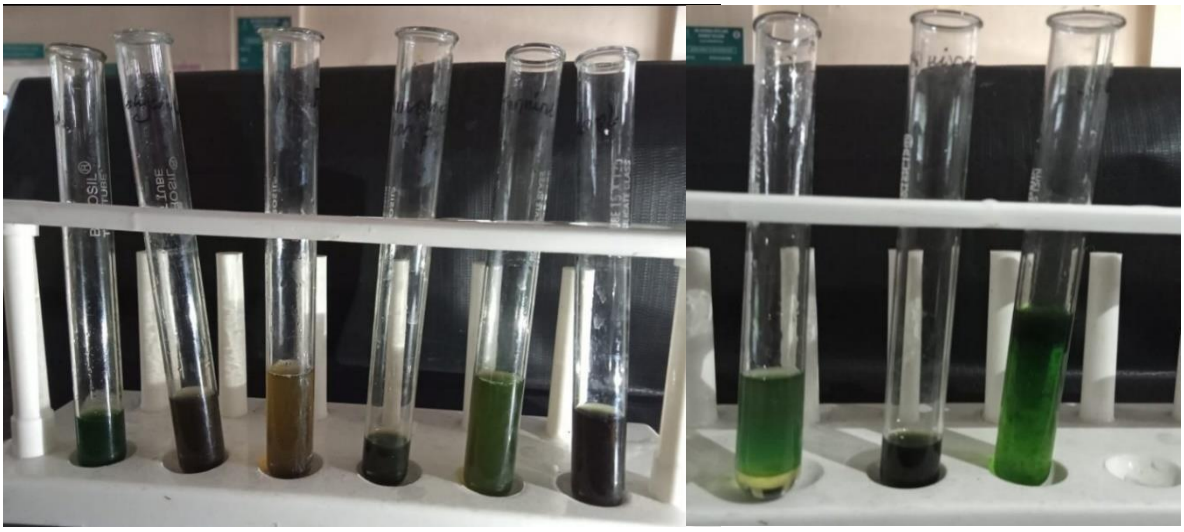

Figure 3: Phytochemical analysis of leaf extract of *Tridax procumbens*

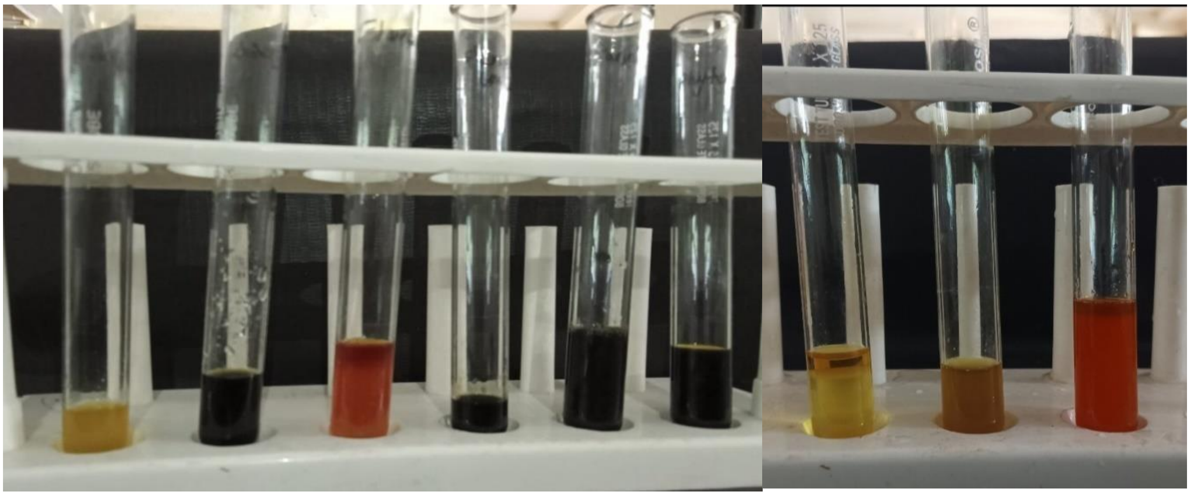

Figure 4: Phytochemical analysis of the Flower extract of *Tagetes erecta*

FTIR Analysis

The FTIR spectrum of the sample exhibited a series of typical peaks of absorption for different functional groups. The wavenumbers found and their corresponding functional group assignments are shown in Table 2. Figure 5 shows the graphical representation of FTIR spectral analysis. The FTIR spectrum of the sample reveals the presence of different functional groups, each of which accounts for considerable biological activity. The broad and intense absorption bands at 3325.28 cm^{-1} and 3819.06 cm^{-1} reveal O–H stretching, equivalent to alcohol as well as to phenolic compounds. This finding agrees with the research of Singh and Mendhulkar, which also found an identical broad peak at 3436.2 cm^{-1} in Abutilon indicum, affirming phenolic content. Similarly, Jain *et al.,* 2016 identified the same peaks at 3389.57 cm^{-1} and 3349.81 cm^{-1} respectively, in *Clitoria ternatea* and *Mentha spicata* and assigned them to antioxidant activity owing to phenolic hydroxyl groups. The bands at 2978.09 cm^{-1} and 2893.22 cm^{-1} are aliphatic C–H stretching vibrations common in fatty acids and hydrocarbon chain molecules. The identical bands occurred in *Mentha spicata*, which confirmed the occurrence of lipid-related compounds possibly conferring membrane-interactive antimicrobial activity (Jain *et al.,* 2016). The most prominent peak at 1651.07 cm^{-1} may be referred to as C=O stretching vibrations of amide bond or conjugated carbonyl compounds like flavonoids that have been largely identified for wound-healing as well as anti-inflammatory activity. This justifies the FTIR analysis results of Abutilon indicum in which a band was seen at 1636.2 cm^{-1} corresponding to amide carbonyl groups.

Furthermore, the bands at 1381.03 cm^{-1} and 1327.03 cm^{-1} represent bending vibrations of CH groups and probable O–H deformation, which confirm the occurrence of carboxylic acids and alkanes. The groups occur in *Mentha spicata* (1384.66 cm^{-1}) and *Clitoria ternatea* (1376.43 cm^{-1}) and are associated with antioxidant activity. Absorption at 1273.02 cm^{-1} due to C–O stretching establishes the presence of esters and ethers and the likelihood of glycosides and terpenoid derivatives as with Abutilon indicum. Intense absorption bands at 1087.85 to 1041.15 cm^{-1} result from C–O–C and C–N stretching vibrations, indicating the presence of ethers, secondary alcohols, and amines. These functional groups were found in *Abutilon indicum* (1047.1 cm^{-1}), *Mentha spicata* (1054.89 cm^{-1}), and *Clitoria ternatea* (1057.61 cm^{-1}). Aromatic ring was indicated by peaks at 879.54 and 686.66 cm^{-1} corresponding to flavonoid backbone structures. The same aromatic peaks were found in *Mentha spicata* at 599.76 cm^{-1} and in *Clitoria ternatea* extracts, affirming the occurrence of flavonoids.

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Generally, the FTIR spectrum of the sample is by other medicinally active plant extracts, which strongly indicates a rich content of phenolic compounds, flavonoids, esters, fatty acids, and aromatic compounds. These bioactive functional groups are known to exhibit a variety of therapeutic activities, viz., antioxidant, antimicrobial, and wound-healing activities. Hence, *Tridax procumbens* and *Tagetes erecta* combination extract has tremendous potential for application in herbal drug products like herbal band-aids, the manner suggested *in Abutilon indicum, Clitoria ternatea,* and *Mentha spicata* studies.

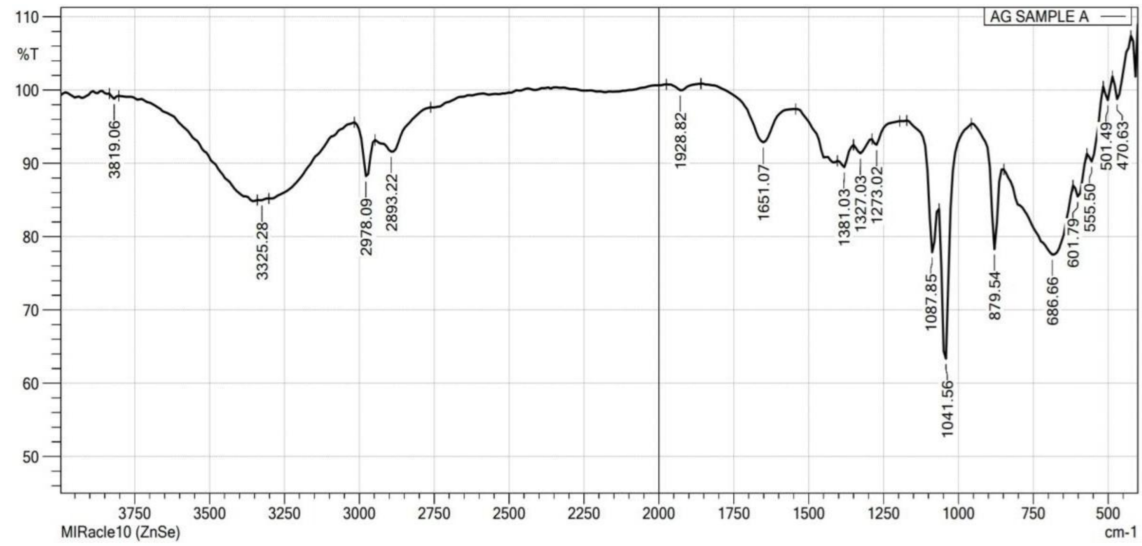


Figure 5: FTIR Analysis

Table 2: FTIR Spectral Analysis Results

S. No.	Wavenumber (cm⁻¹)	Functional Group Assigned	Possible Compound Type
1.	3819.06 / 3325.28	O–H stretch (alcohols/phenols)	Polyphenols, flavonoids
2.	2978.09 / 2893.22	C–H stretch (alkanes)	Aliphatic hydrocarbons, fatty acids
3.	1928.82	Overtone/combinational band	Aromatic ring vibrations
4.	1651.07	C=O stretch (amides, conjugated ketones)	Flavonoids, protein-related compounds
5.	1381.03 / 1327.03	C–H bending / O–H deformation	Carboxylic acids, alkanes

6.	1273.02	C–O stretch (esters, ethers)	Glycosides, terpenoids
7.	1087.85 / 1041.15	C–O–C / C–N stretching	Ethers, alcohols, amines
8.	879.54 / 686.66	Aromatic C–H out-of-plane bending	Aromatic ring compounds
9.	601.79 – 470.63	C–X stretching (halogen compounds)	Minor halogenated metabolites or impurities

Antimicrobial analysis

The antimicrobial effect of *Tridax procumbens* and *Tagetes erecta* extract was studied against *Escherichia coli, Staphylococcus aureus, Pseudomonas,* and *Candida*. The plant extracts of *Tridax procumbens* and *Tagetes erecta* were combined in different ratios, such as 1:1, 2:1, and 1:2, respectively. The zone of inhibition formed for different combinations against various pathogenic organisms is mentioned in Table 3. All the combinations showed the presence of an inhibition zone against the pathogens that have been studied. From table 3, it can be concluded that among all the ratios, the plant extract of *Tridax procumbens* and *Tagetes erecta* in a 2:1 ratio shows a greater antimicrobial effect compared to the 1:1 and 1:2 ratios. Therefore, a 2:1 ratio is taken for further studies. The antimicrobial effect of the coated bandage is shown in Table 4. Phytochemical screening of ethanolic leaf extracts of *Tridax procumbens* and flower extracts of *Tagetes erecta* for the occurrence of diversified bioactive compounds disclosed the existence of diversified bioactive compounds. The bioactive compounds, which were mostly phenolic compounds, flavonoids, and tannins, are generally reported to be antimicrobially active. Against antimicrobial screening, the extracts of *Tridax procumbens* and *Tagetes erecta* in three ratios (1:1, 2:1, 1:2) were screened against *Escherichia coli, Staphylococcus aureus, Pseudomonas*, and *Candida*. The results in Table 2 show that the maximum zone of inhibition was in a 2:1 ratio against all test microorganisms, therefore 11 mm against *E. coli*, 14 mm against *S. aureus*, 13 mm against *Pseudomonas*, and 12 mm against *Candida*. The enhanced antimicrobial activity in the 2:1 ratio is a result of the combined action of phytoconstituents of both plant extracts. Flavonoids and phenolic acids are active against the bacterial membrane, and they cause disruption of membrane integrity and lysis of the cell. Tannins can bind with bacterial protein to complex and inactive key enzymes and transport systems, and as such inhibit microbial growth (Valivittan and Dhasarathan, 2014).

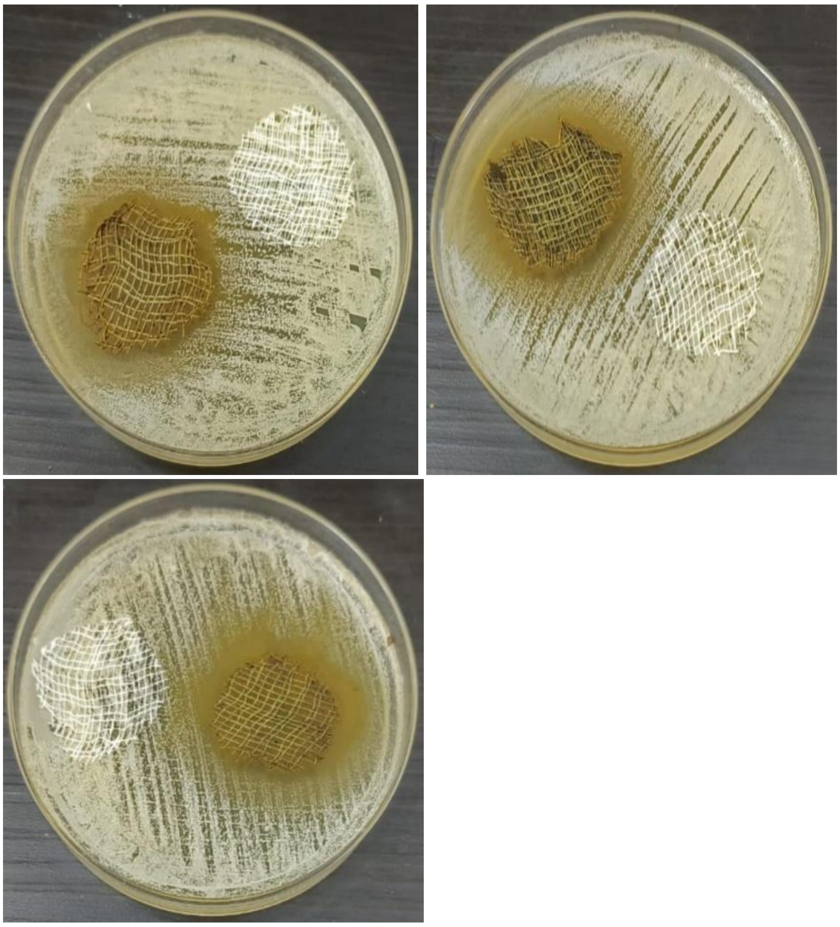
Besides, antimicrobial activity of herbal-coated bandage (Table 3) was tested at the optimal ratio of 2:1 extracts Herbal extract treated bandage in Petri dish gave a satisfactory inhibitory zone of 38 mm for *E. coli*, 37 mm for *S. aureus*, and 35 mm for *Candida*, whereas no inhibitory zone was observed for the control cotton bandage. Petri dish images clearly show the satisfactory inhibitory zones surrounding the herbal-coated cloth. This establishes the effectiveness of extracts containing bandages in inhibiting microbial infection and supports their use on wounds in healing. All these findings combined state that the inclusion of *Tridax procumbens* and *Tagetes erecta* extracts together not only increases the antimicrobial potential but also presents a reliable bio-based option for wound dressing.

Table 3: Antimicrobial effect of herbal extract in various ratios

S. No.	Organism	Zone of inhibition in mm		
		1:1 ratio	2:1 ratio	1:2 ratio
1.	*Escherichia coli*	7mm	11mm	10mm
2.	*Staphylococcus aureus*	8mm	14mm	13mm
3.	*Candida*	10mm	12mm	11mm
4.	*Pseudomonas*	8mm	14mm	13mm

Table 4: Antimicrobial effect of coated bandage with a 2:1 ratio of *Tridax procumbens* and Tagetes erecta

S. No.	Organism	Zone of inhibition in mm	
		Control	Coated cotton cloth
1.	*Escherichia coli*	0mm	38mm
2.	*Staphylococcus aureus*	0mm	37mm
3.	*Candida*	0mm	35mm



Escherichia coli ***Staphylococcus aureus*** ***Candida***

Figure 6: Antimicrobial effect of coated bandage with 2:1 ratio of *Tridax procumbens* and *Tagetes erecta*

Test for antioxidant ability

DPPH scavenging assay is used to analyze the antioxidant ability of the herbal extract. Antioxidant activity of standard ascorbic acid is shown in Table 5, and antioxidant activity of the herbal extract is shown in Table 6. The graph is presented in Figure 7. Antioxidant potential of ethanolic extract of *Tridax procumbens* and *Tagetes erecta* in a ratio of 2:1 was analysed by using DPPH radical scavenging assay. Activity of DPPH scavenging was compared with reference antioxidant ascorbic acid and presented in Tables 5 and 6 and a graphical plot. Ascorbic acid was more effective as a scavenger with different percentages of 72.00% at 10 µg/mL to 90.67% at 100 µg/mL. In comparison, the herbal extract was also increasing with concentration, having the highest inhibition of 85.44% at 100 µg/mL, which is closer to the standard. Increasing antioxidant activity is directly proportional to increasing bioactive compounds in the extract.

DPPH assay confirms the presence of active antioxidant compounds such as flavonoids and phenolic acids in the extract. The molecules transferred hydrogen atoms or electrons to neutralize free radicals and quench the reactive DPPH radicals. IC_{50} of the extract was determined to be around 14.52 µg/mL, i.e., the effective concentration to scavenge 50% of DPPH radicals. Lower IC_{50} indicates higher antioxidant activity (Asadujjaman *et al.,* 2013). The antioxidant activity of plant extracts largely varies according to the concentration of phenolic compounds, which contain redox properties and are, therefore, good reducing agents, hydrogen donors, and singlet oxygen quenchers.

Table 5: Antioxidant activity of standard ascorbic acid

S. No.	Standard Ascorbic acid	Concentration µg/mL	Absorbance OD value	DPPH scavenging activity
1.	Sample 1	10	0.168	72.00
2.	Sample 2	20	0.153	74.50
3.	Sample 3	40	0.131	78.17
4.	Sample 4	60	0.124	79.33
5.	Sample 5	80	0.102	83.00
6.	Sample 6	100	0.056	90.67

Table 6: Antioxidant activity of ethanolic extract of *Tridax procumbens* and *Tagetes erecta* in 2:1 ratio

S. No.	Samples	Concentration µg/mL	Absorbance OD value	o of DPPH scavenging activity
1.	Sample 1	10	0.547	43.37
2.	Sample 2	20	0.411	58.05

3.	Sample 3	40	0.380	61.17
4.	Sample 4	60	0.268	72.43
5.	Sample 5	80	0.185	75.78
6.	Sample 6	100	0.099	85.44

DPPH scavenging activity of Ascorbic acid and Sample

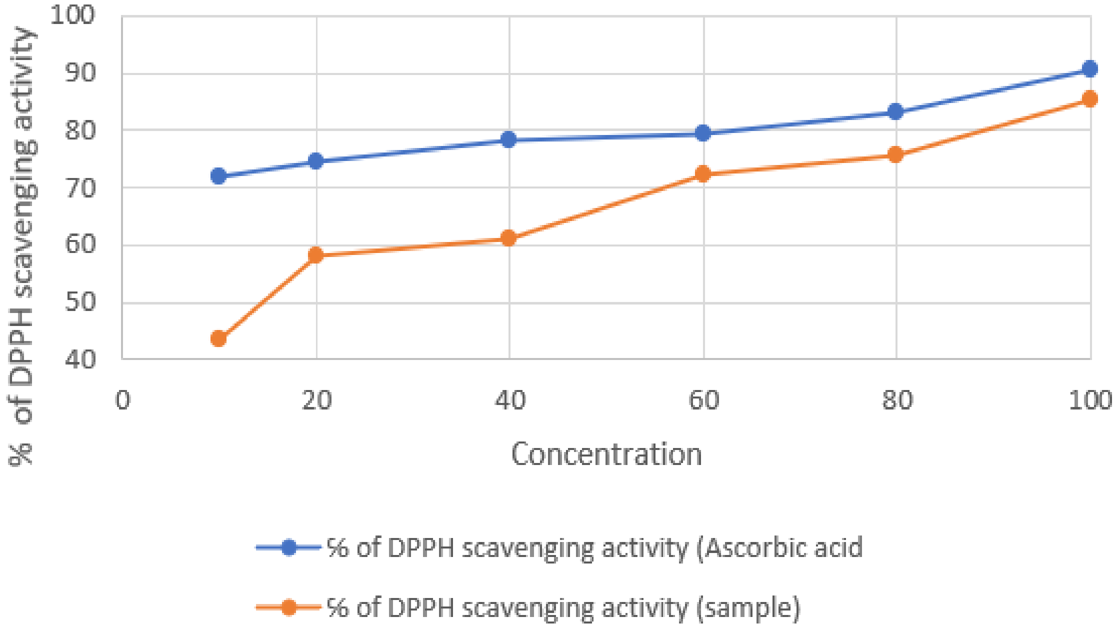


Figure 7: Antioxidant analysis test

Analysis of anti-inflammatory activity

The anti-inflammatory study was done by inhibiting of protein denaturation assay. Tables 7 and 8 demonstrate the results obtained in the test. Figure 8 shows the graphical representation of the anti-inflammatory test. Anti-inflammatory activity of ethanolic extract of *Tridax procumbens* and *Tagetes erecta* in a 2:1 ratio was studied by protein denaturation assay, a standard method of screening anti-inflammatory activities of natural products. Activity of the sample was compared with the activity of aspirin, an anti-inflammatory reference compound. As is evident from Table 6, the inhibition percentage of aspirin was 86.0%, 86.6%, 89.2%, 92.2%, and 93.6% at 10, 20, 30, 40, and 50 µg/mL concentrations, respectively. Whereas, the sample extract of the herb inhibited by 63.0%, 67.0%, 72.0%, 74.8%, and 80.8% for the corresponding concentrations, as is evident from Table 7. The result is graphically represented, with concentration-dependent growth of the inhibitory activity in the standard as well as the sample. Even though the herbal extract was less inhibitory than aspirin, it was also a good anti-inflammatory with highly significant activity at higher doses. This indicates the presence of bioactive phytochemicals that are actually causing inhibition of protein denaturation, an activity that is associated with inflammation.

Anti-inflammatory activity is ascribed to phytochemical compounds like alkaloids, flavonoids, glycosides, saponins, and terpenoids found in the extracts. They stabilize lysosomal membranes, suppress inflammatory mediators, and suppress protein denaturation and thus show anti-inflammatory activity (Valiyath & Dasagapatra, 2014). *Tridax procumbens* and *Tagetes erecta* composite extract thus has a promising natural drug candidates for anti-inflammatory treatment, particularly for topical use in wound healing.

Table 7: Anti-inflammatory activity of standard aspirin

S. No.	Concentration of aspirin in µg/mL	Absorbance OD value	o inhibition
1.	10	0.070	86.0
2.	20	0.067	86.6
3.	30	0.054	89.2
4.	40	0.039	92.2
5.	50	0.032	93.6

Table 8: Anti-inflammatory activity of ethanolic extract of *Tridax procumbens* and *Tagetes erecta* in 2:1 ratio

S. No.	Concentration of sample in µg/mL	Absorbance OD value	o inhibition
1.	10	0.185	63.0
2.	20	0.165	67.0
3.	30	0.140	72.0
4.	40	0.126	74.8
5.	50	0.096	80.8

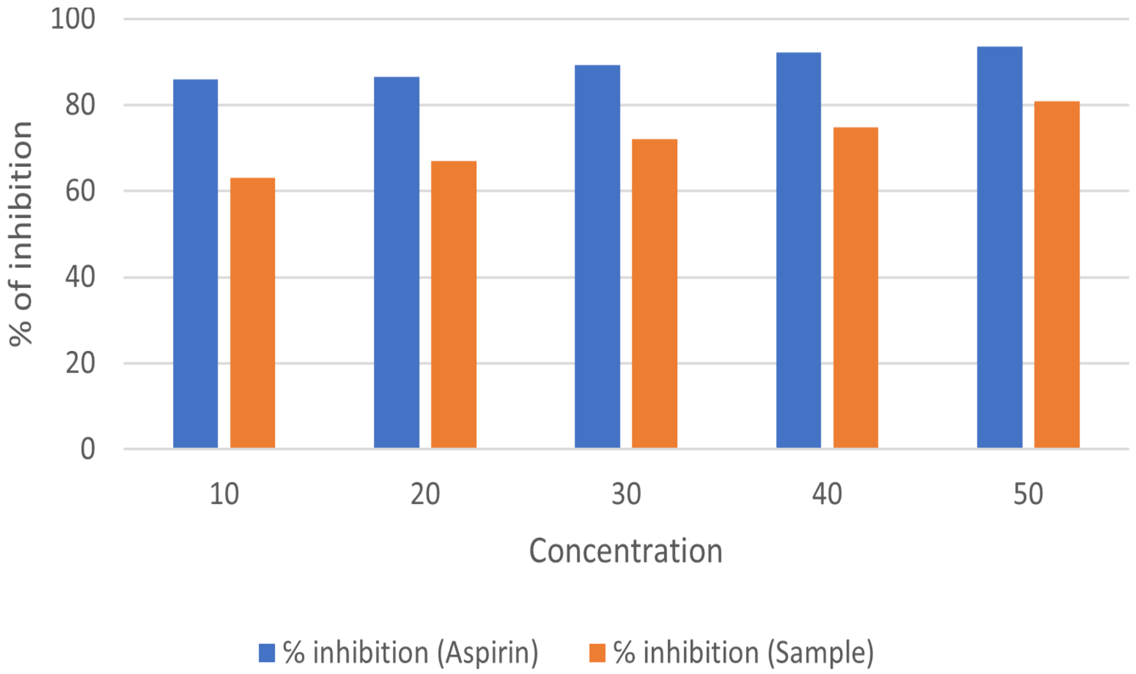


Figure 8: Anti-inflammatory analysis

4.9 Bandage preparation

The antimicrobial test concluded that a 2:1 ratio is best in inhibiting microbial growth. Therefore, the bandage was prepared with a 2:1 ratio of *Tridax procumbens* and *Tagetes erecta,* respectively. The antimicrobial effect of the coated bandage is studied and presented in Figure 9.

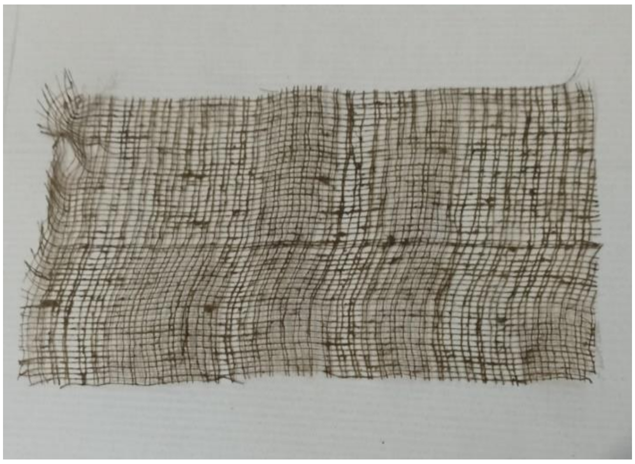


Figure 9: Coated bandage

Evaluation parameters

Evaluating the conc. Liquid extract:

The evaluation parameters of the coated fabric are represented in Tables 9 & 10.

Table 9: The color and pH evaluation of conc. Liquid extract

S. No.	Sample	Color	pH
1.	Ethanolic leaf extract of *Tridax procumbens*	Dark green	8
2.	Ethanolic flower extract of *Tagetes erecta*	Yellow	7
3.	2:1 ratio of *Tridax procumbens* and *Tagetes erecta* extract	Greenish yellow	8

Physical parameters of non-woven fabric loaded with the plant extract:

Table 10: Evaluation of non-woven fabric loaded with the plant extract

S. No.	Parameters	Result
1.	Color	Green
2.	pH	6
3.	Spreadability	Uniformly spread

Conclusion:

The study successfully demonstrates that herbal extracts of *Tridax procumbens* and *Tagetes erecta*, when integrated into a cotton-based bandage, offer a potent, eco-friendly alternative to conventional wound dressings. The 2:1 formulation exhibited notable antimicrobial, antioxidant, and anti-inflammatory activities, all of which are critical for effective wound healing. Phytochemical and FTIR analyses confirmed the presence of key bioactive compounds that contribute to these therapeutic effects. Importantly, the bandage was shown to be biocompatible, non-irritating, and suitable for topical application. By combining traditional medicinal knowledge with modern scientific validation, this research supports the development of a low-cost, biodegradable wound care product that addresses both healthcare and environmental needs. Further exploration into clinical trials and long-term storage stability could pave the way for large-scale adoption in medical practice.

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