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Development and characterization of gelatin-based herbal hydrogels for managing infected wounds

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The present study is aimed at evaluating the antioxidant and antimicrobial properties of aqueous extracts of the plants *Moringa olifera* (drumstick tree or murungai), *Sesbania grandiflora* (vegetable hummingbird or agati), *Basella alba* (malabar spinach or pasalai), and then developing herbal hydrogel with gelatin to improve its pharmacological properties and to use it as a primary dressing for managing infected wounds. The aqueous extracts of murungai, agati, pasalai and the herbal hydrogels has been tested *in vitro* against wound infecting microrganisms by well-diffusion method and micro-dilution method. Both plant extracts and herbal hydrogel are found to inhibit the growth of Gram positive, Gram negative and polymicrobial cultures. According to EN13726, all the three herbal hydrogels manage the simulated exudates effectively. Hence, the hydrogels developed in the present study could be used to manage infection and also moderately exudating wounds.

Keywords: *Basella alba*, Gelatin hydrogel, Herbal hydrogel, *Moringa olifera*, *Sesbania grandiflora*, Vancomycin, Wound healing

1 Introduction

Wounds are the result of injuries in the skin that disrupt the soft tissue. Bacterial infections are a common problem associated with wounds. Healing of a wound is a normal but an intricate biological action which happens in the body in order to repair a damaged tissue. The biological processes are categorized into four different phases, viz. hemostasis, inflammation, granulation and remodelling. During these processes, when the wound is not managed properly, it can get infected by various bacteria which include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* that result in shock, trauma and death[1]. Number of dressings functionalized with antimicrobials is being used by the healthcare practitioners for treating infected wounds. These infections can prolong or impair wound healing, contributing to tissue morbidity and in extreme cases, resulting in sepsis. However, the dressings often fail to achieve other properties required to promote wound healing in spite of their ability to control wound infection. Since an ideal wound dressing is required to promote a moist wound environment, avoid desiccation while absorbing excess exudate and allowing gas exchanges and transpiration, multifunctional dressings have been developed with different textile structures[2].

In recent times, substantial importance is being given to herbal medicine for treating various ailments including skin damage. Several plant extracts and their phytochemicals have been identified as promising alternative for wound healing agents as they pose minimal side effects in contrast to allopathic drugs. In addition, diverse active components, pharmacological properties and ease of access are making the herbal extracts an attractive area of research and development in health care[3]. Commercially herbal extract based medicines are available in the forms of extract/active ingredient coated dressings, herbal oil, powder, ointment, etc and are used for managing infected wounds. As an addition, hydrogel, a type of semi-occlusive wound dressing can be used as permanent or temporary dressings for different type of wounds. This dressing is gaining importance for incorporating herbal extracts as it promotes wound healing by providing moist environment, managing exudate, controlling infection, conforming to the wound bed and mitigating tissue damage. Stability of the extract is also reported to be high at 4˚C when the extract is incorporated in hydrogel[4]. Hence in the present study, an attempt has been made to develop herbal hydrogel with gelatin and the aqueous extract of *Moringa olifera* (drumstick tree or murungai), *Sesbania grandiflora* (vegetable

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hummingbird or agati), *Basella alba* (malabar spinach or pasalai).

The plants are known for their nutritional and therapeutic ingredients and are used to treat various ailments since ancient time. The plant extracts besides other pharmacological properties, are known for their ability to neutralize free radicals and provide excellent support to the body's anti-inflammatory action[5]. Gelatin is a biopolymer derived from the degradation of collagen. Gelatin is one of the biopolymers approved by FDA for drug delivery applications due to its effective dosing property. Moreover, gelatin based hydrogels protect the biomolecules from degradation and release them over extended time periods[6]. In recent years, plants have been used for the treatment of wounds since herbal extracts have proven to be effective in fighting against bacterial infection, and accelerate wound healing. *Moringa olifera, Sesbania grandiflora* and *Basella alba* have been reported to possess anti-microbial, anti-oxidant, anti-cancer and anti-inflammatory activities contributing towards wound healing. Therefore, gelatin based hydrogel dressings are prepared with aqueous extracts of these plants and their biological and mechanical properties are studied in detail and their potential in managing wound is illustrated.

2 Materials and Methods

2.1 Materials

Microbial cultures were procured from ATCC, USA. Gelatin (HiMedia), nutrient medium, antibiotic (vancomycin, HiMedia), other chemicals and solvents were procured from HiMedia Laboratories Pvt. Ltd, Mumbai, India.

2.2 Methods

2.2.1 Collection of Plant and Extraction of Phytochemicals

The leaves of *Moringa olifera* (Drumstick tree), *Sesbania grandiflora* (Vegetable hummingbird), *Basella alba* (Malabar Spinach) were collected from Coimbatore district, Tamil Nadu, India. Hundred gram (100 g) of leaves were shade dried for 12 days and crushed using a hand grinder until the powder became coarse and further used for extraction process. For the extraction, 15 g of the powder was mixed with 150 mL of distilled water and heated for 2 days at 45-55° C. The extract was filtered with Whatman No. 1 paper, dried at 50° C and refrigerated until further use.

2.2.2 Analysis of Phytochemicals

Phytochemicals, such as alkaloids, flavonoids, tannins, anthocyanins, carbohydrates, steroids, phenols, saponins, terpenoids and sugars of aqueous extracts, were analysed as per the standard procedure[7].

Phytochemical composition of methanolic extract of selected plants was studied through GC-MS (Thermo Scientific Co). Phytochemicals were identified by matching the spectra with the Wiley spectral library search programme.

2.2.3 Assessment of Antioxidant Activity

Antioxidant potential of aqueous extract of *M. oleifera, S. grandiflora* and *B. alba* was determined based on their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl), NO (nitric oxide), ferric oxide, ABTS [2,2'-azino-bis(3-ethylben zothiazoline-6-sulfonic acid)] and phosphormolybdenum radicals as per the standard methods[8-12].

2.2.4 Preparation of Herbal and Antibiotic Hydrogel

Gelatin based herbal and antibiotic hydrogel was prepared by dissolving 8 % (w/v) of gelatin in an aqueous extract of selected plants and 2µg/mL of vancomycin respectively. The mixture was stirred with a magnetic stirrer at 70°C and 250 rpm till complete dissolution. Glutaraldehyde was used as a cross linker. The hydrogel was stored at 4°C until further use.

2.2.5 Antibacterial Activity

2.2.5.1 Well Diffusion Method

Aqueous extract of all the plants were tested for their effect on Gram positive *(Staphylococcus aureus*, ATCC 6538*)* and Gram negative *(E.coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 4352) bacteria by using well diffusion method. Briefly, bacterial strains were cultivated in nutrient broth for 24 h at 37 ° C in an incubator. Nutrient agar plates were inoculated by streaking the swab of bacterial strains over the entire sterile agar surface for 2-3 times by rotating the agar plates. Further, the plates were dried at room temperature (37 ° C) under aseptic condition followed by boring of 9 mm diameter wells in them. Extracts and vancomycin, a standard drug, were added in wells. The plates were then incubated at 37°C for 24 h. In the case of herbal hydrogel, instead of well, hydrogel in the size of 9 mm diameter was prepared and placed on the plates. The zone of inhibition of each bacterial strains was measured using a calibrated scale[13].

2.2.5.2 Broth Dilution Method

The test method allows qualitative and quantitative detection of antimicrobial activity of the extracts of *S. aureus, K. pnemoniae, E.coli* and *P. aeruginosa*. For

this test, the overnight culture of bacterial strains was diluted to 1 X 10^5 CFU/mL and was then treated with the aqueous extract of selected plants. Tubes were incubated at 37°C for 24 h. For quantitative estimation, cultures after the treatment were spread plated on nutrient agar and then incubated for 24 h at 37°C. At the end of incubation, colonies were counted using colony counter and the percentage reduction was calculated using the following formula[14]:

$$\% \text{ Reduction} = \frac{\text{Control (cfu/mL)-Treated (Cfu/mL)}}{\text{Control (cfu/mL)}}$$

2.2.6 Evaluation of Primary Wound Dressing Characteristics of Herbal/Antibiotic Hydrogel

Ability of developed hydrogel to functions a primary wound dressing was studied by assessing its potential in managing simulated exudate via absorbency, fluid handling characteristics and dispersion/solubility as per EN13726.

3 Results and Discussion

The present study was aimed at evaluating the antioxidant and antimicrobial activity of the aqueous extract of *Moringa oleifera, Sesbania grandiflora* and *Basella alba* and to develop a herbal hydrogel for managing infected wounds.

3.1 Antioxidant Activity

3.1.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

Antioxidants have been reported to play a significant role in wound healing process by protecting tissues from free radical mediated oxidative damage. DPPH radical scavenging method is commonly used to evaluate the hydrogen donor or free radical scavenging ability of plant extracts[15]. In this study, aqueous extracts of selected plants are scavenged for DPPH radicals in a concentration dependent manner, where the activity is increased with increasing concentration. The highest activity is observed in the aqueous extract of *Moringa oleifera* (97% of inhibition), followed by *Sesbania grandiflora* (95% of inhibition) and *Basella alba* plant (87% of inhibition) (Fig. 1a).

3.1.2 Nitric Oxide Radical Scavenging Activity

Nitric Oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, and is involved in the regulation of various physiological processes. Different concentrations (20-100 mg/mL) of selected plant extracts were investigated for their inhibitory effects on the production of nitric oxide free radical. *S. grandiflora* inhibits more than 95% of NO radicals generation and *M.oleifera* and *B.alba* show 90 % inhibition at 100% concentration. The difference in the inhibitions is analysed (Fig. 1b).

3.1.3 Ferric Reducing Antioxidant Power

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay (FRAP). The flavonoids and phenolic acids present in the medicinal plant exhibit strong antioxidant activity which depends on their potential to form the complex with metal atoms, particularly iron and copper. The reducing property can be a novel antioxidant defence mechanism, possibly through the ability of the antioxidant compound to reduce ferric ion. In the present study, all the extracts show moderate reduction varying between 5% and 32 % at 20% and 100% concentration respectively (Fig. 1c).

3.1.4 Antioxidant Activity by ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]

The formal reduction potentials of ABTS are high enough for it to act as an electron donor for the reduction of oxo species such as molecular oxygen and hydrogen peroxide, particularly at the less-extreme *p*H values encountered in biological catalysis. Similar to ferric reduction, different

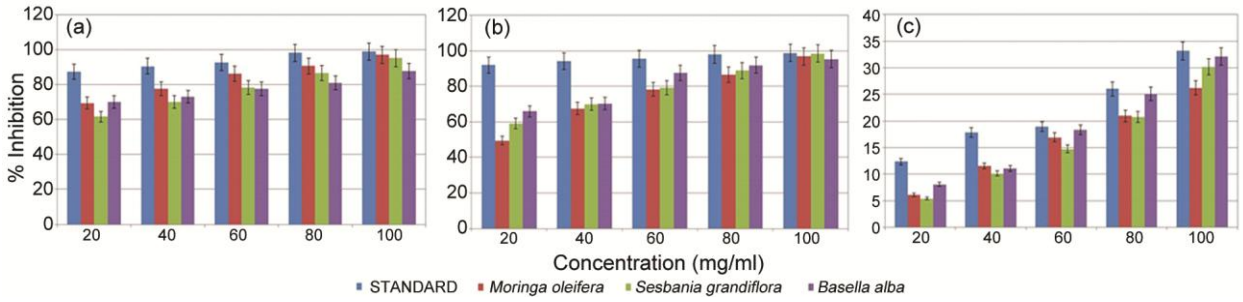

Fig. 1 — Antioxidant activity of the aqueous extract of the selected plants (a) DPPH radical scavenging activity, (b) nitric oxide radical scavenging activity, and (c) ferric reducing antioxidant power

concentrations of selected plant extract exhibit moderate reduction between 3% and 37 % at 40% and 100% respectively.

3.1.5 Phosphomolybdenum Assay

Free radicals, together with secondarily formed radicals, are known to play an important role in the pathogenesis of many chronic conditions. Hence, the study of antioxidant status during a free radical challenge can be used as an index of protection against the development of these degenerative processes in wound healing for therapeutic measures[16]. This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite and this causes a reduction in the intensity of the blue colour. At the lowest concentration of 20 mg/mL, *M.oleifera* exhibits higher reduction. However, at the increasing concentration, the reduction is similar among all 3 plants.

3.2 Antibacterial Activity

The present work elucidates that the aqueous extracts of selected plants possess both Gram positive and Gram negative bactericidal potential and thus can be used for managing infected wounds. The activity of *M.oleifera* varies between 19.5 mm and 26 mm against *E.coli* to polymicrobial culture. *S.grandiflora* is found to be highly effective against *E.coli* (24 mm) followed by polymicrobial culture (19 mm), *S.aureus* (18.5 mm) and *K.pneumoniae* (18.5 mm) and *P.aeruginosa* (16 mm). In the case of *B.alba*, the highest growth inhibition is observed against *K.pneumoniae* with 20.5 mm zone (Table 1 and Fig. 2). On comparing with the standard antibiotic vancomycin, all the plant extracts exhibit comparable activity against test organisms.

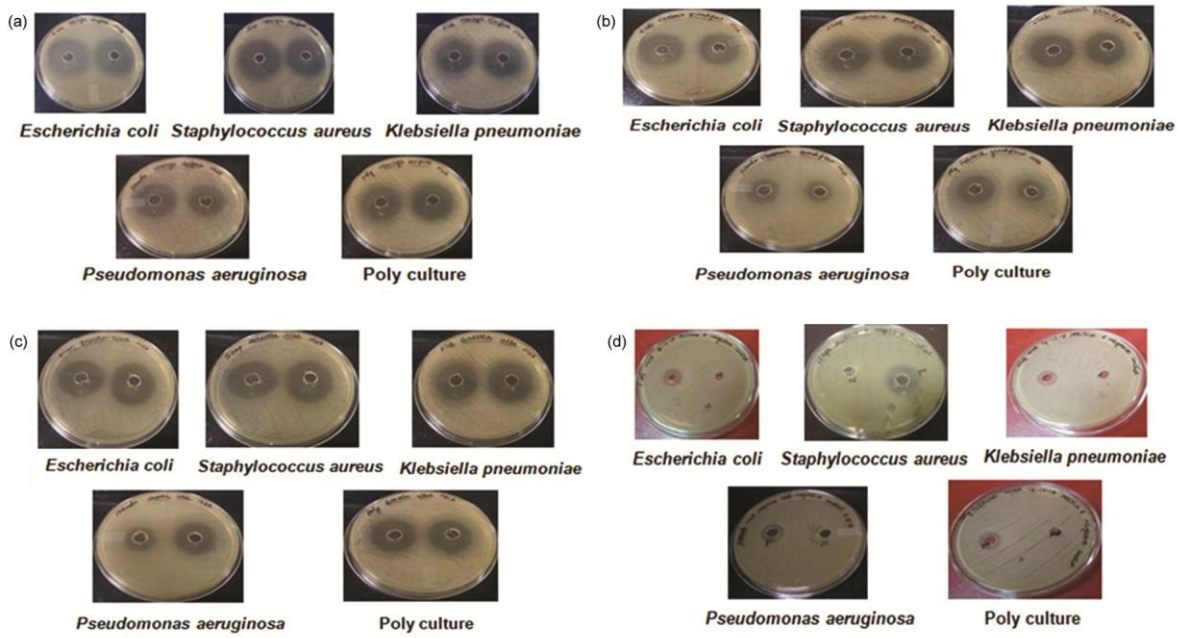

Fig. 2 — Antibaterial activity of selected plant extracts and antibiotic against test organisms (a) *Moringa oleifera*, (b) *Sesbania grandiflora*, (c) *Basella alba*, and (d) Vancomycin

Table 1 — Antibacterial activity of herbal extracts and vancomycin

Bacteria	Zone of inhibition, mm (Qualitative)				% Reduction (Quantitative)			
	M. Oleifera	*S. grandiflora*	*B. alba*	Vancomycin	*M. Oleifera*	*S. grandiflora*	*B. alba*	Vancomycin
E. coli	25	23	20	17	98.2	99.0	97.0	99.0
S. aureus	20	19	17	20	97.8	99.6	98.0	98.4
K. pneumonia	22	19	22	18	> 99.0	99.9	> 99.0	93.5
P. aeruginosa	23	17	16	15	> 99.0	98.6	99.0	99.0
Poly microbial culture	20	20	19	16	> 99.0	98.1	98.0	93.1

* Values are average of duplicates.

Results of broth dilution method further confirms the antibacterial activity of the extract, where the extract of *M.oliefera* completely inhibits the growth of *K.pneumoniae, P.aeruginosa* and polymicrobial culture and exhibits more than 95% reduction against *E.coli* and *S.aureus.* Similarly, extract of *B.alba* shows > 99% reduction against *K.pneumoniae* and > 95 % reduction against other test organisms. Extract of *S.grandiflora* shows its bactericidal activity with more than 98% against all the test organisms. The overall activity of the selected plant extracts is found comparable with that of antibiotic vancomycin (Table 1). The values are depicted using the average of duplicates. As stated by Junior and Zanil (2000), the antibacterial effects can be expressed in term of the zone of inhibition diameter in mm (< 9 mm zone inactive; 9-12 mm moderately active; 13-18 mm active and >18 mm highly active).

Table 2 — Phytochemical screening of aqueous extract of selected plants

Phytochemical analysis (Aqueous extract)	*Moringa oleifera*	*Sesbania grandiflora*	*Basella alba*
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Anthocyanin	+	+	+
Carbohydrates	+	+	+
Steroids	+	+	+
Phenol	+	+	+
Saponin	-	-	-
Terpenoid	+	+	+
Sugar	+	+	+

(+) Presence and (-) Absence.

3.3 Phytochemical Analysis

To identify the bioactive compound responsible for antioxidant/antibacterial activity, the extract was analysed for its phytochemistry. Results show the presence of tannins, alkaloids, flavanoid, terpenoid, steroid, anthocyanin, carbohydrate, etc (Table 2). Tannin is a known biological antioxidant and the concentrated tannins are reported to inhibit and/or destroy filamentous fungi, bacteria and yeast by depressing its adhesion, binding to cell walls, inhibiting the protease activity and preventing their metabolic activity[17]. It is also reported to have a role in metal ion chelation and protein precipitation[18]. Hence, the extract with its rich presence of bioactive substances could have inhibited the growth of microorganisms at both mono and polymicrobial levels as reported earlier[19].

3.4 Gas Chromatography- Mass Spectrometry

Gas chromatography – mass spectrometry (GC-MS) is an analytical method used to identify bioactive substances present in a plant sample. Components present in the extracts of all three plants are compared with the standard at NIST (National Institute of Standards and Technology) library and the details of compound, peak area and their reported biological activity are given in (Fig. 3 and Table 3).

Table 3 — List of phytochemicals identified from *Moringa oleifera*, *Sesbania grandiflora* and *Basella alba* extracts by GC-MS Analysis

Compound	Peak area, %	Biological activity
Moringa oleifera		
5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidazole	14.59	Antimicrobial
Benzene, methyl- (CAS)	2.07	Antimicrobial and cytotoxic
Dodecane (CAS)	4.96	Natural flavoring substance
Benzene, 1,3,5-trimethyl- (CAS)	6.81	Precursor to diverse fine chemicals
Memantine	3.73	Polypharmacophoric agents
Sesbania grandiflora		
2,7-Bis(2,5-di-t-butylphenyl)benzo[lmn][3,8] phenanthroline-1, 3,6,8-tetrone	0.96	Antifungal
1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo [f]quinazoline	1.12	Anti-inflammatory
Isopropyl myristate	14.80	Emollient - softens the skin
1-benzyloxymethyl-1-hydroxymethyl -2,5-cyclohexadiene	20.21	Moisturizer
2,6-Pyrazinediamine (CAS)	0.53	Antibacterial
Basella alba		
5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-Benzimidazole	16.28	Antimicrobial
Memantine	5.05	Polypharmacophoric agents
Benzene, methyl- (CAS)	16.50	Antimicrobial and cytotoxic
(Z)-N-(2-Chloro-2-pentenoyl)piperidine	7.20	Antibacterial
3,7,8-Trimethylpyrido[2,3-d]pyrimidine-2(3H)-,4(8H)-dione	1.51	Anticancer

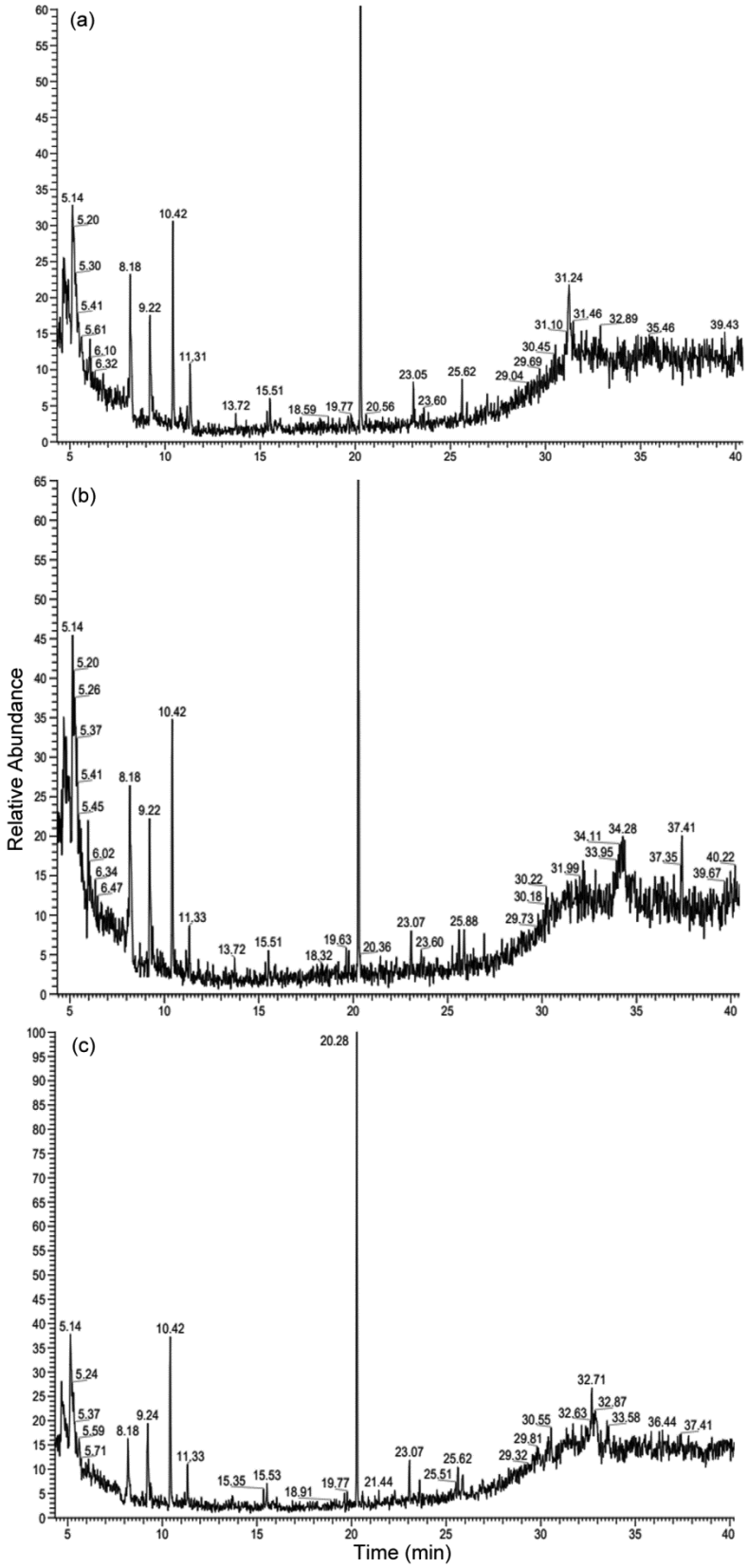

Fig. 3 — GC-MS spectra of selected plants (a) *Moringa oleifera*, (b) *Sesbania grandiflora*, and (c) *Basella alba*

3.5 Preparation of Herbal/Antibiotic Hydrogel and Evaluation of its Primary Wound Dressing Characteristics and Antimicrobial Activity

Various methods are reported for the preparation of hydrogel dressings which include physical (ionic interaction, hydrogen bond, hydrophobic interaction, protein interaction and crystallization) and chemical (conjugation reaction, free radical polymerization reaction, and the enzymatic reaction) cross linking (Table 4)[20]. These methods determine the physical and chemical properties and functions of hydrogel dressings. List of commercially available hydrogel and their application is given in Table 5[21]. In the present study, physical cross linking method has been used to develop gelatin based herbal hydrogel where the gelatin is dissolved in the aqueous extract of selected plant extract (8 % w/v). The interaction between hydrogen of gelatin and different polyphenols of herbal extract is expected to form a stable hydrogel. In the case of antibiotic hydrogel, vancomycin at the concentration of 2µg/mL is added in 8 % gelatin (Fig. 4). The hydrogel so developed is further assessed for its ability in managing wound exudates in accordance to EN 13726.

3.5.1 Assessment of Primary Wound Dressing Characteristics

(i) Fluid affinity

The fluid affinity of hydrogels prepared in this study has been assessed to determine the way in which they influence the moisture content of a wound as per the method described in EN 13726. This standard investigates the fluid affinity by measuring the ability of hydrogel to donate moisture to, or absorb liquid from standard substrates, i.e. agar and gelatin. If a hydrogel has the ability to donate its fluid, it is expected to facilitate rehydration of dry necrotic tissue or slough, thus promoting autolytic debridement. In contrast, if the hydrogel has marked affinity towards the liquid, it can be used to absorb excess wound exudates to liquefy tissue debris after

Table 4 — Preparation method of hydrogels for wound healing

Methods	Preparation	Example
	Physical cross-linking	
Ionic interaction	Dynamic interaction between oppositely charged groups or metal-ligand interaction is an effective way to carry out ionic interactions	Hydroxypropyltrimethyl ammonium chloride chitosan (HACC)/poly(acrylic acid) (PAAc)-Fe^{3+} hydrogel
Hydrogen bond	Hydrogen bond interaction is a dynamic interaction. Hydrogels are prepared by physical cross-linking	Polyacrylamide(PAM)-based hydrogel
Crystallization	Polymer chains are formed by ice crystals	Polyvinyl alcohol (PVA) and hyaluronic acid (HA) hydrogel
Hydrophobic interaction	Hydrophobic interaction is a method of cross-linking hydrogels in water-soluble polymers with hydrophobic end groups, hydrophobic side chains, or hydrophobic monomers	Polyacrylamide (PAAm) and polyacrylic acid (PAAc) hydrogel
Protein interaction	Hydrogels are prepared with natural polymers such as gelatin, collagen, silk fibroin, matrix glue, and so on. Through non-covalent bond interactions between proteins or polypeptides, conditions such as temperature and phase transition are changed to form protein or polypeptide hydrogels	RHC (Rrecombinant human collagen) hydrogel
	Chemical cross-linking	
Conjugation reaction	Hydrogels cross-linked by the conjugation reaction have become a hot spot. The conjugation reaction can be carried out under relatively mild conditions, including the Michael addition reaction, the Schiff's base reaction, and the Diels–Alder addition reaction	Quaternized chitosan (QCS*)* hydrogel
Free radical polymerization	Free radicals can be produced by heating, ultraviolet radiation, high energy radiation, electrolysis, and plasma initiation	Polyacrylamide(PAM)-based hydrogel
Enzymatic reaction	The enzymatic reaction is the cross-linking of natural polymers catalyzed by enzymes such as transglutaminase, tyrosinase, urease, and horseradish peroxidase (HRP)	Hyaluronic acid (HA) hydrogel

Table 5 — Commercially available hydrogel and their application

Company name	Product name	Hydrogel form	Application
DermaGauze™	DermaRite industries	Hydrogel impregnated gauze dressing	• Acute wounds • Chronic wounds • Partial wounds • Full thickness wounds
Restore Hydrogel	Hollister Incorporated	Hydrogel impregnated gauze pad contains hyaluraonic acid and promote wound healing by autolytic debridement	• Partial wounds • Full thickness wound
ActivHeal®	Advanced Medical Solutions Ltd.	It is a primary wound dressing contains 85% water	• Pressure ulcers • Leg ulcers • Diabetic foot ulcers • Cavity wounds
DermaSyn®	Derma Rite industries	It's a primary wound dressing contains vitamin E	• Acute wounds • Chronic wounds • Partial wounds • Full thickness wounds
NU-GEL™	Systagenix	Contains sodium alginate which effectively debrides necrotic tissue and fibrinous slough	• Chronic wound • Diabetic foot ulcers • Venous leg ulcers • Pressure ulcers
Purilon®	Coloplast	Contains calcium alginate and sodium carboxymethyl cellulose with purified water	• Leg ulcers • Pressure ulcers • Non-infected diabetic foot ulcers • First and second degree burns
Woun'Dres®	Coloplast	It contains the polymers like carbomer and collagen with other ingredients	• Dry wounds

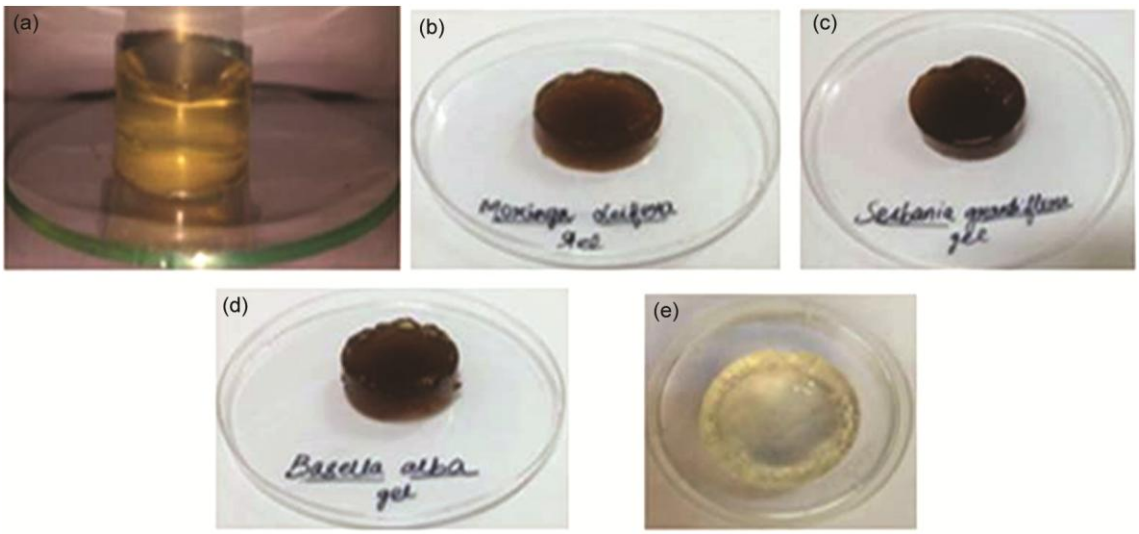

Fig. 4 — (a) Gelatin hydrogel, (b) *Moringa oleifera* gel, (c) *Sesbania grandiflora* gel, (d) *Basella alba* gel, and (e) Vancomycin gel

the autolytic debridement. A hydrogel that combines both absorption and moisture donating properties should therefore be suitable for the treatment of a wider range of wound types. Depending on the relative level of hydration of the tissue and the dressing, hydrogels can either absorb or contribute moisture to the wound. Hydrogels are commonly utilised to aid autolytic debridement and to maintain

moist in the wounds during healing process[22]. The result of the present study shows that the plain hydrogel donates its moisture to the agar and increases its weight from 20 g to 24.9 g (24.5%), whereas all the herbal hydrogels absorb moisture from the gelatin surface and its weight is increased from 5 g to 7.4 g (48 %). From this result, the plain gelatin can be categorised under Type 3a and herbal extract incorporated hydrogels under Type 1e, which can be used for treating moderately exudating wounds.

(ii) Dispersion/Solubility Characteristics of Hydrogel

The test method detects the stability of the hydrogel when it is placed on highly exudating wound. In this method, the hydrogel is immersed in 70 mL simulated exudates and is shaken for 2 min to allow dispersion or dissolution. During shaking, rigidity of all the hydrogels is altered and further dissolved in the simulated exudates during 2 h of incubation. This result suggests that the gel may be dissolved when used on highly exudating wounds and hence can be suitable for the wounds that are moderately exudating.

(iii) Absorbency

Hydrogels are found to take minimum 2 h to maximum of 4 h to absorb the simulated exudates. Absorption of simulated exudates is a key to determine the behaviour of hydrogel when treating low to highly exudating wounds. Since the hydrogel has taken a relatively longer time to absorb simulated exudates, the herbal hydrogel is suitable for managing moderately infected wounds.

3.5.2 Antimicrobial Activity

In the case of chronic wounds, bacteria interfere with the healing process without obvious clinical signs of infection (critically colonized), and resulting in the amputation of affected part in most of the cases. Antimicrobial dressings which include iodine based preparations and silver-releasing agents are widely used, as they possess strong bacteridical effect against both Gram positive and Gram negative bacteria. Some of the commercially available iodide and silver based dressings used for the management of infected wounds include Iodosorb gel (cadexomer iodine) Sorbact®, and Aquacel Ag® (ConvaTec), Acticoat 7 (Smith and nephew), Actiosorb Silver 220 (Johnson and Johnson), Silversorb (medline), and Silvercel (Johnson and Johnson)[23].

Though the dressings are effective in managing infected wounds, healthcare practitioners are always on the search for an ideal wound dressing, as the available dressings are not sufficient enough to provide suitable micro-environment for the wound to heal. Hence, new dressings are often being released into the market by the multinational companies. In the present study, the herbal hydrogel is tested against wound specific bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and polymicrobial culture. All three hydrogels inhibit the growth of Gram positive, Gram negative and polymicrobial culture and the activity is comparable with the extracts of the selected plant. Moreover, compared to antibiotic hydrogel, overall antimicrobial activity is higher in the case of herbal hydrogel, between 96.4 & 9.9%, 93.2 & 98.5% and 90 & 98.5% for *M.oleifera*, *S. grandiflora and B. alba* respectively (Table 6).

Ayurveda, Siddha, and Unani are traditional Indian medical systems that use a variety of herbal plants to treat skin disorders, such as cuts, scars and burns. For a long period of time, these medicinal herbs are being used to cure a variety of skin diseases. The significant advantage of using ethno medicinal plants for wound treatment includes no side effects as compared to the chemical drugs which have their side effects[24]. Plants have been shown to have wound-healing capability, as well as the ability to reduce oxidative stress and inflammation[25].

Plant-based active components have been proposed as a viable therapeutic intervention for the successful management and treatment of wounds, and they have been linked to antibacterial and antioxidant properties. Another factor for wound infection is the

Table 6 — Antibacterial activity of herbal and vancomycin hydrogel (Quantitative)

Bacteria	Antibacterial activity, %			
	M. oleifera gel	*S. grandiflora* gel	*B. alba* gel	Vancomycin gel
Escherichia coli	98.0	97.0	90.0	98.0
Staphylococcus aureus	96.0	98.0	95.0	93.0
Klebsiella pneumonia	> 99.0	98.0	98.0	91.0
Pseudomonas aeruginosa	> 99.0	96.0	96.0	98.0
Polymicrobial culture	> 99.0	93.0	94.0	90.0

susceptibility of growth of bacteria in the wound region in response to infection. When it relates to antibacterial action of herbals, flavonoids and phenolic contents have inhibiting effects on several microbes[26]. Plant-based bioactives have been shown to help in wound care and treatment by speeding up the epithelization process and reducing skin scarring. Wound healing is a homeostatic process that includes re-epithelization and tissue matrices collagen repair. Although it is an innate immune response that occurs on its own, variables such as tissue ROS production, inflammation, and microbial infection slows the process of healing and raises the risk of secondary infection. The currently available medicines do not have a significant influence on tissue repair, necessitating the development of novel drugs with a multimodal approach to tissue repair. Topical wound remedies should have bioavailability, non-toxicity, and efficacy, and plant-derived bioactives would be a preferable alternative. With quick wound contraction, a shorter epithelization phase and dense collagen remodelling, it provides rapid wound healing[27].

The leaves of *M. Oleifera* are known to contain a number of phytochemicals, such as flavonods, saponins, tannins and other phenolic compounds. *M. oleifera* leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria which could partly explain the observed bactericidal activity. *S. grandiflora* contains many enzymatic and non-enzymatic antioxidants due to presence of phytochemicals and could be a good source of dietary antioxidants which play an important role in the prevention of diseases associated with oxidative stress. *B. alba* is a rich source of ascorbic acid & phenolic compounds, and has a high antioxidant potential which may be used against oxidative stress. Findings of the current study suggest that the aqueous extracts of all 3 plants have the potential to inhibit the proliferation of both Gram positive and Gram negative bacteria. The antibacterial activity of the extracts could be due to their rich phytochemicals which could have disrupted the membrane or could have disturbed molecular chemistry of the bacteria. Free radical scavenging potential of the extracts is beneficial in wound healing where the generation of free radicals is expected to be high during inflammatory phase. Thus, scavenging of the free radicals wound assists healing process to proceed with its normal phases of healing, such as hemostasis, inflammation, proliferation and remodelling. The extracts of all 3 plants show concentration dependent antioxidant activity and are found to scavenge all possible free radicals effectively.

Gelatin-based hydrogels have exhibited many attractive aspects for uses and improvements in biomedical applications, including drug delivery devices, tissue engineering scaffolds and wound dressings. Hydrogels prepared from an aqueous extract of plants are quite stable, which is observed in the present study also. Though the plants are different, antioxidant, antimicrobial and primary wound dressing characteristics of aqueous extract of all three herbal hydrogels are found to be similar. Hence, the present study opens a new platform to explore the feasibility of using herbal hydrogel as an alternate to antibiotic hydrogel by investigating its potential using *in vivo* models and through clinical studies.

4 Conclusion

Wound care materials should provide a warm and moist environment for a rapid healing process; in addition, they should prevent the proliferation of bacteria around the wound area. In the present study, an attempt has been made to develop herbal extracts and vancomycin loaded gelatin hydrogel for moist wound healing and to improve the healing of infected wound. Gelatin, a thermo reversible biopolymer is optimized to 8% w/v with 3 different plant extracts and then evaluated for their antimicrobial and primary wound dressing characteristics. Herbal extracts and herbal extract incorporated gelatin hydrogel show appreciable antimicrobial activity against Gram positive, Gram negative and polymicrobial cultures. Herbal hydrogel shows higher affinity towards exudate and is dispersed completely when kept in simulated exudate as compared to antibiotic hydrogel. Results of the study suggest that the herbal hydrogel developed in the study could be used for managing infected and moderately exudating wounds. However, detailed *in vivo* and clinical studies are required to confirm their ability to manage infected wounds.

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