International Journal for Modern Trends in Science and Technology, 7(01): 58-64, 2021

ISSN: 2455-3778 online
DOI: https://doi.org/10.46501/IJMTST070113
Available online at: http://www.ijmtst.com/vol7issue01.html

Preparation of Gelatin Film Incorporated with Extracts of *Hemigraphis Colorata* Used as Wound Dressing

Sandhiya. S[1] | Sabarinath. K[1] | Ishwarya. R[1] | Logeshwaran. V[1] | Kousalya. N[1] | Dr. Arun. P[2]

[1]Student, Department of Biotechnology, Dr. N. G. P. Arts and Science College, Coimbatore, India.
[2]Associate Professor, Department of Biotechnology, Dr. N. G. P. Arts and Science College, Coimbatore, India.

To Cite this Article
Sandhiya. S, Sabarinath. K, Ishwarya. R, Logeshwaran. V, Kousalya. N and Dr. Arun. P, "Preparation of Gelatin Film Incorporated with Extracts of *Hemigraphis Colorata* Used as Wound Dressing", *International Journal for Modern Trends in Science and Technology*, Vol. 07, Issue 01, January 2021, pp.- 58-64.

Article Info
Received on 22-November-2020, Revised on 18-December-2020, Accepted on 28-December-2020, Published on 03-January-2021.

ABSTRACT

Hemigraphis coloratais a medicinal plant originates from the tropical regions of kerala and its native is from the sides of Malesia. It is a perennial herb mostly grown as ornamental plant. It is a tribal plant of the tribes of Kerala. It is commonly called as "murikooti or muryanpacha"H.coloratais used as herbal ointments to cure cut wounds, ulcers and most importantly bleedings. It has many valuable properties like anti-microbial and anti-diabetic activities. It acts as an anti oxidant and exhibit free radical scavenging activity. This study is carried out to prepare a gelatin film using the extracts of H. colorata used as wound dressing.

KEYWORDS:*Hemigraphis colorata, anti microbial activity, gelatin film.*

INTRODUCTION

Wound is explained as disruption of cellular, anatomical and functional continuity of a living tissue, mainly produced by physical, chemical, thermal, microbial, or immunological trauma to the tissue (G.S.Lazarus *et al.*1994). The different processing steps comes under the healing of wound are inflammation, wound contraction, reepithelialization, tissue remodeling and formation of granulation tissue with angiogenesis (Mittal arun *et al.* 2013)

The healing process includes the interaction of cell to cell and cell matrix which allow to proceed in an overlappingfaces. The wound may be an inflammation, loss of blood and so on. The process of wound healing involves inflammation, wound contraction, reepithialization (i.e) formation of new skin, tissue remodeling and formation of granulation tissues. Wound infection is probably the most common reason for impaired **wound healing** Lazarus *et al.* 1994. ***Staphylococcus aureus***,*Streptococcus pyogenes, Corynebacterium* sp., ***Escherichia coli*** and ***Pseudomonas aeruginosa*** are some important organisms causing wound infection (Kumar *et al.* 2006).

Wound healing herbal extracts promote blood clotting, fight infection, and accelerate the healing of wounds. Phytoconstituents derived from plants need to be identified and screened for antimicrobial activity for management of wounds. The study must be conducted after obtaining approval of the Ethics Committee and according to the guidelines for care and use of animals. The prepared formulations of herbal extract can be evaluated by various physicopharmaceutical parameters. The wound healing efficacies of various herbal extracts have been evaluated in excision, incision, dead

space, and burn wound models. *In vitro* and *in vivo* assays are stepping stones to well-controlled clinical trials of herbal extracts (Rupesh Thakur et al. 2011).

Transparent film dressings provide a moist, healing environment; promote autolytic debridement; protect the wound from mechanical trauma and bacterial invasion; and act as a blister roof or "second skin." Because they're flexible, these dressings can conform to wounds located in awkward locations such as the elbow.

In our studies the wound healing property of *Hemigraphis colorata* plays a vital role in treating and curing the wounds.

India has wide variety of herbal plants which are used as medicines by the peoples to cure diseases these types of medications used by us is known as phytopharmaceuticals or phytotherapeutic agents. In such wide varieties of herbal plants in this paper we are going to know about the properties of *Hemigraphis Colorata.*

Hemigraphis colorata is an herbal plant mostly grown as an ornamental plant. Its origin is from tropical regions of Malay Archipelago and its native is from theSouth EastAsiaand also the cascades over Northern Queensland. The characterizations of Hemigraphis colorata are as follows; It is a perennial herbal plant form about 15 to 30 cm. It is a prostate herb which has toothed leaves and purple citations in leaves. The colour of the leaves is greyish green andhas small white flowers. The flowers are white in colour and have five lobes. It is bell shaped.

The phytopharmaceutical properties of *Hemigraphis colorata* are: It is most commonly used to treat bleeding wounds, cuts and inflammation and internally used to cure ulcers, hemorrhoids, diuretics, gall stones, anemia and diabetes mellitus.

PLANTPROFILES

Scientific classification:

Kingdom : Plantae
Order : Lamiales
Family : Acanthaceae
Genus : *Hemigraphis*
Species : *colorata*

A wound dressing material was successfully prepared from alginate, a natural polymer capable of forming into hydrogels, and asiaticoside (PAC), a substance from the plant *Centella asiatica* which has commonly been used in traditional medicine to heal wounds (Panprung Sikareepaisan et al. 2010).

Biological dressings are frequently employed clinically, but they are associated with some disadvantages (high antigenicity, limited supplies, poor adhesiveness and risk of cross-contamination). Synthetic dressings have a long shelf life and create minimal inflammatory response without any risk of pathogen transmission. Biologic–synthetic dressings are bilayered structures with high polymeric and biological content (P. Bruin *et al.* and S. Suzuki *et al.* 1990).

Gelatin is a natural biopolymer derived from controlled hydrolysis of the fibrous insoluble collagen present in the bones and skin and has good biocompatibility and biodegradability. It is an effective biomaterial for using as wound dressing since it can absorb wound exudates and provide moist environment to a wound leading to acceleration of wound healing (Gomez-Guillen and others 2011). Collagen gives skin its healthy and youthful appearance. As people age, they lose collagen. Their skin becomes less firm, and wrinkles and lines develop.Gelatin may be a natural way to boost collagen and improve the skin's appearanceGelatin has been one of the most well-studied protein based materials because of its excellent film-forming property and its usefulness as an outer film to protect food by acting as a barrier to gases, and it is produced at relatively low cost all over the world (R. Núñez-Flores et al. 2013).

Gelatin-based films usually present good mechanical resistance and high elasticity but are also sensitive to environmental conditions, as

relative humidity, and are affected by several factors such as pH, heat treatment, addition of plasticizers, ion concentration, protein concentration, and its molecular conformation (I. S. Arvanitoyannis 2002). An alternative to enhance the gelatin-based films properties that has attracted the interest of researchers is related to the reinforcement of films with nanoparticles, producing a material often called bio-nanocomposites or only nanocomposites. However, gelatin itself has no antimicrobial activity to prevent wound infection. Silver nanoparticles (Vimala and others 2010) and Zinc oxide nanoparticles (Li and others 2010) successfully incorporated to gelatin films as antimicrobial agent. Natural phenolic monoterpenes derived from herbal medicines also can be added to the gelatin films as antimicrobial agent to improve its antimicrobial activity.

Here in this study the gelatin films with antioxidant and antimicrobial activities were prepared from gelatin solutions containing different concentrations of ethyl acetate extracts of *Hemigraphis colorata* to formulate wound dressing hydrogel film to check its efficacy as an ideal wound dressing film.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The fresh plant leaves were collected from Kerala district and transferred to the laboratory. Taxonomic identities of plant were confirmed by Botanical Survey of India, Coimbatore, Tamil Nadu, India.

Preparation of Leaf extract

The fresh leaves collected were washed thoroughly under tap water and shade dried at room temperature for 2-3 weeks. The dried leaves were coarsely powdered with the help of an electric blender. The extraction were carried out by Soxhlet extraction with ethyl acetate as a solvent for 24 hours. The extract were dried until all the ethyl acetate evaporated and mixed with DMSO and transferred to sterile air tight bottles and stored at 4˚C until required for use.

Leaf extract dissolved in DMSO

The evaporated extract leftover was weighed and dissolved in pure DMSO. (i.e 100mg of plant extract in 1ml of pure DMSO then dilutions were made in 5%DMSO).

Phytochemical Studies with plant extract

Preliminary qualitative analysis of the aqueous extract was carried out by employing the standard conventional methods (Reshma Rajeev K *et al.*2018).

Test for Carbohydrates

To 2 ml test solution add 2 drops of the molish reagent. The solution is the poured slowly into test tube containing 2ml of concentrated sulphuric acid.So that two layers form. The formation of a purple product at the interface of the 2 layers indicated the presence of carbohydrates.

Test for Protein

It is used to determine the presence of peptide bounds in protein. To 3ml of test sample add 3% sodium hydroxide and few drops of 1% copper sulphate. The solution turns from blue to violet (purple) indicated the presence of protein.

Test for Starch

Mix 3ml test solutions. A few drops of dilute iodine solutions. Blue colour appear. It disappears on boiling and reappears on cooling.

Test for Steroids

To 2ml of extract add 2ml chloroform & add 2ml concentrated sulphuric acid. Shake well; chloroform layer appear red and acid layer show greenish yellow fluroscence which indicated the presence of steroid.

Test for amino acid

To 5ml of test sample solution add a few drops of 40% sodium hydroxide &10% lead acetate boiled the solution formation of black precipitate indicated the presence of amino acid.

Test for Glycosides

To the extract add Glacial acetic acid, few drops of 5% ferric chloride and concentrated sulphuric acid are added and observed for a reddish-brown coloration at the junction of the two layers, and bluish green colour in the upper layer which indicated the presence of glycoside

Test for Flavonoid

To 2ml of extract add few drops of ammonia solution. A yellow coloration was observed for the presence of flavonoid

Test for Alkaloid

To 0.5g of each extract add 5ml of 1% aqueous hydrochloric acid and kept in water bath, 1ml of filtrate is to be treated with Mayer's reagent. Formation of a yellow coloured precipitate indicated the presence of alkaloids

Test for Tannin

To 0.5ml of extract 1ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannin and green black for catecholic tannin.

Test for Saponins

To 1ml extract add 2ml distilled water and shake it persistent foam indicated the presence of saponin.

Test for Terpenoid

2ml of extract was mixed with 2ml chloroform in a test tube. To this 3ml concentrated sulphuric acid was carefully added alone the wall of the test tube, an interface with reddish brown colouration confirmed the presence of terpenoid.

PREPARATION OF WOUND DRESSING HYDROGEL FILM

Hydrogel is simply a hydrophilic polymeric network cross-linked in some fashion to produce an elastic structure. Thus, any technique which can be used to create a cross-linked polymer can be used to produce a hydrogel. It is an excellent source for providing moisture to a dry lesion, hydrogel dressings act fast to help cool down a wound. For preparation of hydrogel, Gelatin is used as gel base or thickener and glycerol as a plasticizer.

10g of gelatin powder was dissolved in 100ml of boiling water (i.e 10% gelatin (w/v)). To these varied concentrations of plant extracts were added and 25% of glycerol (v/v) was added as a plastizicer. After proper mixing the formulation was poured into a sterile plastic petriplate and kept it for about 48 hours at room temperature for proper film formation.

Anti bacterial assay for the extract and film

Bacterial strains:*Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas* sps isolated from the clinical sample obtained from KMCH Laboratory, Coimbatore used for our study. Strains were brought to pure culture on Nutrient agar plates and maintained at 4°C.

Well diffusion method

The well diffusion method was adopted according to kavanagh, (1972) to assess the anti bacterial activity of the prepared extracts and extract loaded gelatin films. Muller hinton agar plates were prepared and a well was cut using sterile cork borer. The wells were filled with different extracts of 50µl, 75µl and 100µl respectively and allow diffusing of plant extract into the medium for about 45minutes and then kept in an incubator at 37°C. After 24 hours, the agar plates were examined for inhibition zones, and the zones were measured in millimeters. Microbial growth was determined by measuring the diameter of zone of inhibition. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

RESULT AND DISCUSSION

Collection and Authentication of Plant Material

Plant leaves were collected from Kerala district and transferred to the laboratory. The taxonomic identities of Plant were confirmed by botanical Survey of India, Coimbatore, Tamil Nadu, India.

Preparation of Leaf extract

Fresh leaves collected were washed thoroughly under tap and shade dried at room temperature for 2-3 weeks. The dried leaves were coarsely powdered the help of an electric blender. The extractions were carried out by Soxhlet extraction with ethyl acetate as a solvent for 24 hours. The extract were transferred to sterile air tight bottles and stored at 4˚C until required for use.

Phytochemical analysis with plant extract

The phytochemical analysis of the ethyl acetate extracts of *Hemigraphis colorata* was done. The presence of carbohydrate, glucoside, steroids, amino acids, glycosides, flavonoids, terpenoids, tannin and coumarins were observed as shown in table 1. Sheu *et al.* (2012) observed the presence of phenols, saponins, flavonoids, terpenoids and carboxylic acid in the aqueous extract of *H.colorata.*

Preparation of wound dressing hydrogel film

To prepare the film 10g of gelatin powder was dissolved in 100ml of boiling water (i.e 10% gelatin (w/v)). To these varied concentrations of plant extracts were added and 25% of glycerol (v/v) was added as a plastizicer. After proper mixing the formulation was poured into a sterile plastic petridish and kept it for about 48 hours at room temperature for proper film formation.

The proper film formation was done by adding 20ml of 10% gelatin, 8 ml of glycerol and 5 ml of extract.

Anti bacterial assay for the extract

The bacteria such as *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas* sps was used for the studies. The antibacterial activity of hydrogels and extracts was quantatively assessed by the presence or absence of inhibition zone and zone diameter as shown in table 2. The Standard antiobiotic disc (Gentamycin 30mg) showed zone of inhibtion of, 10mrn, 8mm and 10 mm respectively for the bacterias such as *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas* sps. The zone of inhibition for the extract was 7.5mm, 8 mm, 9.5mm. The extract showed high activity against clinical pathogens like *Pseudomonas aureginosa* and *Klebsiella pneumonia* the lesser activity against *Staphylococcus aureus* when compared to the standard antibiotic agent.

Table 1.Phytochemical analysis of plant extracts

Phytochemical test	Result
Carbohydrates	Positive
Proteins	Negative
Starch	Negative
Glucoside	Positive
Steroids	Positive
Amino acids	Positive
Glycosides	Positive
Flavonoids	Positive
Alkaloids	Negative
Saponins	Positive
Terpenoids	Positive
Tannins	Positive
Coumarins	Positive

Table 2.zone of inhibition of extract

Organisms	Zone of inhibition(mm)		
	Positive control (Gentamycin)	Negative control (water)	Extract
Klebsiella pneumonia	10	0	7.5
Staphylococcus aureus	8	0	8
Pseudomonas aureginosa	10	0	9.5

Figure 1.Anti bacterial activity of the extract against *pseudomonas*

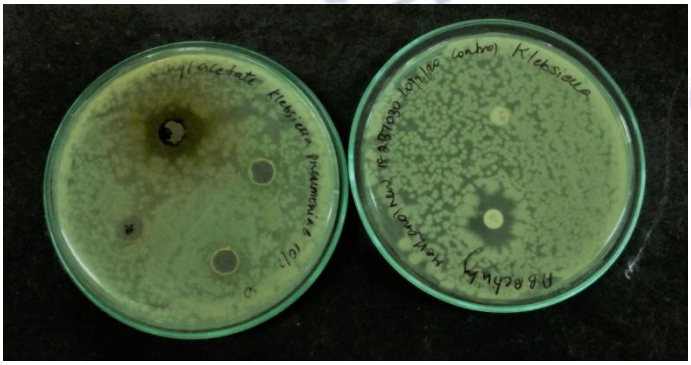

Figure 2.Anti bacterial activity of the extract against *klebsiella pneumoniae*

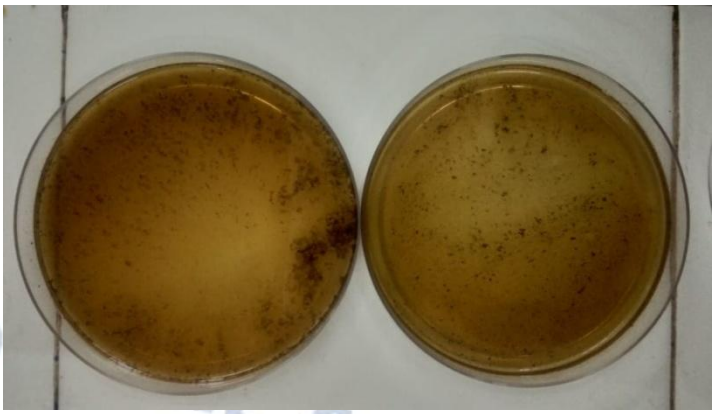

Figure 3. Hydrogel

CONCLUSION

The present study was an attempt to prepare an herbal wound dressing hydrogel from the leaf extract of *Hemigraphis colorata*. The sample plant was collected from Kerala district and subjected for soxhlet extraction for the aqueous extraction from the leaves. This extract was subjected to phytochemical analysis and hydrogel preparation. The extract and hydrogel was tested for antibacterial activity.

The investigations for phytochemical analysis revealed the presence of flavonoids, saponins and phenolic compounds. The antibacterial activity revealed that the hydrogel showed high activity against the selected pathogens like *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas* sps. In future hydrogel may be tested for its wound healing properties. Here by in this studies the proper formulation of hydrogel and its anti microbial activity was studied.

ACKNOWLEDGEMENT

The author express their gratitude towards the host institution Dr.N.G.P Arts and Science College and DST-FIST Scheme, DBT Star scheme, Management, Principal, Deans, Head of the department,Guide and other all other staffs of Department of Biotechnology for rendering all the facilities and support. Communication no:DrNGPASC 2020-21 BS009

REFERENCES

[1] Abeer Temraz and Walid H EL- Tantawy. Characterization of antioxidant activity of extract from Artemisia vulgaris. Pak. J, Pharm. 2008; 21:321-326.

[2] Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *Journal of advanced research*, *6*(2), 105-121.

[3] Annapoorna, M., Kumar, P. S., Lakshman, L. R., Lakshmanan, V. K., Nair, S. V., & Jayakumar, R. (2013). Biochemical properties of Hemigraphis alternata incorporated chitosan hydrogel scaffold. *Carbohydrate*

polymers, *92*(2), 1561-1565.
Jones, A., & Vaughan, D. (2005). Hydrogel dressings in the management of a variety of wound types: A review. *Journal of Orthopaedic nursing*, *9*, S1-S11.
[4] Arun, M., Satish, S., & Anima, P. (2013). Herbal boon for wounds. *wounds*, *6*(7), 8.
[5] Bruin P, Jonkman MF, Meijer HJ, Pennings AJ. A new porous polyetherurethane wound covering. J Biomed Mater Res 1990;24:217–26
[6] Das, U., Behera, S. S., Singh, S., Rizvi, S. I., & Singh, A. K. (2016). Progress in the development and applicability of potential medicinal plant extract- conjugated polymeric constructs for wound healing and tissue regeneration. *Phytotherapy research*, *30*(12), 1895-1904.
[7] M. Devipriya. Review on pharmacological activity of Hemigraphis colorata (Blume).International journal of herbal medicine, 2013; 1(3): 120-121.
[8] Ferguson, M. W., & O'Kane, S. (2004). Scar–free healing: from embryonic mechanisms to adult therapeutic intervention. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1445), 839-850.
[9] Gayathri V, Lekshmi P, Padmanabhan RN. Antidiabetes and hypoglycaemic properties of Hemigraphis colorata in rats. International J. Pharam Science. 2011; 4(2):224-328.
[10] Gómez-Estaca, J., Gómez-Guillén, M. C., Fernández-Martín, F., & Montero, P. (2011). Effects of gelatin origin, bovine-hide and tuna-skin, on the properties of compound gelatin–chitosan films. *Food Hydrocolloids*, *25*(6), 1461-1469.
[11] Gupta, S. S., Singh, O., Bhagel, P. S., Moses, S., Shukla, S., & Mathur, R. K. (2011). Honey dressing versus silver sulfadiazene dressing for wound healing in burn patients: a retrospective study. *Journal of cutaneous and aesthetic surgery*, *4*(3), 183.
[12] Han, G., & Ceilley, R. (2017). Chronic wound healing: a review of current management and treatments. *Advances in therapy*, *34*(3), 599-610.
[13] Huang, X., Zeng, Z., & Zhang, H. (2013). Metal dichalcogenide nanosheets: preparation, properties and applications. *Chemical Society Reviews*, *42*(5), 1934-1946.
[14] Islam, R., Nazifa, T. H., Yuniarto, A., Uddin, A. S., Salmiati, S., & Shahid, S. (2019). An empirical study of construction and demolition waste generation and implication of recycling. *Waste Management*, *95*, 10-21.
[15] Jayakumar, R., Prabaharan, M., Kumar, P. S., Nair, S. V., & Tamura, H. (2011). Biomaterials based on chitin and chitosan in wound dressing applications. *Biotechnology advances*, *29*(3), 322-337.
[16] Joy, R., John, F., & George, J. (2019). Preparation and Characterisation of Niosomal Emulsions as Novel Drug Delivery Vehicle Derived from Natural Seaweeds. In *Role of Novel Drug Delivery Vehicles in Nanobiomedicine*. IntechOpen.
[17] V.D.Jadhav, G.Talele Swati, A.Bakliwal Akshada and G.N Chaudhari. Formulation And Evaluation Of Herbal Gel Containing Leaf Extracts Of Tridax Procumbens. Journal of Pharmaceutical and Bio Sciences, 2015; 3(1): 65-72.
[18] Kamoun, E. A., Kenawy, E. R. S., & Chen, X. (2017). A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *Journal of advanced research*, *8*(3), 217-233.
[19] Kanitakis, J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. *European journal of dermatology*, *12*(4), 390-401.
[20] Kashyap, A. K., Reddy, N. P., Chaitanya, R. K., & Karnati, R. (2013). Ethyl acetate extract of Hemigraphis colorata leaves shows anti-inflammatory and wound healing properties and inhibits 5-lipoxygenase and cyclooxygenase-1 and 2 enzymes. *Journal of Medicinal Plant Research*, *7*(37), 2783-91.
[21] Kavoosi, G., Dadfar, S. M. M., & Purfard, A. M. (2013). Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing. *Journal of Food Science*, *78*(2), E244-E250.
[22] Kumar, A., Roberts, D., Wood, K. E., Light, B., Parrillo, J. E., Sharma, S., ... & Gurka, D. (2006). Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine*, *34*(6), 1589-1596.
[23] Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Percoraro, R. E., Rodeheaver, G., & Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound repair and regeneration*, *2*(3), 165-170.
[24] Nandakumar, V. G., Suresh, S., Sreekala, C. O., Sudheer, S. K., & Pillai, V. M. (2017). Hemigraphis colorata as a natural dye for solar energy conversion. *Materials Today: Proceedings*, *4*(2), 4358-4365.
[25] Núñez-Flores, R., Giménez, B., Fernández-Martín, F., López-Caballero, M. E., Montero, M. P., & Gómez-Guillén, M. C. (2013). Physical and functional characterization of active fish gelatin films incorporated with lignin. *Food Hydrocolloids*, *30*(1), 163-172.
[26] Panasci, K. (2013). Burns and Wounds. *Acute Care Handbook for Physical Therapists*, *7*, 283.
[27] Prakashbabu, B. C., Vijay, D., George, S., Kodiyil, S., Nair, S. N., Gopalan, A. K., ... & Ravindran, R. (2017). Wound healing and anti-inflammatory activity of methanolic extract of Gmelina arborea and Hemigraphis colorata in rats. *Int. J. Curr. Microbiol. App. Sci*, *6*(8), 3116-3122.
[28] Quave, C. L., Plano, L. R., Pantuso, T., & Bennett, B. C. (2008). Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant Staphylococcus aureus. *Journal of ethnopharmacology*, *118*(3), 418-428.
[29] Radhika, P. V. (2017). Arun Kumar KV Herbal Hydrogel for Wound Healing: A Review. *International Journal of Pharma Research and Health Sciences*, *5*(2), 1616-22.
[30] Rathish Nair, Sumitra VC Antibacterial activities of some medicinal plants of the western region of India. Turk J 2007; 31:231-236.
[31] Sabrin, F., Alam, A. M. S., Islam, M. R., Al Mamun, M. E., & Chowdhury, J. A. (2019). Effects of Aqueous Extract of Basella alba L. leaves on Blood Cell Count in Rats. *Bangladesh Pharmaceutical Journal*, *22*(1), 73-78.
[32] Sashiwa, H., Kawasaki, N., Nakayama, A., Muraki, E., Yamamoto, N., Arvanitoyannis, I., ... & Aiba, S. I. (2002). Chemical modification of chitosan 121: synthesis of organo-soluble chitosan derivatives toward palladium absorbent for chemical plating. *Chemistry letters*, *31*(6), 598-599.
[33] Sasidharan, S., & Pottail, L. (2020). Anti-bacterial and skin-cancer activity of AuNP, rGO and AuNP-rGO composite using Hemigraphis alternata (Burm. F.) T. Anderson. *Biocatalysis and Agricultural Biotechnology*, 101596.

[34] Sheu, J. B. (2007). An emergency logistics distribution approach for quick response to urgent relief demand in disasters. *Transportation Research Part E: Logistics and Transportation Review*, *43*(6), 687-709.
[35] Sikareepaisan, P., Ruktanonchai, U., & Supaphol, P. (2011). Preparation and characterization of asiaticoside-loaded alginate films and their potential for use as effectual wound dressings. *Carbohydrate Polymers*, *83*(4), 1457-1469.
[36] Sorg, H., Tilkorn, D. J., Hager, S., Hauser, J., & Mirastschijski, U. (2017). Skin wound healing: an update on the current knowledge and concepts. *European Surgical Research*, *58*(1-2), 81-94.
[37] Srivastava, J., & Lambert, J. N Vietmeyer (1996) Medicinal plants: An expanding role in development. *World Bank technical paper*, *320*.
[38] Subramoniam, A., Evans, D. A., Rajasekharan, S., & Nair, G. S. (2001). Effect of Hemigraphis colorata (Blume) HG Hallier leaf on wound healing and inflammation in mice. *Indian journal of pharmacology*, *33*(4), 283-285.
[39] Suzuki S, Matsuda K, Isshiki N, Tamada Y, Ikada Y. Experimental study of newly developed bilayer artificial skin. Biomaterials 1990;11:356–60.
[40] VP Silja, K samitha Varma & KV Mohanan, Ethnomedical plant knowledge of the *mullu kuruma* tribe of wayanad distirct, Kerala. Calicut Kerala-35, 2008 vol. 7(4) 604-612.
[41] Thakur, R., Jain, N., Pathak, R., & Sandhu, S. S. (2011). Practices in wound healing studies of plants. *Evidence-based complementary and alternative medicine*, *2011*.
[42] Vimala, K., Mohan, Y. M., Sivudu, K. S., Varaprasad, K., Ravindra, S., Reddy, N. N., ... & MohanaRaju, K. (2010). Fabrication of porous chitosan films impregnated with silver nanoparticles: a facile approach for superior antibacterial application. *Colloids and Surfaces B: Biointerfaces*, *76*(1), 248-258.
[43] Vimala, K., Sivudu, K. S., Mohan, Y. M., Sreedhar, B., & Raju, K. M. (2009). Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly (acrylamide) and carbohydrates: a rational methodology for antibacterial application. *Carbohydrate polymers*, *75*(3), 463-471.
[44] Yadav, RNS and Munin Agarwala. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011; 3(12): 10-14.