IJBPAS, April, 2023, 12(4): 1632-1644

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COUNTERMEASURE FOR BACTERIA CAUSING WOUND INFECTION

ASHA Y[1*], PRATHIBA G[2], JOLDY H[1] AND BALAKUMAR V[3]

1: Department of Microbiology, Sathyabama Institute of Science and Technology, Chennai-600119, Tamil Nadu, India

2: Department of Microbiology, Sathyabama Dental College and Hospital, Chennai-600119, Tamil Nadu, India

3: Department of Prosthodontics, Sathyabama Dental College and Hospital, Chennai-600119, Tamil Nadu, India

***Corresponding Author: Yumnam Asha: E Mail: yumnamashalang@gmail.com**

Received 8th May 2022; Revised 16th June 2022; Accepted 27th Aug. 2022; Available online 1st April 2023

https://doi.org/10.31032/IJBPAS/2023/12.4.7053

ABSTRACT

The goal of this research was to determine the antibacterial activity of four different medicinal plant extracts including *Tridax procumbens*, *Nigella sativa, Acalypha indica, Vitex negundo* as well as the phytochemicals present in the extract. In this study, ethanol and methanol solvents were used for the plant extraction. The antibacterial activity of crude extracts of four plants was investigated using agar well diffusion method against *Staphylococcus aureus, Enterococci* sp. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella sp.,* and *Proteus sp.* by agar well diffusion method. Standard antibiotic Ciprofloxacin is used as a positive control. The best plant extract was analyzed qualitatively for the presence of phytochemicals. Both the methanolic and ethanolic extract of *Nigella sativa* seeds showed higher zone of inhibition against gram-positive bacteria viz. *Staphylococcus aureus, Enterococci* when compared to gram-negative organisms. Among the four plant extracts, *N. sativa* seeds extract showed effective inhibition against the tested organisms. Phytochemical analysis revealed that tannin, saponin, alkaloids, flavonoids, phenol were present in the methanolic extract of *N. sativa* but terpenoids was absent. *N.*

sativa seeds extract might be helpful in preparing the alternative drug against the bacteria causing wound infection which are cost-effective and non-toxic to human.

Keywords: Plant extracts, antibacterial activity, methanol, ethanol, wound pathogens

INTRODUCTION

Wound provides a suitable site for the bacterial infection which causes pain, swelling, redness to the skin and delays rate of healing **[1]**. Some of the most common bacterial pathogens associated with wound infection includes *S. aureus, Enterococci sp., E. coli, P. aeruginosa, Klebsiella sp., Proteus sp., etc.* **[2]**. These bacteria can slow wound healing process by interfering with normal clotting mechanisms, altering white blood cell's function, formation of poor-quality granulation tissue, lowering connective tissue tensile strength and weakening epithelialization **[3]**.

The emergence of antibiotic resistant organisms and their spread is becoming a major threat to the world population. Infectious diseases are the second largest cause of fatality in the world recording for more than 13 million deaths annually **[4]**. Overuse of antibiotics, use of unprescribed antibiotics and continuous use of topical antimicrobial agents has led to the condition where the pathogens become resistant to the used antibiotics **[5]**. Since the multidrug resistant strains have been increased, the choice of therapy often becomes very limited **[4]**. The reason for becoming multi-drug resistant in the wound infection is due to the misuse and overuse of antibiotics. New therapy and expensive antibiotics were also increases due to increase in resistance to the used antibiotics **[6]**. Moreover, usage of continuous antibiotics leads to severe complications such as nausea, hypersensitivity reactions, gastrointestinal disorders, nephrotoxicity and continuous use of topical antimicrobial agent may result in a fungal infection. Because of such concern, the necessity to find an effective, safer and natural alternative way is increased.

An alternative way is the use of phytobiotics that can be used as a promising therapeutic agent in the treatment of infected wounds **[6]**. Plants have been used for both therapeutic and nutritional purposes for centuries **[7]**. Herbal medicine has been found to be effective against infectious diseases for over the years **[8]**. Medicinal plants which contain a wide range of biological properties are used for the treatment of various diseases across the world **[7]**.

Plants contain secondary metabolites including alkaloids, phenolic compounds, flavonoids, saponin and tannin which are reported to possess multiple biological activities such as antibacterial, free-radical scavenging ability, anti-inflammatory, anti-

carcinogenic effect **[9]**. Phenolic compounds are important secondary metabolites because it has free-radical scavenging ability **[10]**. Flavonoids contain various properties such as antimicrobial, anticancer, anti-inflammatory and anti-histamines activities **[11]**. Tannins are a heterogenous group of polyphenols which are found to be responsible for antioxidant and antibacterial activities **[12]**. Alkaloids possess anticancer, antibacterial properties and responsible for many healing properties in traditional medicine. Saponin is a potential medicinal phytochemical **[9]**.

Many researchers recommended the use of methanol and ethanol solvent for the extraction of wide variety of compounds from the plants **[13]**. Ethanol is a suitable solvent for the extraction of polyphenols. Methanol is found to be more effective in the extraction of polyphenols having low molecular weight **[14]**.

Tridax procumbens is a wild herb which is found all over India. It possesses several potential therapeutic activities such as antimicrobial, anticoagulant, antioxidant, wound healing property, insecticidal and anti-inflammatory activity **[15]**. *Nigella sativa* is an herbaceous plant. It is also called as Black seeds. Its seeds have been reported to contain many pharmacological activities including anti-parasitic, antimicrobial, antioxidant and anti-inflammatory activities **[16]**. *Acalypha indica* is an herb widely distributed across India and reported to be effective in the treatment of asthma, pneumoniae, rheumatoid arthritis etc. **[17]**. Rural people used paste of leaves to treat skin diseases **[18]**. *Vitex negundo Linn*. is an important plant with various medicinal properties and it is distributed across India. It is commonly known as Chinese chaste tree and nochhi in Tamil. It has bitter, pungent, thermogenic, expectorant, stomachic and digestive properties. The extracts of the plant possess anti-inflammatory, antimicrobial, antiseptic, antipyretic, diuretic properties **[19]**. Natural products from the higher plants are generally known for its fast cure, inexpensive, non-toxic and cost-effective, which may lead to the search of new drug. Hence, the present study is focussed to study the antibacterial activity of methanolic and ethanolic extracts of four medicinal plants against the wound associated bacteria and to analyse phytochemicals present in the plant extract having higher zone of inhibition.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *Tridax procumbens (Vettukkaaya), Acalypha indica (kuppaimeni), Vitex negundo (Nocchi illai)* were collected and *Nigella sativa* seeds were bought from shop in and around Chennai.

Plant extract preparation

The collected medicinal plant leaves and seeds were carefully washed with running tap water to remove the dust **[20]** and allowed it to shade dry for 10 days **[21]** and powdered using grinder. Then the powdered leaves and seeds were subjected to extraction using methanol and ethanol solvents separately with the ratio of 10:100 (25g in 250 ml) in an Erlenmeyer flask and then kept in shaker at room temperature for 3 days. Then the solution was strained using muslin cloth and filtered through Whatman no.1 filter paper **[22]**. The filtrates were evaporated to dryness to obtain the concentrated extract.

Bacterial strains:

A total of 6 bacterial strains were used for the experiment for the antibacterial sensitivity test. Bacteria such as *S. aureus, Enterococci sp., E. coli, P. aeruginosa, Proteus sp., Klebsiella sp.* were obtained from Basic Institute of Medical Science, Taramani, Chennai.

Media used for culturing: Muller Hinton Agar, Brain Heart Infusion broth.

Determination of the antibacterial activity of the different plant extract:

Agar well diffusion method was performed to evaluate the antibacterial activity of the different plant extract **[23]**. A stock solution of 1000mg/ml was prepared.100µl, 200µl of the stock solution of plant extracts were added into the well using micropipette. Standard antibiotic Ciprofloxacin (5µg/ml) was used as a positive control. All the experiments were done in triplicates. The resulting data represented as mean ±SD (standard deviation). Results are examined using student's t-test and found to be significant at p-value<0.05.

Qualitative Phytochemical Analysis:

The plant extract with high antibacterial activity was used to analyse for its phytochemical's presence. The tests were performed by the following protocols.

Tannin test: 1ml of the plant extract was added in 5ml of distilled H_2O and 2 drops of $FeCl_3$ solution were then added. Presence of tannins was indicated by the colour change to bluish/greenish to black colour **[24]**.

Saponin test: Extract was combined with 2ml of H_2O and agitated vigorously. Persistent foam formation for 10mins indicates the presence of saponin **[25]**.

Flavonoid test: To 50mg of extract, a few drops of sodium hydroxide was added. The intense yellow colour changes to colourless when dil. acid was added into it. This indicates the presence of flavonoids **[26]**.

Phenol test: 3-4 drops of $FeCl_3$ solution was added to the extract. Bluish black colouration denotes the presence of phenol **[27]**.

Terpenoids test: To 0.2 g of plant extract, 2 ml $CHCl_3$ and 3 ml of conc. sulphuric acid

was added. Reddish-brown coloration implies the presence of terpenoids **[28]**.

Alkaloid test: Meyer's reagent (Potassium Mercuric Iodide) was added to the plant extract. Pale-yellow precipitation denotes for the alkaloid's presence **[27]**.

RESULTS

Antibacterial activity of Plant extracts

Four plant extracts (*Tridax procumbens*, *Acalypha indica, Vitex negundo* and *Nigella sativa* seeds) were investigated to evaluate the antibacterial activity against the wound associated bacteria such as *S. aureus*, *Enterococci* sp., *E. coli, P. aeruginosa, Klebsiella sp., Proteus sp.* **Figure 1** shows the antibacterial activity of *N. sativa* seeds extract. Graphical representation of zone of inhibition of ethanolic and methanolic extracts of four plants and ciprofloxacin against the test organisms were shown in **Figure 2 and Figure 3** respectively. The comparison of the antibacterial activity of ethanolic and methanolic extracts was shown in **Figure 4**.

The methanolic and ethanolic extract of *Tridax procumbens* revealed sensitive against all the tested organisms. *T. procumbens* methanolic extract showed larger zone of all the organisms than the ethanolic extract.

The methanolic and ethanolic extract of *Acalypha indica* revealed sensitive against *Staphylococcus aureus*, *Enterococcus sp*., *Escherichia coli, P. aeruginosa,* but resistant to *Klebsiella* sp*., Proteus sp.*

The methanolic and ethanolic extract of *Vitex negundo* showed sensitive against *Staphylococcus aureus*, *Enterococcus sp*., *Pseudomonas sp., Proteus sp.* but resistant to *Escherichia coli* and *Klebsiella* sp. *V. negundo* ethanolic extract showed bigger zone than the methanolic extract.

Both the methanolic and ethanolic extract of *Nigella sativa seeds* showed sensitive against all the tested organisms. Methanolic extract gives larger zone than the ethanolic extract.

Phytochemical analysis:

Results of the phytochemical screening of the methanolic extract of *Nigella sativa* seeds extracts in qualitative basis were summarized in the **Table 1**.

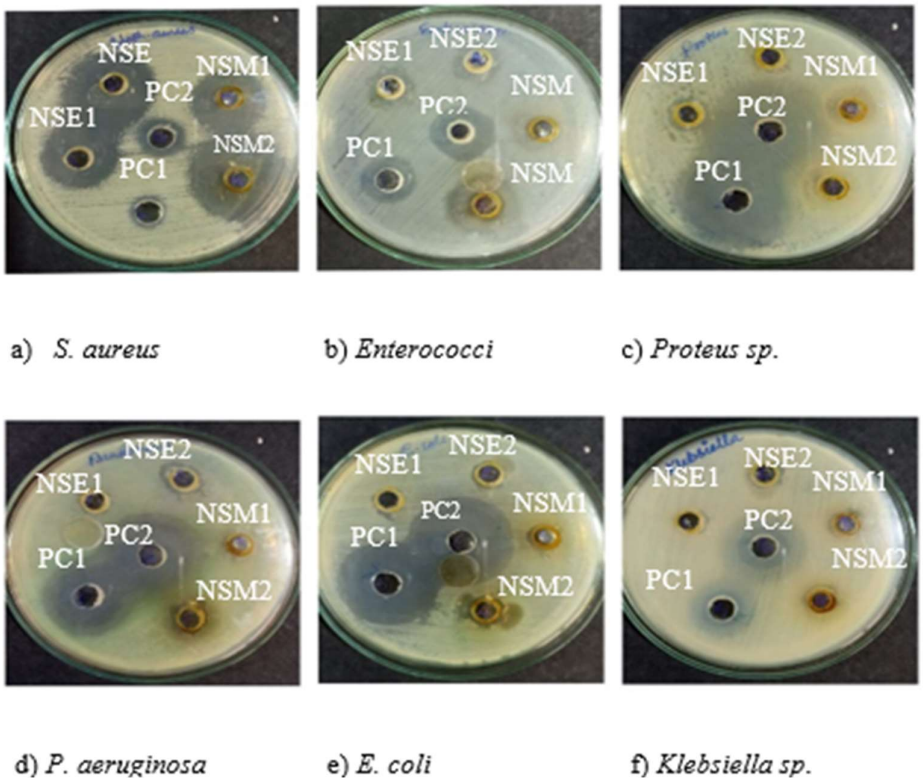

Figure 1: Antibacterial activity test.
NSE1- *N. sativa* ethanolic extract (100µl), NSE2- *N. sativa* ethanolic extract (200µl), NSM1- *N. sativa* methanolic extract (100µl), NSM2- *N. sativa* methanolic extract (200µl), PC1- Positive control- (100µl), PC2- Positive control- (200µl)

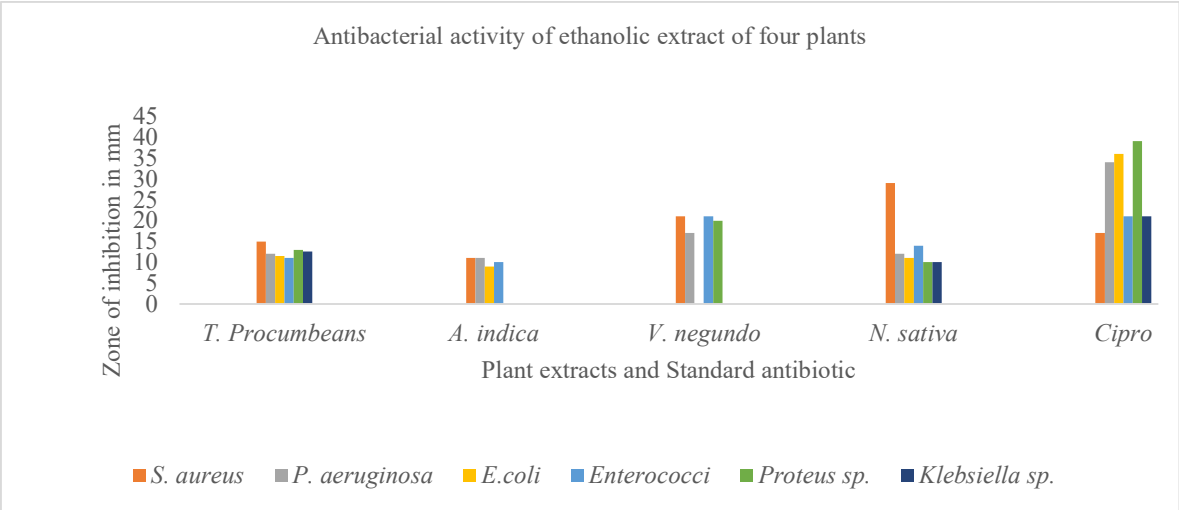

Figure 2: Antibacterial activity of ethanolic extract of four plants and ciprofloxacin against the test organisms

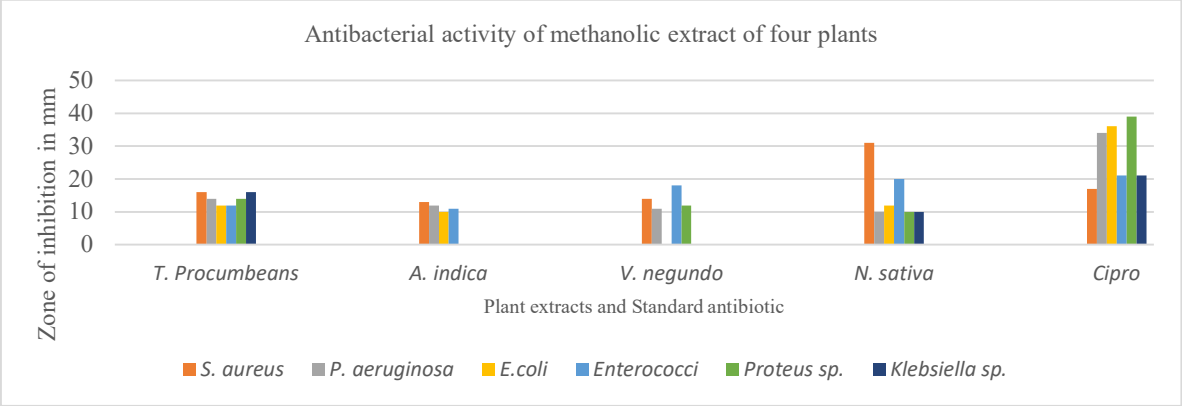

Figure 3: Antibacterial activity of methanolic extract of four plants and ciprofloxacin against the test organisms

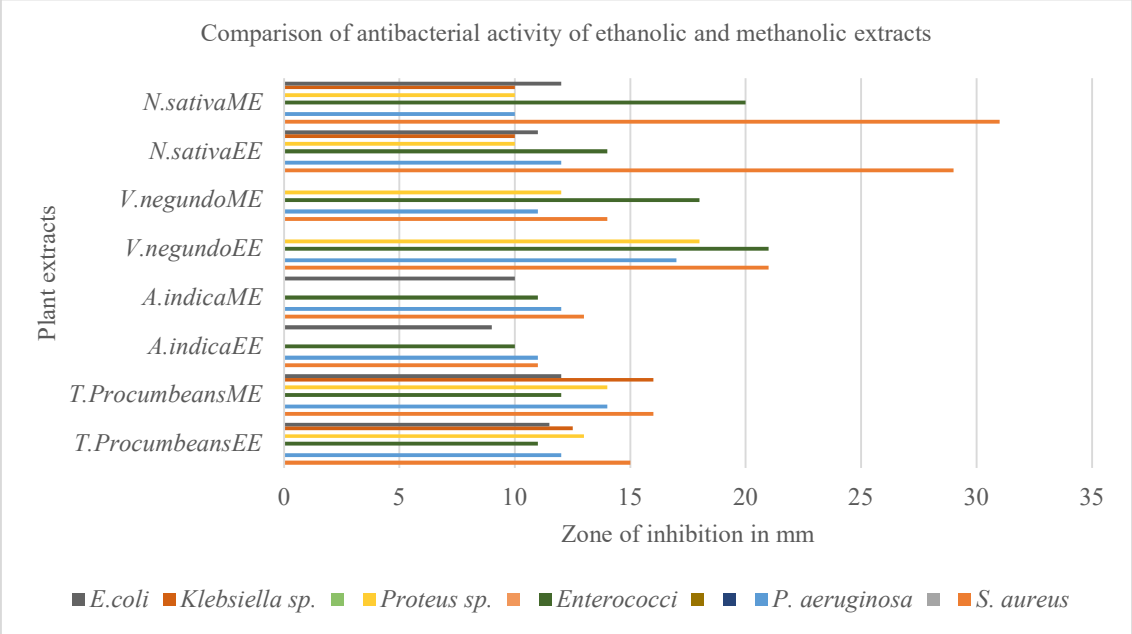

Figure 4: Comparison of antibacterial activity of ethanolic and methanolic extracts.

Table 1: Phytochemical analysis of the methanolic extracts of *N. sativa*.

Phytochemicals	Methanol
Tannin	+
Saponin	+
Alkaloids	+
Terpenoids	_
Flavonoids	+
Phenol	+

DISCUSSION

In this study, the methanolic and ethanolic extracts of four medicinal plants such as *Tridax procumbens, Nigella sativa, Acalypha indica* and *Vitex negundo* were examined for their antibacterial activity against the wound associated bacteria such as *S. aureus, P. aeruginosa, E. coli, Klebsiella sp., Proteus sp., Enterococci sp*. Present study revealed the ethanolic and methanolic extract of *T. procumbens* showed sensitive to all tested organisms.

According to Bharathi V *et al*., the methanolic extract of *T. procumbens* revealed significant zone against *S. aureus, Klebsiella* and *E. coli* **[7]**. In the present study, the methanolic extract of *A. indica*, it showed sensitive to *S. aureus*, *Enterococci, P. aeruginosa*, *E. coli*. Previous study also reported that the methanolic extract of A. indica produced remarkable zone of inhibition against *S. aureus* and *P. aeruginosa* **[17]**. In the present study, ethanolic and methanolic extracts of *V. negundo* produced good zone of inhibition against *S. aureus, Enterococci, Proteus*, *P. aeruginosa* but resistant to *E. coli* and *Klebsiella*, however, Kurapatti P *et al*., 2017 reported that the ethanolic extract of *V. negundo* showed significant zone against *S. aureus* and *Klebsiella* and less activity against *E. coli, P. aeruginosa* and maximum activity against *S. aureus* in case of methanolic extract **[29]**. Ethanolic and methanolic extracts of *N. sativa* showed sensitive against all the tested organisms. *N. sativa* extract was found to be more effective against gram-positive organisms such as *Staphylococcus aureus* and *Enterococci* sp. than the gram-negative organisms Previously it was reported that the methanolic extract of *N. sativa* showed remarkable zone against *E. faecalis*, *E. coli, P. aeruginosa,* but resistant to *S. aureus* **[30]**. *N. sativa* seeds extract exhibited larger zone of inhibition than the other plant extracts. Methanolic extract was found to be more effective than ethanolic extract in all plant extracts except *A. indica* which ethanolic extract is more effective than methanolic extract.

Results of phytochemical analysis of methanolic extract of medicinal plant *N. sativa* revealed the presence of tannin, saponin, alkaloids, flavonoids and phenol. Previous study also revealed that the methanolic extract of *N. sativa* contains tannin, saponin, alkaloids **[31]**. Volatile oil of *N. sativa* seeds has been reported to possess antimicrobial activity **[32]**. This study also revealed that *N. sativa* seeds extract possess antibacterial activity against all the test organisms.

CONCLUSION

The present study concludes that *Nigella sativa* seeds extract have higher antibacterial activity against all the test organisms when compared to the other plant extracts. Phytochemical analysis of the extract gives an information about the secondary metabolites which is responsible for the antibacterial activity. Hence, methanolic extract of *N. sativa* seeds extract was found to have significant effect against wound associated organisms such as *S. aureus, P. aeruginosa, E. coli, Klebsiella sp., Proteus sp.* and *Enterococci* sp. and can be used as an alternative drug for the wound infections which are cost-effective and non-toxic to human. Further

investigation should be done for the toxicity of the *Nigella sativa* seeds extract.

ACKNOWLEDGEMENT

The authors would like to thank Sathyabama Institute of Science and Technology for providing the facilities to perform this research work. We would also like to express our sincere thanks to the Department of Microbiology, Institute of Basic Medical Sciences, Taramani for providing the bacterial strains.

REFERENCES

[1] Leonard J. How to recognize and treat an infected wound. *Medical News Today.* 2019. Available online: https://www.medicalnewstoday.com/articles/325040.

[2] Tom, I.M., Ibrahim,M.M., Umoru, A.M., Umar,J.B., Bukar, M.A., Haruna, A.B. and Aliyu, A. Infection of Wounds by Potential Bacterial Pathogens and Their Resistogram. *Open Access Library Journal*. 2019; 6(7):1-13. DOI: https://doi.org/10.4236/oalib.1105528.

[3] Annan K, Houghton P. Antibacterial antioxidant and fibroblast growth stimulation of aqueous extracts of Ficus asperifolia Miq. and Gossypium arboretum L., wound-healing plants of Ghana. *Journal of Ethnopharmacology*. 2008; 119:141-144. DOI: 10.1016/j.jep.2008.06.017.

[4] Reenaa M. Antimicrobial activity of Tridax procumbens against multidrug resistant Pseudomonas aeruginosa isolated from burn case. *Indian Journal of Applied Microbiology*. 2016; 19:58-65. http://www.ijamicro.com/abstractview.php?ID=15&vol=19-1-2016&SNo=7 .

[5] Antimicrobial resistance. What causes AMR? *Australian Government.* Available online: https://www.amr.gov.au/about-amr/what-causes-amr.

[6] Yasodha T, Jeevitha, Yogesh, Ramanam. Efficiency of phytobiotics of Indian medicinal plant Tridax procumbeans L. against wound infecting bacteria. *MOJ Current Research & Reviews.* 2018; 1:278-280. DOI: 10.15406/mojcrr.2018.01.00046.

[7] Bharathi V, Varalakshmi B, Gomathi S, Shanmugapriya A, Karpagam T**.** Antibacterial activity of Tridax procumbens Linn. *International Journal of Pharma Sciences and Research.*2012;3:364-366. IJPSR12-03-04-09.doc

[8] Jain A, Rao D V, Batra A, Jian A. A Study on Antimicrobial Potential of Tridax procumbens (L.) against Clinical isolates. *International Journal of Pharmaceutical Sciences and Research.* 2015; 6:1330-1335. DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(3).1330-35

[9] Nithya TG, Jayanthi J, Ragunathan MG. Antioxidant activity, Total phenol, Flavonoid, Alkaloid, Tannin, and Saponin contents of Leaf Extracts of *Salvinia molesta* D. S. Mitchell (1972). *Asian Journal of Pharmaceutical and Clinical Research.* 2016; 9:200-203. https://www.researchgate.net/publication/352561795_Antioxidant_activity_total_phenol_flavonoid_alkaloid_tannin_and_saponin_contents_of_leaf_extracts_of_Salvinia_molesta_D_S_Mitchell_1972

[10] Naczk M, Shahidi F. Extraction and analysis of phenolics in food. *Journal of Chromatography A*. 2004; 1054:95-111. https://doi.org/10.1016/j.chroma.2004.08.059

[11] Montoro P, Braca A, Pizza C, De Tommasi N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. *Food Chemistry*. 2005; 92:349-355. https://doi.org/10.1016/j.foodchem.2004.07.028

[12] Fraga-Corral M, Otero P, Cassani L, Echave J, Garcia-Oliveira P, Carpena M, Chamorro F, Lourenco-Lopes C, Prieto M A, Simal-Gandara J. Traditional Applications of Tannin Rich Extracts Supported by Scientific Data: Chemical Composition, Bioavailability and Bio accessibility. *Multidisciplinary Digital Publishing Institute (MDPI).*2021;10:251. doi: 10.3390/foods10020251

[13] Sharma A. 2014. Which is the best solvent for herbal extraction? Available online:

[14] https://www.researchgate.net/post/Which_is_the_best_solvent_for_herbal_extraction/53901f41cf57d770178b45b0/citation/download.

[15] Do Q D, Angkawijaya A E, Tran-Nguyen P L, Huynh L H, Soetaredjo F E, Ismadji S, Ju Y H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*. 2014; 22:296-302. https://doi.org/10.1016/j.jfda.2013.11.001

[16] Veena Gayathri Krishnaswamy and Christina**.** Antibacterial Activity of different parts of Tridax procumbens against Human Pathogens. *International journal of Current Research and Academic Review.* 2015; 3:211-218.

[17] Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU**.** Antibacterial activity of Nigella sativa against clinical isolates of methicillin resistant Staphylococcus aureus. *Journal of Ayub Medical College, Abbottabad.* 2008; 20:72-74. Anti bacterial activity of Nigella sativa against clinical isolates of methicillin resistant Staphylococcus aureus - PubMed (nih.gov)

[18] Govindarajan M, Jebanesan A, Reetha D, Amsath R, Pushpanathan T, Samidurai K. Antibacterial activity of Acalypha indica L. *European Review of Medical and Pharmacological Sciences*.2018; 12:299-302. Antibacterial activity of Acalypha indica L - PubMed (nih.gov)

[19] Indira P D. A. Studies on Antimicrobial Activity of Acalypha indica along with Preliminary *Phytochemical screening. International Journal of Life Science & Pharma Research*. 2015; 5:34-38. https://www.ijlpr.com/admin/php/uploads/239_pdf.pdf

[20] Anbuselvan S. Phytochemical evaluation and antimicrobial Activity of Vitex negundo (L.) leaves extracts. *International Journal of Research in Pharmaceutical and Nano sciences.* 2013; 2:44-56. PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF VITEX NEGUNDO (L.) LEAVES EXTRACTS (ijrpns.com)

[21] Rajaselvam J, Benila smily J, Meena R. A Study of Antimicrobial Activity of Acalypha Indica Against Selected Microbial Species. *International Journal of Pharma Sciences and Research.* 2012;3. IJPSR12-03-09-005.docx

[22] Das M, Goswami S. Antifungal and Antibacterial Property of Guava (Psidium guajava) Leaf Extract: Role of Phytochemicals. *International Journal of health sciences and Research.* 2019; 9:39-45.

https://www.ijhsr.org/IJHSR_Vol.9_Issue.2_Feb2019/7.pdf

[23] Mummed B, Abraha A, Feyera T, Nigusse A, Assefa S. In Vitro Antibacterial Activity of Selected Medicinal Plants in the Traditional Treatment of Skin and Wound Infections in Eastern Ethiopia. *BioMed Research International* 2018; 2018:1-8**.** https://doi.org/10.1155/2018/1862401

[24] Balouiri M, Sadiki M, Ibnsouda S K. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6:71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[25] Pius O Ukoha, Egbuonu A.C. Cemaluk, Obasi L. Nnamdi, Ejikeme P. Madus. Tannins and other phytochemicals of the Samanaea saman pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry.* 2011; 5:237-244. Ukoha *et al* pdf (academicjournals.org)

[26] Nigussie D, Davey G, Legesse B A, Fekadu A, Makonnen E. Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. 2021; 21(2):1-10. doi: 10.1186/s12906-020-03183-0

[27] Bandiola B**.** Extraction and qualitative phytochemical screening of medicinal plants: A brief summary. *International journal of pharmacy.* 2018; 8:137-143. (PDF) Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary (researchgate.net)

[28] Rathi S, Jalwal P, Kharb M. Evaluation of Phytochemical constituents and Antioxidant potential of hydro-alcoholic extract of seeds of Petroselinum crispum. Research Journal of Pharmacy and Technology.2021;14(10):5391-6. DOI: 10.52711/0974-360X.2021.00940

[29] Alamzed M, Khan M R, Ali S, Shah S Q, Rashid M U. Antimicrobial properties of extracts and compounds isolated from Berbers jaeschkeana. *Bangladesh Journal of Pharmacology.* 2013; 8:107-109.

[30] Kurapatti P, Murugesan K, Anbalagan S, Sankareswaran M. Phytochemical Analysis and Antibacterial Activity of Vitex

negundo Leaf Extracts against Clinically Isolated Bacterial Pathogens. 2017; 46:183-187. (PDF) Phytochemical Analysis and Antifungal Activity of Vitex negundo Leaf Extracts Against Clinically Isolated Fungal Pathogens (researchgate.net)

[31] Khan A U, Ali S, Rehman A U, Ali H, Ahmad T, Wagar M, Niaz Z. Antibacterial activity of Nigella sativa and Piper nigrum. Asian Journal of Natural and applied sciences. 2013; 2:74-80. (PDF) ANTIBACTERIAL ACTIVITY OF NIGELLA SATIVA AND PIPER NIGRUM (researchgate.net)

[32] Ishtiaq S, Ashraf M, Hayat M Q, Asrar M. Phytochemical Analysis of Nigella sativa and its Antibacterial Activity against Clinical Isolates Identified by Ribotyping. *International journal of agriculture & biology*. 2013; 15: 1511–1156. (PDF) Phytochemical Analysis of Nigella sativa and its Antibacterial Activity against Clinical Isolates Identified by Ribotyping (researchgate.net)

[33] Salman M T, Khan R A, Shukla I. Antimicrobial activity of Nigella sativa Linn. Seed oil against multi-drug resistant bacteria from clinical isolates. Natural Product Radiance. 2008; 7(1):10-14. (PDF) Antimicrobial activity of Nigella sativa Linn. seed oil against multi-drug resistant bacteria from clinical isolates (researchgate.net)