Effect Of Heat Treatment On Antimicrobial Activity Of Medicinal Plant Extract Against Atcc 25923 Staphylococcus aureus

Francis A. L[1], S. Karthick Raja Namasivayam[2*], G. Bhuvaneshwari[3], Catherine Thamayanthi[3]

[1]Department of Research & Innovation, Saveetha School of Engineering, SIMATS, Chennai, India

[2]Professor, Department of Research & Innovation, Saveetha School of Engineering, SIMATS, Chennai, India

[3]Department of Microbiology, Saveetha Medical College and Hospital, Chennai, India

DOI: 10.47750/pnr.2023.14.03.049

Abstract

Since prehistoric times till the present and most probably in the future, medicinal plants will take the spotlight in the pharmaceutical industry because of their rich important natural sources of antioxidants for novel drugs discovery and development. Plants synthesise several bioactive compounds that exhibit a high antioxidant capacity. Furthermore, because of their anti-inflammatory and antimicrobial properties, these antioxidant compounds that have higher heat stability and plays a vital role in plants by maintaining stress tolerance which have a very high connection to human immune system and health. The main focus of this research is to study the effect of heat treatment on phytochemical constituents and their actions against ATCC 25923 Staphylococcus aureus. The plants (Azadirachta indica, Acalypha indica, Vitex negundo, Aloe vera, Justicia adhatoda, Rosa damascene, and Citrus sinensis) were selected and processed. The selected plant extracts were then subjected to phytochemical screening and anti-microbial studies. After the preliminary screening, a part of the respective plant extracts was tested for the effect of heat treatment on them by phytochemical screening and anti-microbial studies. By comparing the results of both the treated and untreated groups, we were able to conclude that there was no antibacterial activity observed in the treated groups because of the inactivation of the bioactive compounds during the treatment (autoclaving it at 121 °C with 15 psi for 30 min), which was confirmed additionally by repeating a phytochemical test. The compounds that were thermally unstable were inactivated by the effect of the heat on them.

Keywords: Plant extracts, Bioactive compounds heat treatment, phytochemical screening, and antibacterial activity.

INTRODUCTION

According to ethnopharmacological research findings, the development of modern treatment systems is typically focused on pharmaceutical discovery from plant sources. Furthermore, the biological activity potential of natural medicines has been explored in multiple preclinical and clinical studies, indicating unique biological effects of a phenomenal range of plant-derived compounds in various chemical groups [1]. The Indian subcontinent is home to around 15,000 varieties of flowering plants. Around 1500 new species of beneficial and medicinal plants have been found. Only 900 of these are considered medicinal herbs, some of which are also food plants. [2] Traditional plant knowledge is vital not only for biodiversity conservation but also for the improvement of healthcare and medicine.

Medicinal plants offer varied therapeutic characteristics that are habituated to treat chest and stomach discomfort, constipation, digestive issues, menstrual bleeding, and liver illnesses. [3] [4]. Infectious illnesses have repeatedly been identified as one of the primary sources of global health threats. In the last few decades, the medicinal properties of plant extracts and their constituents have been demonstrated to exhibit antiulcer, antifungal, anti-inflammatory, antimalarial, antimutagenic, antioxidant, immunomodulatory, antibacterial, antihyperglycemic, anticarcinogenic, antiviral, and other properties plant extracts and their constituents have been demonstrated to exhibit pharmacological properties [5]

Medicinal plants include a high concentration of phytochemicals that can be structurally refined and processed into novel medications. [6] Antibiotic resistance among microorganisms necessitates continual drug revelation, and the moderate to astringent negative side effects of synthetic medication use on humans has given birth to the injunctive authorization for alternatives such as probiotics and phytomedicine. [7] Over the years, knowledge of their medicinal powers has been passed down within and among human cultures. Herbal formulations are the best alternative because of their phytochemicals, which are naturally found in plants and have demonstrated biological paramountcy by playing a paramount role in antimicrobial action through inhibition or killing processes [8]. Traditional medicine derived from medicinal plants continue to be prolific sources for novel medications, which accounts for roughly 90% of the newly discovered drugs in pharmaceuticals industry. Traditional medicine perpetuates to offer health care to more than 80% of the world's population, categorically in underdeveloped countries. There is a desideratum to ensure that what is understood is utilized for financial advantage and to promote the health of our people. [9]. There is a strong potential for traditional medicines because there are approximately half a billion plants on the entire planet, the majority of which are still being investigated for their therapeutic benefits, and the room for development of medical activities could be crucial in the treatment of current and emerging studies.[10]

To deter infections and predators, plants manufacture and utilise a variety of metabolic chemicals. Utilizing plant-predicated metabolites to unearth novel bioactive molecules that might be acclimated to engender incipiently potent antimicrobial medicines to treat multidrug-resistant diseases is a potential strategy. These stable compounds have a specific role in addition to providing protection since they are linked to multiple metabolic processes both within and outside of various portions of the plants that have a range of well-known therapeutic benefits. Several Stable bioactive chemicals and their derivatives have been used as medications to treat diseases such as cancer, hypertension, immunosuppression, neurological disorders, and fungal, viral, and bacterial infections, with some of them currently in clinical trials or on the market. Importantly, these chemicals have shown excellent effects in combating the magnification of antibiotic resistant bacteria and enhancing the efficacy of old antibiotics via synergistic interaction, thereby truncating resistance development.[11] Metabolite instability is a well-documented cause of variation in biological samples. Herbal plants and their derivatives have been extensively established to play key roles in current medicine development. Natural resources for engendering novel medications include medicinal plants [12, 13, 14]. Data on the antibacterial activity of several plants, which were previously thought to be empirical, have now been scientifically proven, with a growing number of reports on pathogenic microorganisms resistant to antimicrobials. [15]. In this research, the effect of treatment of plants extracts is studied by using antibacterial activity. This study helps us to identify the importance of metabolite stability because it contains the unknown microbial properties.

MATERIALS AND METHODS

PLANT MATERIAL ACQUISITION AND PROCESSING

Young and vigorous leaves Azadirachta indica, Acalypha indica, Vitex negundo, Aloe vera, and Justicia adhatoda leaves were amassed from a plant nursery near ECR and enclosed in a sterilized resealable plastic bag before being transported to the facility for further processing. Rosa damascene petals were bought from a plant nursery, and Citrus sinensis outer peel was obtained directly from a local fruit merchant. To abstract surface impurities, accumulated leaves, petals, and outer peel were washed with tap dihydrogen monoxide, then sterile distilled dihydrogen monoxide. The washed material was dried for 12 hours at 600°C to 800°C in a sultry air furnace. The respective plant materials were engendered by homogenising fine dried plant powder in a mixer. The fine powder was filtered through a 25-mesh filter, resulting in a fine and uniform powder that was stored in an airtight plastic container until further use.

EXTRACTION OF PLANT MATERIAL

Utilizing distilled water, dried plant powders of the respective plants were yare. 100 g of powdered leaves in 1 litre of dihydrogen monoxide in a flask, and were filtering through a Whatman No. 1 paper filter. The extracts were filtered and chilled to a volume of 500 ml each in separate quantifying glass borosilicate bottles.

ESTIMATION OF TOTAL YIELD

A weighing scale was used to weigh the filtrate produced from each extracted sample. The powder products were weighed, and the results were utilised to calculate the extraction yield.

Extraction yield % = Final weight of plant Extract / Initial weighed of plant dried material X 100

PHYTOCHEMICAL ANALYSIS

Phytochemicals analysis such as alkaloids, flavonoids, glycosides, reducing sugar, saponins, steroids, phenols, terpenoids, anthraquinones, tannins, and phlobatannin were tested for the untreated and treated groups by using the phytochemical method. [16,17,18,19]

EFFECT OF HEAT TREATMENT ON PLANT EXTRACT

The known volume of the respective plant extract (25 ml with 0.5µl/ml of final concentration) which contains the active bioactive compound was moved to a sterilized screw-capped vial which was subjected to autoclaving (1210 for 30 mins at 15 Psi). To prevent the heat loss a layer of mineral oil was added on the top of the extract. After autoclaving the samples were incubated at an ambient condition. The changes in the phytochemical constituents were also checked as discussed earlier. [20]

ANTIBACTERIAL ACTIVITY

BACTERIAL STRAINS

The bacterial strain ATCC 25923 Staphylococcus aureus was acquired from Thandalam, Saveetha Medical College and Hospital in Chennai. The strain was grown on Trypticase soy Agar slants and kept at 8-10 degrees Celsius. The inoculum was created in nutrient broth and used for future research.

WELL DIFFUSION ASSAY

The well diffusion experiment was used to investigate the anti-bacterial activity. After incubation, the inoculum was evenly dispersed utilizing sterilized cotton swabs over sterile Mueller Hinton (MH) Agar Media (Hi-media, India). Wells were bored with a cork borer and aliquots of corresponding plant extract for the treated and untreated groups (25 µl, 50 µl, 75 µl, and 100 µl were engendered from concentrated stock) were placed into the wells. The plates were incubated at 37°C for 24 hours. Each treatment was replicated three times. After the incubation time, the zone of inhibition was quantified. The effect of the heat treatment on the antimicrobial studies was calculated by the following formula: efficacy

$$Antimicrobial\ efficacy(\%)$$

$$= \frac{Zone\ of\ inhibition\ in\ the\ untreated\ group - zone\ of\ inhibition\ in\ the\ treated\ group}{Zone\ of\ inhibition\ in\ the\ untreated\ group} X\ 100$$

RATE OF KILL DETERMINATION

The rate of killing of the selected bacterial species for aqueous extract was tenacious utilizing a modified plating procedure in which the extract was mixed into 10 mL Mueller Hinton broth in McCartney bottles at 1/2, 1 MIC, and 2 MIC. One control and one test was taken, where one with the first bottle was with Mueller-Hinton broth without extract that was infected with test organisms, and the other one was Mueller-Hinton broth that coalesced with the extract at the test concentrations but did not contain the test organisms. The inoculum density of about 106 cfu/mL was used to inoculate 10 mL volumes of both the Test and control borosilicate bottles. The bottles were agitated on a shaker at 37°C at 350 rpm. At 0hr, 4hr, and 8 hr, a 100 mL aliquot was removed from the culture medium for the plate count technique to measure cfu/mL by plating out 25 mL of each of the dilutions. Emerging bacterial colonies were counted after 24 hours at 37°C, cfu/mL was calculated, and the count was compared to the count of the culture control without the extract.[21]

RESULTS AND DISCUSSION

Plants produce a vast range of secondary metabolites, which are found in larger quantities within the plants. Secondary metabolites act as chemical barriers to keep diseases and herbivores at bay. The secondary metabolites with antimicrobial properties are working in a coordinated manner in order to avoid pathogen infection. The stability of these metabolites greatly influences the pharmacological efficacy of medicinal plants. As a result, it is critical to investigate the role of various physicochemical parameters in antibacterial activity. With this goal in mind, the current study looked at how treatment affected the antibacterial activity of medicinal plant extracts.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The therapeutic potential of Traditional plant species' is based on phytochemical components which have particular pharmacological impact on the human body. The phytochemical features of medicinal plants were studied in order to demonstrate and isolate the medicinal lead compounds, and components from different parts of the plant. The phytochemical features of plants can be used to identify their distinct biological activity. [22]. The qualitative phytochemical analysis of the aqueous plant extracts for treated and untreated sets were performed. [Tables 1&2]. Plant knowledge has proven extremely useful in the creation of many medications.

Table I: Phytochemical analysis for Aqueous extract (Untreated)

Phytochemical analysis for Aqueous extract (Untreated)								
S.no	**Plant Name** / **Phytochemical compounds**	*Citrus sinensis*	*Justicia adhatoda*	*Aloe vera*	*Vitex negundo*	*Acalypha indica*	*Azadirachta indica*	*Rosa damascene*
1	Alkaloids	+	+	+	+	+	+	-
2	Flavonoid	+	+	+	+	+	+	+
3	Glycosides	+	+	+	+	+	+	-

4	Reducing sugar	+	+	+	+	+	+	+
5	Saponin	+	-	+	+	+	-	+
6	Steroids	-	+	+	-	-	+	-
7	Phenols	+	+	+	-	+	+	+
8	Terpenoid	+	-	+	-	-	-	+
9	Anthraquinone	+	-	+	+	+	+	+
10	Tannin	+	+	+	-	-	+	+
11	Phlobatanins	-	+	-	-	-	+	-

Table I: Phytochemical analysis for Aqueous extract (Untreated) which shows us the presence or absence of Bioactive compounds by (+) which indicates the presence of phytochemical compounds in plants; (-) which indicates the absence of phytochemical compounds in plants

Table II: Phytochemical analysis for Aqueous extract (treated)

Phytochemical analysis for Aqueous extract (Treated)								
S.no	Plant Name / Phytochemical compounds	*Citrus sinensis*	*Justicia adhatoda*	*Aloe vera*	*Vitex negundo*	*Acalypha indica*	*Azadirachta indica*	*Rosa damascene*
1	Alkaloids	-	-	-	-	-	-	-
2	Flavonoid	-	-	-	-	-	-	-
3	Glycosides	+	+	+	+	+	+	-
4	Reducing sugar	-	-	-	-	-	-	-
5	Saponin	+	-	+	+	+	-	+
6	Steroids	-	-	-	-	-	-	-
7	Phenols	-	-	-	-	-	-	-
8	Terpenoid	-	-	-	-	-	-	-
9	Anthraquinone	+	-	+	+	+	+	+
10	Tannin	+	+	+	-	-	+	+
11	Phlobatannin	-	-	-	-	-	-	-

Table II: Phytochemical analysis for Aqueous extract (treated) which shows us the presence or absence of Bioactive compounds by (+) which indicates the presence of phytochemical compounds in plants; (-) which indicates the absence of phytochemical compounds in plants

Plants are considered to be a rich source of a variety of compounds that can be employed in the pharmaceutical manufacturing process. Alkaloids are crucial secondary metabolites with pharmacological potential. [23] Citrus sinensis, Justicia adhatoda, Aloe vera, and Vitex negundo were shown to be unaltered in the untreated groups. It was observed in all types of plants in the treated group, including Rosa damascene, Acalypha indica, and Azadirachta indica, because it was thermally unstable.

Flavonoids are antioxidant-rich natural compounds generated in various sections of plants. When reactive oxygen species (ROS) are produced, flavonoids have the capacity to regulate their build up through scavenging ROS. As a result, many antioxidant compounds play a crucial role in plants stress resistance and have a significant implication in human health because of their anti-inflammatory and antibacterial effects. [24]. It was found in nearly all of the plants. Because flavonoids are heat-sensitive, they were not discovered in any of the treated plants. Heat applied directly at 75 °C can damage enzyme function and impede the flavonoid production process. [25]

Medicinal plants contain high levels of naturally occurring glycosides. Several of these glycosides have anti-cancer properties. Glycosides extracted from pharmaceutical plants have been isolated and administered as alternative therapies for a diverse range of malignancies. They are also thought to be heat stable. [26] The glycosides were found in all untreated and treated plants except Rosa damascene.

Sugars impact all phases of the plant life cycle, interact with other signalling molecules, including phytohormones, and govern plant growth and development due to their position as energy and carbon sources as well as their regulatory roles. A high sugar content in plant tissues improves the plant's immunological response to fungal infections. Sucrose and monosaccharides have been shown to boost effective defence systems in plants against fungal infections. Reducing sugars are extremely unstable over 450 Celsius [27]. Reducing sugars were identified in all untreated groups, but not in most plants due to their unstable nature.

Saponins are a diverse family of secondary metabolites. Many plants used in traditional medicines worldwide contain saponins, which can often account for their therapeutic action. It is believed that the natural role of these compounds in plants is to protect against attack by potential pathogens, which would account for their antimicrobial activity. Although saponins are extremely toxic to cold-blooded animals, their oral toxicity to mammals is low. Saponins are a broad class of secondary metabolites. It is thought that these chemicals' natural job in plants is to defend against possible infections, which would explain for their antibacterial effect. Although saponins are particularly poisonous to cold-blooded species, they have a modest oral toxicity in mammals and are heat stable at high temperatures. [28] Saponins were identified in virtually all of the plants in both the untreated and treated groups, with the exception of Justicia adhatoda and Azadirachta indica.

Crops manufacture a wide and diversified range of secondary metabolites, represented by phenols and polyphenols, for physiological purposes in their life cycle. Plants' manufacture of phenol and polyphenol chemicals has grown to assist them cope with a variety of abiotic and biotic challenges such as salt, heavy metals, drought, temperature, UV radiation, and disease development. [29] Except for Vitex negundo, phenols are found in all of the plants in the untreated set. In the treated group, it was not found in any plant because they thermally unstable above 900C [30]

Because of their evolutionary and biochemical selection for biological activity in animals, specialised plant terpenoids have found serendipitous uses in medicine. While certain plant terpenes, like -carotene, phylloquinone, and tocopherols, are vital (pro)vitamins in the human diet, others, like phytosterols and essential oils, give nutritional advantages or function as anti-oxidants. [31] Terpenoids compounds were found in the untreated groups of Citrus sinensis, Aloe vera, and Rosa damascene but not in the other plants. Terpenoids are unstable when heated over 1000 degrees Celsius, hence it was not seen in any of the treated plants.

The metabolites of the anthraquinone class and their derivatives are noted for their chemical diversity and various biological/pharmacological effects. Anti-inflammatory, antifungal, enzyme inhibitory, antibacterial, anti-obesity, anti-biofilm formation, anti-fouling, antiviral, antiparasitic, anti-angiogenesis, antioxidant, algicidal, insecticide, anticoagulant, and cytotoxic properties were demonstrated by the compounds. Anthraquinones are relatively stable and disintegrate at 2560 degrees Celsius [32]. Except in Justicia adhatoda, the anthraquinone metabolite was detected in both the untreated and treated groups.

Plant tannins have been discovered to have a range of biological roles as a natural polyphenolic compound. The potential value of plant tannins as feed additives in animal production is immense; plant tannins have a wide range of effects against pathogenic bacteria and distinct mechanisms of action against different organisms. They are extremely heat stable and begin to degrade around 196.91° [33]. Tannins were found in all plants except Vitex negundo and Acalypha indica in both the untreated and treated groups.

Plant sterols and steroid hormones, the brassinosteroids (BRs), are compounds that exert a wide range of biological activities. They are essential for plant growth, reproduction, and responses to various abiotic and biotic stresses.

Brassinosteroids (BRs) are plant sterols and steroid hormones that have a variety of biological actions. They are required for plant growth, reproduction, and resistance to a variety of abiotic and biotic stressors. They are unstable because they deteriorate at temperatures exceeding 700°C. [34] Only Justicia adhatoda, Aloe vera, Azadirachta indica, and Rosa damascene had compound steroids for treated groups. The steroid was inactivated and not seen in the treated groups.

Phlobatannin has been found to have anti-inflammatory and analgesic wound healing effects. They are heat sensitive and deteriorate between 700 and 800 Celsius [35]. For the untreated group, the component phlobatannin is found solely in Justicia adhatoda. No phlobatannin-treated plants were found in the treatment group.

YIELD CALCULATION

The yield of the plant material was weighed initially and finally for the Aqueous plant extract before being treated with heat. The following formula was used to calculate:

Extraction yield %= Final weight of plant Extract / Initial weighed of plant dried material X 100

The highest extraction yield was observed in Vitex negundo, Justicia adhatoda and Acalypha indica followed by the remaining plants which was between 9-10%. [Figure 1]

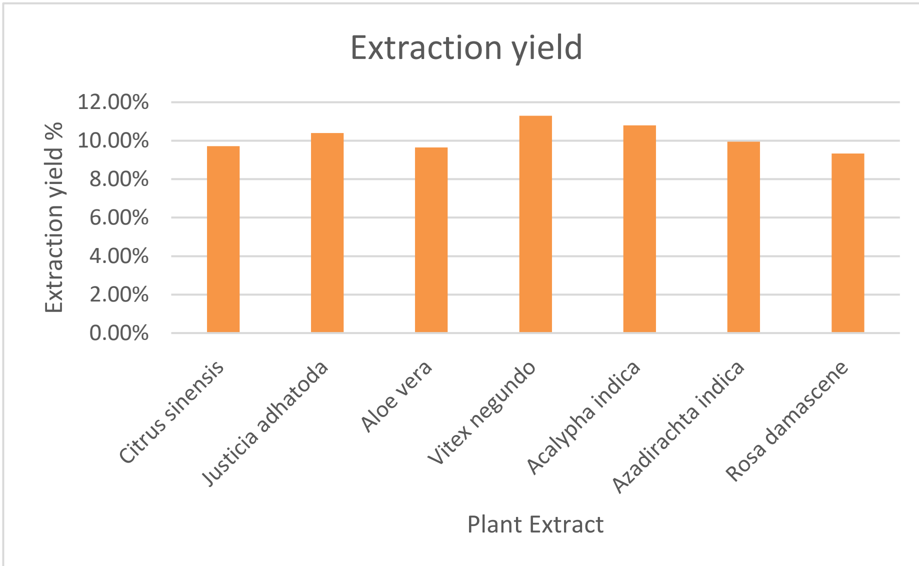

Figure 1: Total Extraction yield obtained

WELL-PLATE DIFFUSION ASSAY

The diffusion of agar wells methodology is frequently used to evaluate the antibacterial activity of plants or microbiological extracts and it was done in triplicates. The heat-treated aqueous extracts of Azadirachta indica, Acalypha indica, Vitex negundo, Aloe vera, Justicia adhatoda, Rosa damascene, and Citrus sinensis were shown to be ineffective and non-inhibitory for ATCC 25923 Staphylococcus aureus, as shown in the Figure 2A and 2B. On the contrary, the untreated groups showed the zone of inhibiton.

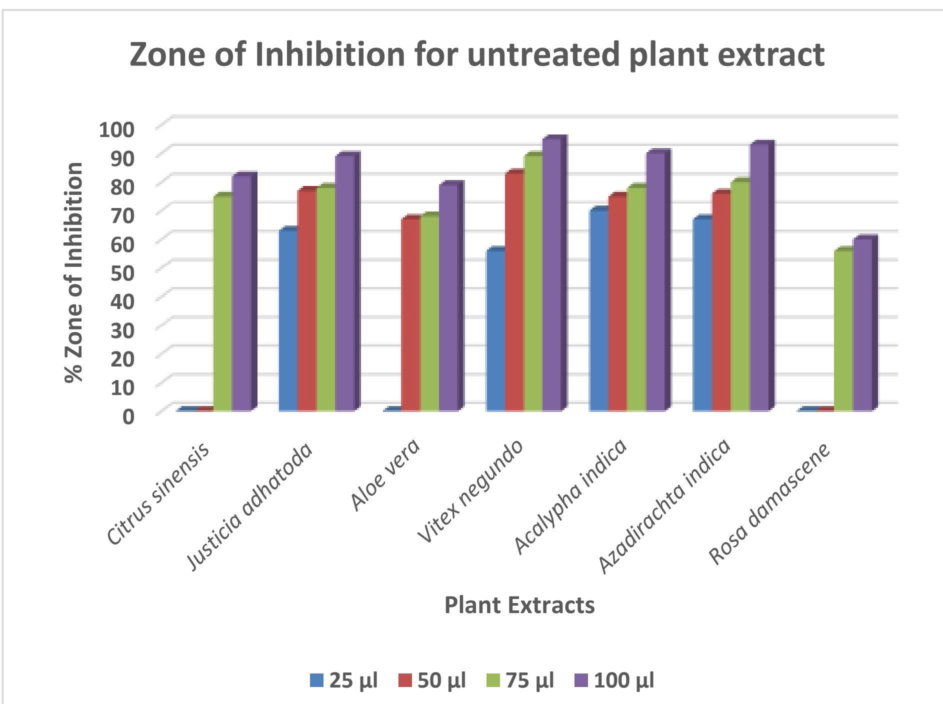

Figure 2A: Zone of Inhibition for untreated plant extract

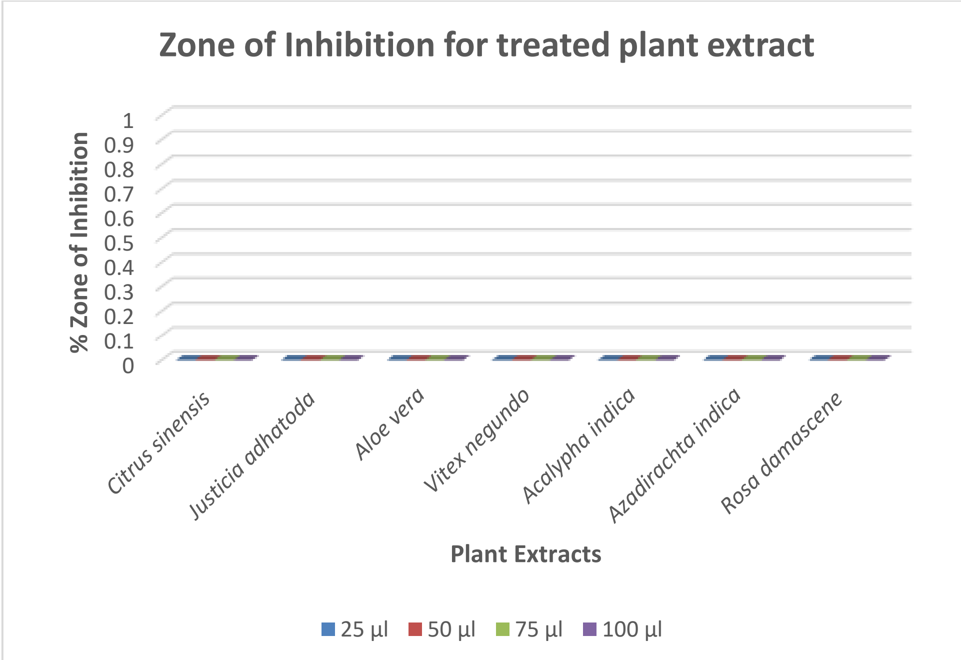

Figure 2B: Zone of Inhibition for treated plant extract

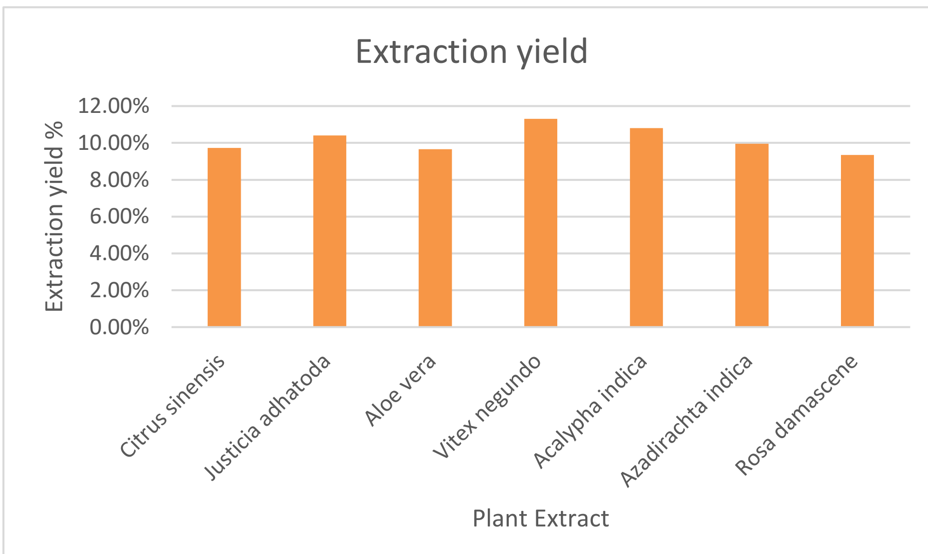

Figure 3: Total Extraction yield obtained

DETERMINATION OF RATE OF KILL

The study was conducted to understand the basic pharmacodynamic information on the relationship between medicinal plant extracts concentrations and the growth of ATCC 25923 Staphylococcus aureus, which has contributed to a better understanding for the current and future drug administration for diseases caused by Staphylococcus aureus. The findings of the time-kill experiment showed no transmutations in viable colonies, designating that the extract exhibited no substantial bactericidal or bacteriostatic action. Thus, we can conclude that the plant extracts have lost they stability during the process of autoclaving.

Conclusion

Antibiotic activity in medicinal plants offers new promise for overcoming the difficult difficulties faced by rising evidence of antibiotic resistance. Plants contain bioactive compounds that increase the ability of resistance against bacterial pathogens. The pharmacological activity of the medicinal plant extracts is heavily depended on the physico chemical characteristics of metabolites the loss of pharmacological activity is mainly due to the metabolite that are subjected to inactivation. In this study, we subjected the test aqueous plant extract to autoclaving and analogue the effect on anti-bacterial activity against

Staphylococcus aureus. The results clearly indicated all the autoclaved plant extract showed no zone of inhibition against the tested bacterial strains. On the contrary, the plant extract which was untreated showed zone of inhibition. The following results are novel, as per our knowledge that this heat treatment procedure is being used for the first time. These findings are being supported by the experiment carried out by Nagwa M. Atef et al., who has produced the extract with water and observed anti-bacterial activity [36], confirming that bioactive compounds plant an important role in suppressing harmful microorganisms.

ACKNOWLEDGEMENTS

We would like to acknowledge the staff and head of the Microbiology department of Saveetha medical college and hospital for their support. We would also like to thank the white lab from Saveetha dental college for their support.

References

1. Süntar, I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Rev 19, 2020, 1199–1209.
2. Santhan P. A field study on Indian medicinal plants. J Med Plants Stud 2020;8(4):198-205.
3. Damask rose and marigold: prospective industrial crops. Kaul, V. K. ; Virendra Singh ; Bikram Singh Journal of Medicinal and Aromatic Plant Sciences 2000,22 (1B),.313-318
4. Journal of Pharmacy and Pharmacology, February 2020, 72 (2),161–174,
5. Rathod VS, Pawar PS, Gorde AB. A review on pharmacological importance of azadirachta indica. World Journal of Pharmaceutical Research,2022 ,11(4) 412-419
6. Harriet U. Ugboko, Obinna C. Nwinyi, Solomon U. Oranusi, Toluwase H. Fatoki, Conrad A. Omonhinmin, "Antimicrobial Importance of Medicinal Plants in Nigeria", The Scientific World Journal, 20200, 20(20), 10.
7. Attah Friday, Otalu Otalu, Cletus A. Ukwubile, Otuoze Victor Onoruoyiza, Garba Daniel Edisha. “Use of Medicinal Plants as Antimicrobial Agents: A Review of the Successes Story.” International Journal of Medicinal Plants and Natural Products (IJMPNP), 2020, 6(3),28-33
8. Balakrishnan K, Manoharan a. 2022. Preliminary phytochemical, biochemical and ftir spectroscopic analysis of kuppaimeni chooranam (acalypha indica linn). World Journal of Pharmaceutical Research,2022, 11(7),1213-1218
9. Moshi, MJ. “Current and Future Prospects of Integrating Traditional and Alternative Medicine in the Management of Diseases in Tanzania | Tanzania Journal of Health Research.” Tanzania Journal of Health Research, 2005 Sep;7(3):159-67.
10. R., Singh. “Medicinal Plants: A Review:: Science Publishing Group.” Journal of Plant Sciences, May 2015, 3(1),50-55
11. Keita, K., Darkoh, C. & Okafor, F. Secondary plant metabolites as potent drug candidates against antimicrobial-resistant pathogens. SN Appl. Sci. 2022;4(8):209.
12. Pirintsos, Stergios, et al. “From Traditional Ethnopharmacology to Modern Natural Drug Discovery: A Methodology Discussion and Specific Examples.” Molecules, June 2022, 27 (13),4060.
13. Zamora-Mendoza, Lizbeth, et al. “Antimicrobial Properties of Plant Fibers.” Molecules, Nov. 2022, 27(22), 7999.
14. Secretariat. “Traditional Medicine.” World Health Organization, 13 Dec. 2013, apps.who.int/gb/ebwha/pdf_files/EB134/B134_24-en.pdf.
15. Vaou, Natalia, et al. “Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives.” Microorganisms, Sept. 2021, 9 (10), 2041.
16. S A, M Sankareswaran , M M, B E, E F. Phytochemical Analysis and Antifungal Activity of Vitex negundo Leaf Extracts Against Clinically Isolated Fungal Pathogens. Indian Journal of Applied Microbiology. December 2017,20(2),119-125
17. Pawar s, kamble v. 2017. Phytochemical screening, elemental and functional group analysis of vitex negundo l. Leaves. Int j pharm pharm sci,. June 2017, 9(6):226
18. Pawar RS, Jain A, Sharma Preeti , Chaurasiya Pradeep Kumar , Singour PK.. In Vitro Studies on Sida Cordifolia Linn for Anthelmintic and Antioxidant Properties. Scientific research, June 2011, 2(2)149–153.
19. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants’ extracts. Afr j tradit complement altern med 2011,8:1–10.
20. Nadolny, Zbigniew, and Grzegorz Dombek. “Thermal Properties of Mixtures of Mineral Oil and Natural Ester in Terms of Their Application in the Transformer, Oct. 2017, 19,6
21. Afolayan, Anthony Jide, and Olufunmiso Olusola Olajuyigbe. “In Vitro Antibacterial and Time-Kill Evaluation of the Erythrina Caffra Thunb. Extract Against Bacteria Associated With Diarrhoea.” Hindawi, Nov. 2012,
22. Agidew, M.G. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bull Natl Res Cent (2022). 46, 87
23. Roy, Arpita. A review on the alkaloids an important therapeutic compound from plants. International Journal of Plant Biotechnology, 2017, 3. 1-9.
24. Dias, M.C.; Pinto, D.C.G.A.; Silva, A.M.S. Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules 2021, 26, 5377.
25. Zhang X, Wang X, Wang M, Cao J, Xiao J, Wang Q. Effects of different pretreatments on flavonoids and antioxidant activity of Dryopteris erythrosora leave. PLoS One, Jan. 2019 2;14(1)
26. Khan, Haroon & Saeedi, Mina & Nabavi, Seyed & Mubarak, Mohammad & Bishayee, Anupam. Glycosides from Medicinal Plants as Potential Anticancer Agents: Emerging Trends Towards Future Drugs. Current medicinal chemistry. 2019;26(13):2389-2406.
27. Jeandet P, Formela-Luboińska M, Labudda M, Morkunas I. The Role of Sugars in Plant Responses to Stress and Their Regulatory Function during Development. Int J Mol Sci. May 2022 5;23(9):5161.
28. Desai, Sapna & Desai, D.G. & Kaur, Harmeet. Saponins and their biological activities. Pharma Times. 2009,41. 13-16
29. Palistha Tuladhar, Santanu Sasidharan, Prakash Saudagar, Role of phenols and polyphenols in plant defense response to biotic and abiotic stresses, Biocontrol Agents and Secondary Metabolites, 2021, 419-441
30. Antony A, Farid M. Effect of Temperatures on Polyphenols during Extraction. Applied Sciences 2022, 12(4), 2107;
31. Bergman ME, Davis B, Phillips MA. Medically Useful Plant Terpenoids: Biosynthesis, Occurrence, and Mechanism of Action. Molecules 2019, 24(21), 3961;
32. Hafez Ghoran S, Taktaz F, Ayatollahi SA, Kijjoa A. 2022. Anthraquinones and Their Analogues from Marine-Derived Fungi: Chemistry and Biological Activities. Marine Drugs 2022, 20(8), 474;
33. Tong Z, He W, Fan X, Guo A. 2021. Biological Function of Plant Tannin and Its Application in Animal Health. Frontiers ,2021,8
34. Vriet, Cécile & Russinova, Eugenia & Reuzeau, Christophe. (2012). Boosting Crop Yields with Plant Steroids. The Plant cell. 2012 Mar;24(3):842-57.
35. Wadood, Abdul. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochemistry & Analytical Biochemistry.

2013,2(4), 1-4
36. Atef, N.M., Shanab, S.M., Negm, S.I. et al. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. Bull Natl Res Cent (2019). 43, 144.